Drug-Resistant Tuberculosis in Children

Thesis for Doctorate in Philosophy

James Alexander Seddon

Department of Clinical Research
Faculty of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine

Supervisors and collaborators

Supervisors

Professor Peter Godfrey-Faussett (London School of Hygiene and Tropical Medicine)
Professor Simon Schaaf (Stellenbosch University)
Professor Anneke Hesseling (Stellenbosch University)

Collaborators

Professor Nulda Beyers (Stellenbosch University)
Professor Robert Gie (Stellenbosch University)
Dr. Katherine Fielding (London School of Hygiene and Tropical Medicine)
Professor Don Enarson (The International Union Against Tuberculosis & Lung Disease)
Dr. Helen Cox (Médecins sans Frontières)
Dr. Jennifer Hughes (Médecins sans Frontières)
Dr. Cheryl McDermid (Médecins sans Frontières)
Dr. Stephanie Thee (Charité, Universitätsmedizin)
Mr. Adam Ebrahim (Brooklyn Chest Hospital)
Miss Kayleen Jacobs (Brooklyn Chest Hospital)
Mr. Rory Dunbar (Stellenbosch University)
Dr. Heather Finlayson (Tygerberg Children's Hospital)
Professor Peter Donald (Stellenbosch University)
Professor Gert Vlok (Stellenbosch University)
Mrs. Klassina Zimri (Stellenbosch University)
Miss Zingiwe Mramba (Stellenbosch University)
Mr Wayne Green (Stellenbosch University)
Professor Robert Warren (Stellenbosch University)
Professor Tommie Victor (Stellenbosch University)
Ms Annemie Jordaan (Stellenbosch University)
Professor Ben Marais (University of Sydney)
Dr. Marianne Willemse (Brooklyn Chest Hospital)
Dr. Douwe Visser (VU University Medical Centre)
Miss Margaux Bartens (VU University Medical Centre)
Professor Marceline van Furth (VU University Medical Centre)
Professor Johan Schoeman (Stellenbosch University)

Other collaborators

Provincial Government of the Western Cape
City of Cape Town Health Authority
Médecins sans Frontières
The International Union against Tuberculosis and Lung Disease
United States Agency for International Development

Declaration

I, James Alexander Seddon, confirm that the work presented in this thesis is my own. Where work has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:

Date: 29 NOVEMBER 2012

Abstract

The number of children globally who develop drug-resistant tuberculosis is unclear, in part due to diagnostic challenges and limited resistance testing, and in part because recording and reporting is not comprehensive. Large numbers of children, however, are exposed to drug-resistant bacilli each year and it is clear that the very young and those immune-compromised are vulnerable to developing disease.

Few studies have looked at the progression from exposure to infection or from infection to disease in the child contacts of adults with drug-resistant tuberculosis. It is uncertain which factors influence this progression and also whether any interventions can prevent it. Finally, few studies have analysed the presentation, treatment and outcome of children with disease.

This thesis starts by reviewing what is published regarding drug-resistant tuberculosis in children. This includes systematic reviews of the management of children exposed to drug-resistant tuberculosis as well as the management of those with multidrug-resistant tuberculosis disease. It reviews what is known regarding the second-line tuberculosis drugs in children and then clarifies the definitions that are used throughout the rest of the work.

The thesis then systematically examines each of the stages from infection to disease with a series of inter-related studies. The first study attempts to quantify the burden of drug-resistance in the context that the work is carried out. The following study investigates the risk factors for infection and prevalent disease in children exposed to a multidrug-resistant tuberculosis source case. This is followed by two studies which explore the transmission of drug-resistant bacilli from adults to children. The identification and referral patterns and obstacles to referral for exposed children are examined through operational studies that include qualitative and quantitative components. A descriptive cohort study assesses the toxicity and efficacy of a standardised preventive treatment regimen given to child contacts. The final part of the thesis includes a series of studies to investigate the treatment of drug-resistant tuberculosis disease in children and the adverse effects of the second-line medications.

Table of Contents

Tables

Figures

Acknowledgements

None of this work would have been possible without the support of the children and families living with drug-resistant tuberculosis in the Western Cape. On a daily basis these families deal with challenging living conditions and a difficult diagnosis. They cope with complicated and unpleasant treatments and generally face it all with a bright smile and a sense of humour. I am continually amazed and touched by the resilience of the human spirit.

The work would also not have been possible without the financial support of the United States Agency for International Development, working through TREAT TB and the International Union Against Tuberculosis and Lung Disease. The Sir Halley Steward Trust also generously provided a fellowship for me, allowing me to live and work in Cape Town.

It feels a very long time since I started out on this journey and I would not be here without the huge amounts of help and support that I have received. The first person to thank is Peter Godfrey-Faussett. He was there at the beginning and has supported me throughout. Constantly probing, usually sceptical and always curious. Never to accept anything at face value and never to tolerate anything other than the highest of standards.

I owe an enormous debt of gratitude to Simon Schaaf. A kind, generous, caring, thoughtful and modest man who always finds time for colleagues and juniors but most of all, he always finds time for patients and their families. I have learnt so much, not only about how to be an academic but also how to be a better doctor. The work in this thesis is a continuation of the work that he has been carrying out tirelessly for over twenty years and he has helped and supported me through all of it.

To Anneke Hesseling who has been a wonderful supervisor, colleague and friend. She is one of the hardest working, most energetic and most enthusiastic people that I have ever met, mentoring countless students and juniors and supporting so many others in their careers. She has an amazing breadth of insight into so many aspects of research and her advice is always hugely appreciated.

The next person that I would like to thank is Nulda Beyers, who leads the Desmond Tutu TB Centre with warmth, laughter and amazing good humour. She made it possible for me to go to Cape Town and has supported me in every aspect of my time there, both in work and outside.

The environment at the Centre is unique in its nurturing, supportive and inclusive nature – all a direct result of Nulda's leadership. Rob Gie has provided help and support at every turn. He is always insightful and is usually the best person to turn to for advice or when you need to look at a problem with a new perspective. He is a wonderful clinician and a thoughtful and generous person. Peter Donald is a father figure for many. He inspired others to study paediatric tuberculosis when no one else in the world was interested, pulling people along with his energy, humility and passion. I feel hugely privileged to have sat in Brooklyn Chest Hospital on many blustery days, discussing life and work and the finer points of history with him. Don Enarson has given support, advice and wonderful insight. He provides such a critical scientific and epidemiological perspective and never stops asking important questions. Katherine Fielding has kept me on track in the world of statistics and has been incredibly generous with her time and assistance. The design, conduct and analysis of my studies would have been considerably worse without her help and input.

There are three very special people without whom I would have achieved only a fraction of what I have managed to achieve. Klassina Zimri has been the most amazing study nurse, manager, co-ordinator and general star. She is hard-working, generous, thoughtful, kind and always ready with a solution to the most recent challenge. Zingiwe Mramba kept us all on track with her amazing organisation, dedication and good humour. She is a highly intelligent and motivated lady who solved so many problems with her insight and initiative. Wayne Green worked tirelessly driving to all corners of the province, never complaining, always positive and always gracious. He is a proper gentleman who would never give up if there was still work to be done. The rest of the Desmond Tutu TB Centre, and the paediatric team in particular, have been wonderful colleagues and have become great friends.

My uncle Keith McAdam and my father David Seddon, both hugely experienced academics, provided me with advice, suggestions and direction throughout. They read my work and provided critical input and support. The rest of my family and friends have been a tremendous source of strength and kindness and it would not have been possible to have completed everything without such a wonderful network. Particular thanks go to my mother, Elspeth McAdam, step-father, Martin Watson, newly acquired parents in John and Di Morley and my siblings Michael, Daniel and Olivia.

The final person to thank is the woman who started this journey with me when we met in Cape Town at the end of 2008. The most kind, generous and supportive woman I have ever met, enormously intelligent and the opinion I listen to above all others. Thank you, Lauren.

Abbreviations

AAP	American Academy of Pediatrics
ABR	Auditory brainstem response
AOR	Adjusted odds ratio
ASHA	American Speech and Hearing Association
ATS	American Thoracic Society
BCG	Bacille Calmette–Guérin
BCH	Brooklyn Chest Hospital
BMI	Body mass index
cART	Combination antiretroviral therapy
CDC	Centers for Disease Control and Prevention
CHW	Community health worker
CI	Confidence interval
C_{max}	Maximum serum concentration
CR	Chest radiograph
CSF	Cerebrospinal fluid
CT	Computerised tomography
DMID	Division of Microbiology and Infectious Diseases
DOT	Directly observed therapy
DPOAE	Distortion product otoacoustic emissions
DQ	Developmental quotient
DR	Drug-resistant
DST	Drug susceptibility test
EBA	Early bactericidal activity
EPTB	Extrapulmonary tuberculosis
FBC	Full blood count
FNAB	Fine needle aspiration biopsy
GCS	Glasgow coma score
HIV	Human Immunodeficiency Virus
HMR	Isoniazid monoresistant
IDSA	Infectious Disease Society of America
IGRA	Interferon gamma release assay
IM	Intramuscular
IQR	Interquartile range

IRIS	Immune reconstitution inflammatory syndrome
IV	Intravenous
LFT	Liver function tests
LPA	Line probe assay
LRT	Likelihood ratio test
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
MPC	Mutant prevention concentration
MRI	Magnetic resonance imaging
MSF	Médecins Sans Frontières
MUAC	Mid upper arm circumference
NAAT	Nucleic acid amplification test
NICE	National Institute of Health and Clinical Excellence
NTM	Non-tuberculous mycobacteria
OAE	Otoacoustic emissions
OR	Odds ratio
PAS	*Para*-aminosalicylic acid
PCR	Polymerase chain reaction
PI	Protease inhibitor
PTA	Pure tone audiometry
RFLP	Restriction fragment length polymorphism
RMR	Rifampicin monoresistant
RR	Rate ratio
SD	Standard deviation
t½	Half life
fT_4	Free thyroxine
TB	Tuberculosis
TBM	Tuberculous meningitis
TCH	Tygerberg Children's Hospital
t_{max}	Time to maximum serum concentration
TSH	Thyroid stimulating hormone
TST	Tuberculin skin test
WFA	Weight-for-age
WHO	World Health Organization
XDR	Extensively drug-resistant

Introduction

The burden of drug-resistant tuberculosis infection and disease in children

Determining the global burden of tuberculosis (TB) is difficult as reporting is inconsistent and tests for infection and disease are imperfect. Traditional estimates suggest that a third of the world population may be infected with TB[1] and that each year nine million people develop the disease, with an estimated 1.7 million dying from it.[2-3] Challenges in determining the scale of the paediatric TB epidemic are compounded as the diagnosis of *Mycobacterium tuberculosis* infection and disease in children is even more problematic than in adults. The tuberculin skin test (TST) and commercial interferon gamma release assays (IGRAs) have limited sensitivity and specificity to detect *M. tuberculosis* infection in children[4] and due to the paucibacillary nature of paediatric TB, together with difficulties in obtaining clinical samples, microbiologically confirmed TB disease is typically obtained in less than 30% of children with radiological/clinical evidence of intra-thoracic pathology.[5] The diagnosis of TB relies on a constellation of history, examination, immunology, radiology and bacteriology, all with limited sensitivity and specificity.[6] As even children with culture-confirmed TB are frequently not recorded and reported in TB registers,[7] the total number of children reported with TB is likely an under-estimate of the actual burden.

Drug-resistant (DR) TB presents a challenge to international public health. It is a man-made problem arising through ineffective treatment regimens and failures of health programming.[8] DR-TB is defined as *M. tuberculosis* resistant to any first-line TB medication but once the bacilli are resistant to rifampicin and isoniazid, they are said to be multidrug-resistant (MDR).[9-10] Extensively drug-resistant (XDR) TB is caused by bacilli that are, in addition, resistant to a fluoroquinolone and an injectable second-line TB medication.[11-12] The World Health Organization (WHO) estimates 650,000 prevalent cases of MDR-TB each year[13] and cases have now been seen in most countries in the world;[14] in some areas of Eastern Europe and Central Asia multidrug resistance is seen in more than 30% of all TB cases. Although in sub-Saharan Africa and Southern Asia the proportion is lower, the total number of cases is high due to high overall TB burden. South Africa has one of the highest incidences of TB in the world, with rates in the townships surrounding Cape Town exceeding 1,000 per 100,000.[2] Over two percent of TB cases are MDR and this figure has risen in the last decade reflecting increasing drug resistance, increasing detection of drug resistance or a combination of the two.[2, 15] To diagnose

DR-TB either the bacilli must be demonstrated to grow in the presence of an antibiotic (phenotypic resistance) or to possess genetic mutations known to be associated with drug resistance (genotypic resistance). It is therefore not possible to diagnose DR-TB from sputum smear microscopy alone. In many regions of the world drug resistance testing is unavailable and DR-TB is therefore not diagnosed, reported or treated.

As paediatric TB is under-reported and as DR-TB is challenging to determine, it is not a surprise that the number of reported cases of paediatric DR-TB is limited. Prior to the studies presented in this thesis, only two hundred children had been described with MDR-TB in the medical literature. As over half a million cases of MDR-TB are estimated to occur each year globally[13] and as children comprise up to 20% of the total TB burden in high TB incidence settings,[16-18] the burden of children with DR-TB is significantly under-reported.

Tuberculosis pathophysiology and immunology

Following exposure to aerosolised *M. tuberculosis* some children will become infected. Once infection has occurred the adaptive immune system recognises the bacilli; it may clear the infection, fail to contain it or reach an equilibrium in which the immune system is unable to eradicate the infection but prevents it from progressing. For those with *M. tuberculosis* infection, a proportion will, at some point in the future, progress to TB disease.[19] The risk for this is greatest in the first couple of years following infection[20] and for young children, 90% of those progressing to disease do so in the first year.[21] From data collected prior to the era of chemotherapy, it is clear that a proportion of children infected with *M. tuberculosis* will develop chest radiology changes that spontaneously resolve without treatment. However, throughout the thesis, TB disease is defined as either symptomatic illness or chest radiology changes consistent with TB. Children with *M. tuberculosis* infection, therefore, have no clinical symptoms or signs and radiology that is normal. Traditionally the only means of detecting *M. tuberculosis* infection was through a history of exposure and a positive TST such as the Mantoux or Tine test. The crude antigen mixture used, however, does not completely differentiate between Bacillus Calmette-Guérin (BCG), *M. tuberculosis* and environmental, non-tuberculous mycobacteria (NTM).[22] It can take up to three months following infection for an individual to mount a response and the response is affected by human immunodeficiency virus (HIV) infection,[23] malnutrition and other immunosuppressive states such as viral infections, steroid use or neoplastic disorders.[20] Sensitivity and specificity are difficult to measure in the absence of a gold standard but when sensitivity is measured against confirmed

TB disease, results are variable. Newer tests, IGRAs, measure either the interferon-γ released by T-cells or the number of T-cells which release interferon-γ, after stimulation by *M. tuberculosis*-specific antigens such as early-secreted antigenic target 6-kDa protein (ESAT-6) culture filtrate protein 10 (CFP-10) or TB antigen TB7.7. Large numbers of studies have examined these *in vitro* tests and in some contexts they seem to show higher sensitivity in confirmed TB cases or against an exposure gradient.[24] Specificity seems less affected by prior BCG vaccination or NTM exposure.[25] IGRAs, like the TST, are unable to differentiate between *M. tuberculosis* infection and TB disease. The evidence base regarding the role of IGRAs in children is expanding[26] but data from high-burden settings are limited, especially in HIV-infected children.[4] Few studies have included individuals exposed to MDR-TB.[27] Meta-analyses of the existing data suggest that IGRAs currently offer little in addition to the TST for screening TB-exposed children, particularly in low resource settings.[4] This is reflected in current international guidelines.[28-30]

The epidemiology of drug-resistant tuberculosis in children

The development of TB requires exposure to *M. tuberculosis*, subsequent infection, and finally, progression to disease.[31] The risk of moving from one state to the next is determined by multiple microbiological, immunological, social and cultural factors.[31] In the absence of accurate tests of infection and disease, it is important to understand the epidemiology of each of the exposure, infection and disease stages, and risk factors determining progression between each stage. If these factors are identified, interventions can be targeted to those at the highest risk of progression to the next stage. As children serve as a sentinel marker of ongoing *M. tuberculosis* transmission and failing TB control, by identifying risk factors for infection and disease in children the broader epidemic can be better understood.

Infection rather than just exposure is required for disease to develop. Exposed children with a positive TST are five times more likely to develop disease than those with a indeterminate or negative TST, suggesting that those with a positive TST were more likely to have been infected.[32] This may also be the case for the IGRAs but so far there is little evidence to inform their predictive utility in children. Due to the limitations of these tests, as well as a time delay in hypersensitivity conversion, the results must be combined with a clinical assessment of the likelihood that infection has occurred. This will include an evaluation of the infectiousness of the source case as well as the risk of transmission. Although initial animal models implied that isoniazid-resistant mycobacteria were less infectious and pathogenic than drug-susceptible

organisms,[33-34] studies in humans are less clear.[21, 35-38] An association exists between certain strain types and drug resistance[39] and different strains demonstrate different virulence patterns.[40-41] The overall picture, however, remains uncertain.

For drug-susceptible TB, patients with sputum microscopy smear-positive disease are between two and three times more likely to cause infection in contacts than those with sputum microscopy smear-negative disease,[42-43] with higher bacterial loads more infectious.[42, 44-45] Although HIV-infected individuals with culture-confirmed TB are more often sputum smear-negative than HIV-uninfected individuals,[46] there is little evidence that they are less infectious.[47-48] More extensive pulmonary disease, affecting multiple zones on a chest radiograph, is associated with increased infectiousness, independent of mycobacterial load.[42, 49-50] The implication, therefore, is that when assessing a contact it is vital to understand the extent of lung involvement in the source case.

The risk of a contact becoming infected depends on the physical proximity of the source case to the contact, the daily extent of the interaction, environmental factors, as well as the duration of the exposure.[51] First degree relatives are up to five times more likely to cause infection in the contact than more distant relatives,[50] especially when the relative is female.[45] Those sleeping in the same room are up to three times more likely to infect contacts than those sleeping in different rooms,[42, 49-50, 52-54] with physical proximity of sleeping showing a graded response.[49-50] The odds of infection in the contact is up to four times higher in families where smokers live,[43, 55] and is increased where rooms are crowded or where ventilation is poor.[56] The length and frequency of cough of the source case has been shown to affect the risk of infection in the contact.[57] This is particularly relevant to MDR-TB as source cases have often been previously treated with ineffective first-line regimens and the diagnosis has been delayed, consequently increasing exposure time.[58-59]

From studies that examined the natural history of tuberculosis, conducted prior to the chemotherapy era, it is known that infected infants (i.e. <12 months) have a 50% life-time risk of progression to disease. Children from one to two years have a 20-30% risk, those from three to five a risk of 5%, those five to ten years only a 2% risk and adolescents an adult-like risk (5-10%).[60-61] Adults with HIV and TB infection have a 7-10% annual risk of developing TB,[62] an effect modulated by combination antiretroviral therapy (cART) and immune status. Children with HIV are more than twenty times as likely to develop TB as those HIV-uninfected[63] and children with malnutrition have been shown to be more vulnerable than those adequately nourished.[43]

The origin of infection in child contacts is dependent on background TB prevalence. A number of studies have demonstrated that TB contacts subsequently developing disease usually do so with the same strain as that of the identified source case.[21, 37, 64-65] Other studies, however, have shown that in high prevalence regions many contacts develop disease of a different strain to their identified source case implying infection from someone else within the community.[66-69] Older studies have shown that although household source cases are important, many children have evidence of infection without a known household source case.[53, 70] It is possible that in the same household there is more than one TB case, each with a different strain and it is also possible for a source case to be infected with multiple strains.[71-72] In reality, in low prevalence regions the identified source case is likely to be the origin of the infection,[73] whereas in high prevalence areas, a combination of household and community sources of infection is likely to occur.[66] The nature of the interaction between the source case and the contact is also important and the more intense the interaction the more likely the strains will be concordant. Younger children are therefore more likely to be infected with the same strain as the known source case as opposed to older children who interact more with the community. When planning preventive treatment in the presence of multiple possible strains, however, a balance between the most likely and the most dangerous outcomes should be considered.

The evolution of drug-resistance in *M. tuberculosis* strains

At every division of *M. tuberculosis* there is a small probability of a genetic mutation arising that will confer resistance to a TB medication. Therefore, at any one time, within a large untreated population of *M. tuberculosis*, mycobacteria will exist which possess such mutations. Monotherapy with only one drug will exert a selective advantage onto those strains, allowing them to prosper with drug-susceptible strains dying. Eventually, the entire population will possess that mutation and will be resistant to that medication. Isoniazid and rifampicin are the two most important medications used to treat *M. tuberculosis* and the rate of spontaneous mutation to create resistance to isoniazid is 1 in 10^6 cell divisions and rifampicin 1 in 10^8.[74] The use of a multidrug regimen should ensure that those mutants resistant to one of the medications can be killed by one of the others in the regimen. A population of 10^{14} bacilli would be required to create the mathematical possibility of a mutation to both isoniazid and rifampicin. Even in cavitary TB, with high bacillary load, the number of organisms cannot reach this level.

Therefore, for resistance to develop to a multidrug regimen, monotherapy must be inadvertently given. This occurs when serum levels are sub-therapeutic, treatment is intermittent, chaotic or only some of the drugs in the regimen are taken. Resistance usually develops first to isoniazid as this medication is the most bactericidal and therefore causes the greatest selective advantage. In addition mutations to isoniazid occur more frequently than to rifampicin. This leads to isoniazid mono-resistant *M. tuberculosis*. If rifampicin is then given as monotherapy (or in combination with other medications given imperfectly) resistance to rifampicin will develop. Resistance to only rifampicin was rare prior to the HIV epidemic. However, and for reasons that are still not entirely clear, it is now see more frequently, especially in those HIV-infected.

Drug resistance can be acquired as described above, through sequential, selective pressure in the face of inadequate therapy, where a previously drug-susceptible organism develops resistance within one human host. Alternatively, resistance can be transmitted where mycobacteria, already resistant, are transmitted to a new host. Additionally, a combination of the two can occur when one individual receives a mycobacterium already resistant to one or more medications and then in the face of inadequate treatment develops resistance to further antibiotics. It is unclear what proportion of drug resistance in tuberculosis is transmitted and what is acquired. Children usually have transmitted resistance as disease is normally paucibacillary, making acquired resistance less likely.

The relationship between strain type, drug resistance and virulence is complex. One study has demonstrated a relationship between Beijing strain and both HIV infection and drug resistance in adults with tuberculous meningitis (TBM).[75] However, another study found no association between strain type and either presentation or outcome in an investigation of children with TBM.[76] Further investigations have demonstrated a relationship between strain type and disease phenotype in children[77] and in adults[41, 78] and a number of studies have demonstrated that strain type, and Beijing specifically, is associated with drug resistance.[39, 79-81]

The management of children with drug-resistant tuberculosis infection

If a child with *M. tuberculosis* infection can be given effective treatment to prevent the progression to disease, the child is spared a TB disease episode. This has clinical implications for the individual, reducing morbidity, mortality and avoiding lengthy, unpleasant and potentially costly treatment with associated adverse events. It also has implications for the

community as children provide a reservoir for future disease propagation, as well as direct onwards transmission in those children who develop infectious TB.[82] The first trials of isoniazid as preventive therapy for TB were carried out over fifty years ago,[83] and isoniazid has been demonstrated to reduce the risk of progressing from infection to disease in HIV-positive and HIV-negative children following exposure to drug-susceptible TB.[84-86] The majority of international agencies and National TB Programmes advise providing children less than five years and all HIV-infected children with isoniazid daily for six months following exposure to an infectious case of drug-susceptible TB.[16]

Following exposure to a source case with MDR-TB it is unclear how vulnerable children should be managed.[87] Although concordance between putative source cases and child contacts is not complete, many clinicians are uncomfortable treating a child exposed to an MDR organism with isoniazid. Cases have been reported of children exposed to MDR-TB developing TB disease on isoniazid preventive therapy.[88-89] Resistance caused by an *inhA* promoter region mutation may be overcome by giving isoniazid at a high dose (15-20mg/kg) but this will not treat all strains.[90-92] Drugs other than isoniazid and rifampicin have not been proved to be effective in preventing the progression from infection to disease and concerns exist regarding the potential toxicity of other agents, given to well children without TB disease. The Centers for Disease Control identified the need for a preventive therapy trial for contacts of MDR-TB in 1992.[93] Since then numerous international agencies and experts have recommended that studies assessing MDR-TB preventive therapy should be a global public health priority.[6, 94-98]

The management of children with drug-resistant tuberculosis disease

A microbiologically confirmed diagnosis is only made in about 20% of children with clinical evidence of TB.[5] When extensive efforts are employed this figure can increase, but rarely to above 50%.[99] It is therefore recommended that children should be treated for clinically presumed, as well as confirmed, TB, based on symptoms, signs and radiology.[16] Many clinicians are wary of making a presumed diagnosis of MDR-TB in children, however, due to perceptions that the treatment is associated with significant adverse events, is long and traumatic for children, and may involve prolonged hospital admission. The balance between making a confirmed diagnosis (specific but not sensitive) vs. a presumed diagnosis (sensitive but perhaps not specific) is influenced by such assumptions.

Adults with TB usually have multibacillary disease with extensive tissue damage and lung cavities. Microbiological diagnosis is common. Treatment recommendations for adults with MDR-TB include an injectable medication for six to eight months and a total duration of therapy of 18-24 months.[100] Durations shorter than this are associated with increased risk of failure and relapse. Children, however, have a different spectrum of disease. Whilst older children (older than 8 years) may have adult-type disease,[82] younger children commonly have limited, paucibacillary disease, including intra-thoracic or extra-thoracic lymph node disease.[101] Children metabolise drugs differently to adults, have a different spectrum of adverse effects and different psychosocial, developmental and educational needs. However, generally, the advice is for children to be treated in a similar way to adults. Some experts suggest that it may be possible to treat limited disease less aggressively but evidence to support this is limited.[16, 102-103]

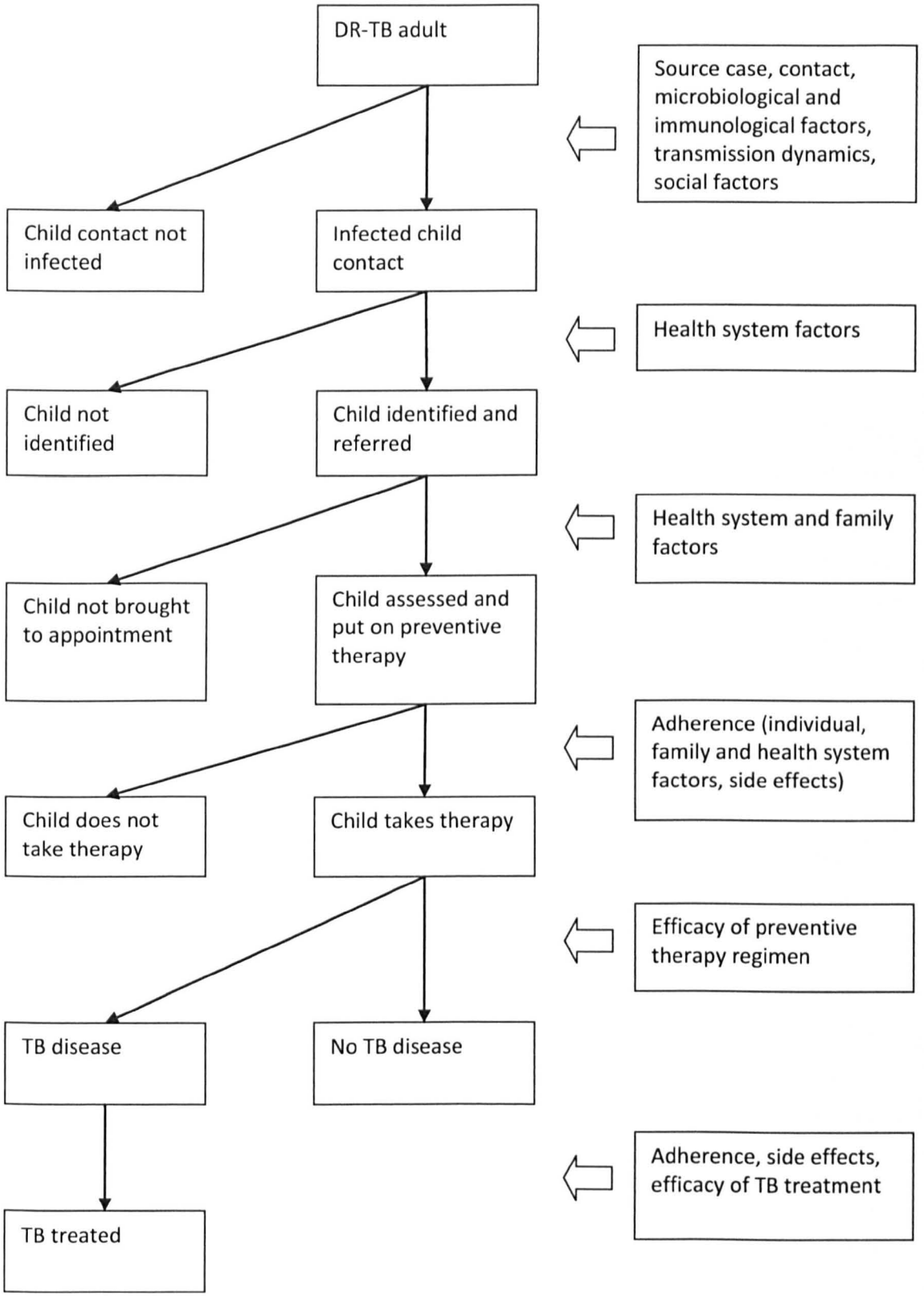
Drugs used to treat drug-resistant tuberculosis infection and disease

The treatment of DR-TB infection and disease necessitates the use of second-line TB agents. Despite their approval more than forty years ago, there are major gaps in our knowledge of the pharmacokinetics of TB drugs in children, particularly of the second-line agents.[104] The pharmacokinetics of TB drugs is modulated by several factors. Age is an important variable as young children achieve lower serum concentrations for most first-line TB drugs compared to adults when given at the same mg/kg dosages.[105-108] Other potentially important determinants include malabsorption and immune-compromise resulting from HIV infection,[109-110] poor nutritional status[111] and variable pharmacogenetics.[106, 112] Recent global interest in paediatric TB resulted in a critical review of existing treatment recommendations and a number of new recommendations have been made regarding the appropriate dosing of first-line TB drugs in children;[113] however, there is scant evidence on which to base dosing guidelines for the second-line TB drugs. Toxicity is a major concern, but paediatric data are limited. Co-administration with cART may potentiate drug toxicity or result in drug–drug interactions that compromise the efficacy or safety of the TB regimen or cART. Knowledge of the effects of age, HIV co-infection and concomitant cART in children on the pharmacokinetics of second-line TB agents is limited.

The challenges to treating MDR-TB in children are only partly due to the uncertainties surrounding the activity and safety of the available drugs. The second-line drugs are rarely produced in paediatric formulations or appropriate tablet sizes, necessitating breaking,

splitting, crushing or grinding. Hence dosing may be inaccurate and sub-therapeutic or toxic levels are possible. The taste of the medications is often unpalatable. A number of the drugs cause vomiting and diarrhoea which may affect the amount absorbed and causes further uncertainty about the dosing. The daily pill burden can be vast as the child may require multiple TB medications, cART, other antibiotics as well as supplements of vitamins and calories. Adherence can be challenging in children either too young to understand or not old enough to cooperate. Treatment for MDR-TB in children should always be given under directly observed therapy (DOT) but in reality, in many settings, responsibility is often given to the caregiver who is given a week or a month's supply of drugs. Caregivers may well be the source case, however, and may have chronic medical problems themselves, have defaulted treatment, or have additional problems such as drug or alcohol abuse. There is an established relationship between TB and alcohol in many contexts and populations[114] and in treatment cohorts of MDR-TB alcohol and drugs are common and associated with both default[115] and poor prognosis.[116]

Conceptual framework



Aims and structure of thesis

The aim of this thesis was to examine each of the different stages from exposure to DR-TB through to the treatment of disease, as outlined in the conceptual framework.

The first step was to carry out a review of literature relevant to the different components of the cascade. I have therefore examined the literature regarding the management of children exposed to DR-TB, the management of children with DR-TB disease and finally the characteristics of the second-line TB drugs. The next step was to define the terms that I proposed to use in the studies that were to be carried out.

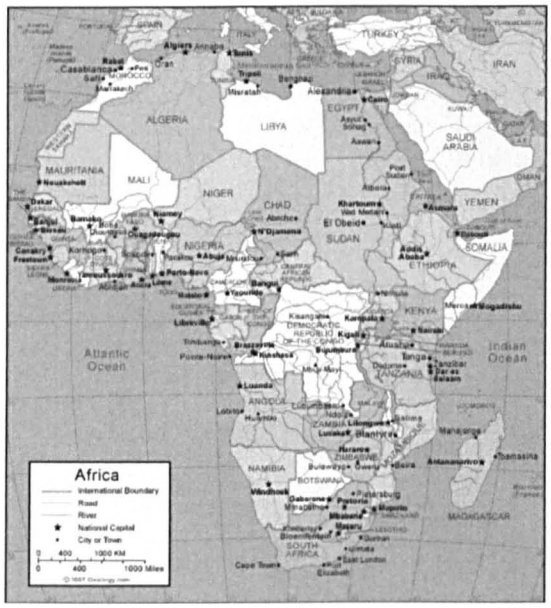
The main component of the thesis was to carry out original research to examine the different stages in the progression from exposure to disease treatment. Through a series of inter-related studies, the reasons for progression from one stage to the next in the cascade were explored and interventions examined. Within this, the first study aimed to quantify the burden of drug resistance in the context in which the research is to be carried out. Epidemiological risk factors for infection and disease were then explored in child contacts of MDR-TB. Investigations into transmission dynamics were examined in two studies and then two operational research studies explored what proportion of child contacts access care and why so many fail to do so. The toxicity, tolerability and efficacy of a multidrug preventive therapy regimen were studied and finally a series of investigations explored the treatment and adverse effects of DR-TB disease treatment. Following the original research, the final step in the thesis is to draw together conclusions from what had been discovered and identify suggestions for changes in policy and practice, together with areas for future research.

The majority of the elements in the thesis, both the literature reviews and original research studies, have been written up as individual articles for submission to peer-review academic journals. However, to provide a coherent description of the body of work, one thesis has been produced. Where sections of the thesis are drawn from articles that have been written, this will be noted and published/submitted articles are available at the end. Text presented in the thesis is only drawn from articles that I have written myself. However, all articles benefitted from critical input from other authors.

All research studies in the thesis were approved by the Ethics Committees of both the London School of Hygiene and Tropical Medicine and Stellenbosch University.

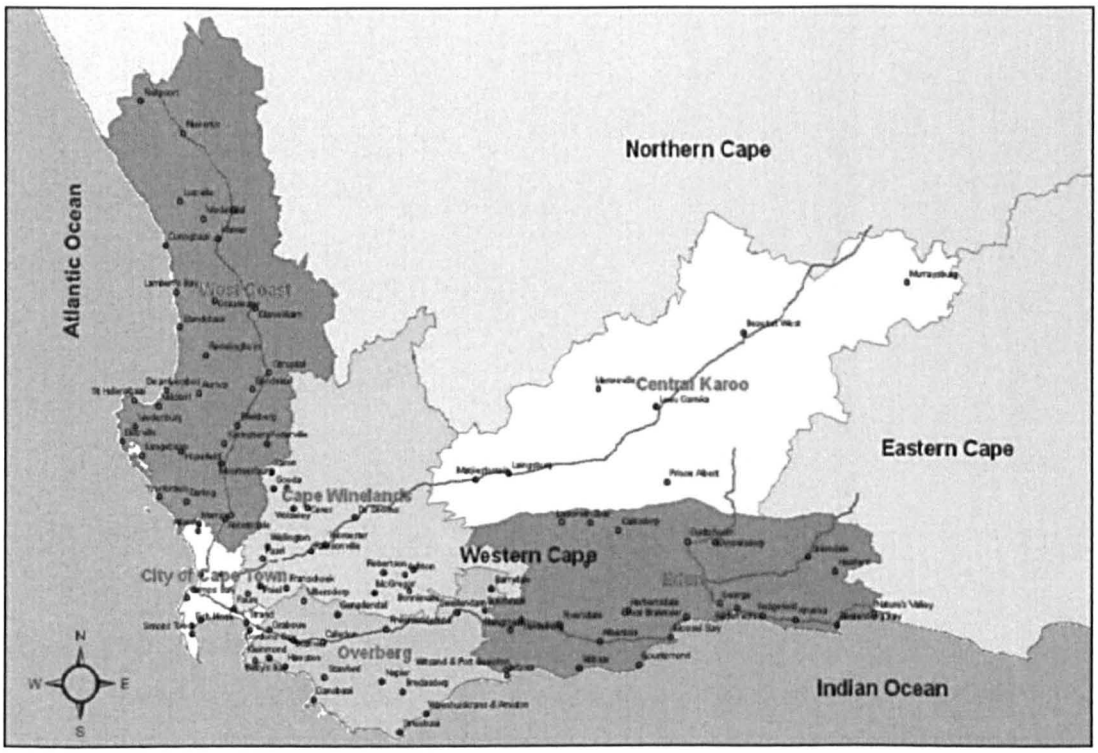
Context and environment of work

The Republic of South African is located at the southern tip of the African continent. The population was just over 50 million in 2011[117] and the country is comprised of Black African (Zulu, Xhosa, Basotho, Bapedi, Venda, Tswana, Tsonga, Swazi, Ndebele), White (mainly of European ancestry), Indian and Coloured (a heterogeneous group of mixed ancestry) ethnic groups. The antenatal HIV prevalence was 29.3% in 2008[118] and the national TB incidence was 948 cases per 100,000 in 2007.[118]



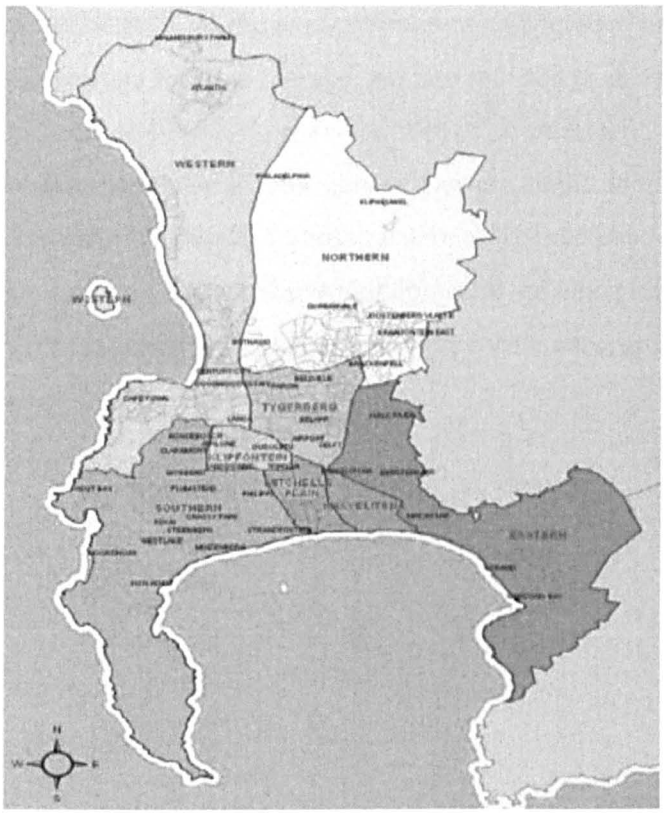
South Africa is divided into nine provinces, of which the Western Cape is situated in the south-west corner of the country.

The population of the Western Cape was 5.3 million in 2011.[117] The HIV antenatal prevalence was 16.9%[119] in 2009 and the TB notification rate was 976 per 100,000.[118] Within the Western Cape, the province is divided into six health districts. The largest is the metropolitan health district called the City of Cape Town with a population of 3.4 million.



The City of Cape Town health district is further subdivided into eight sub-districts of roughly half a million population each. One of these, Khayelitsha, is a peri-urban township, and is located on the outskirts of the City. Khayelitsha is a poor sub-district and has a predominantly Xhosa-speaking population.[120] In Khayelitsha the TB notification rate was nearly 1600 per 100,000 in 2008 with an antenatal HIV seroprevalence of 31.1%.[121] Médecins sans Frontières (MSF), working with local health authorities in one township of Cape Town, Khayelitsha, since 2007, are piloting a decentralized model for the identification and treatment of MDR-TB patients. This involves a package of care that includes counselling, support, sensitization, education, adherence support groups, active case finding of contacts and psychosocial care. [122] Each year, there are about two hundred adults diagnosed with MDR-TB from the Khayelitsha sub-district. A paediatric outreach DR-TB clinic has been running since December 2008 in

Khayelitsha where, once a month, a specialist visits the community clinic. New patients are assessed and for those who can be managed in the community, follow-up of both exposed and diseased patients occurs.



In all sub-districts, once an adult has been diagnosed with sputum positive MDR-TB, contact tracing should occur to identify any individuals at risk in the household. In practice this occurs inconsistently and many vulnerable children are not identified or referred. At the beginning of 2010, a professional nurse was appointed in each of the eight sub-districts to oversee this process. One of their tasks is to carry out a household assessment on all new adults with MDR-TB at diagnosis to discuss infection control, adherence and follow up. Another task is to identify any exposed children who are less than five years and those HIV-infected and refer them to their nearest clinic (roughly one hundred exist in the City of Cape Town Health district) to be seen by the local doctor before referral to the regional paediatric DR-TB clinic. This DR-TB clinic takes place at Tygerberg Children's Hospital (TCH), a large provincial, academic hospital, and as an outreach service, conducted within Khayelitsha on a monthly basis. The children are referred by faxing a standardised referral form and ringing to make an appointment. At the DR-TB clinic, the child is assessed by a specialist to rule out disease using history, examination and plain film chest radiography (CR; antero-posterior and lateral). If the child is found to be well, but is deemed to have had significant exposure, they are started on preventive therapy.

The provincial policy is to provide ethambutol (20-25mg/kg daily), ofloxacin (15-20mg/kg daily) and high dose isoniazid (15-20mg/kg daily) for six months. Children are seen routinely at two, four, six and twelve months where they are examined and have a chest radiograph taken.

Any children in the Western Cape who are confirmed or suspected of having MDR-TB disease, and children failing effective first-line therapy, are also referred to this specialist children's DR-TB clinic. Children are assessed and, if necessary, started on treatment. If the child requires a daily injectable medication for the intensive phase they are admitted to Brooklyn Chest Hospital (BCH), a specialist residential TB hospital with a sixty bed paediatric capacity. Otherwise they are managed as outpatients. Children who are being followed-up after discharge from BCH to complete their treatment in the community return to the DR-TB clinic for appointments.

Literature Review

Literature review 1: managing child contacts of drug-resistant tuberculosis

Concepts from the following topic have been written as articles:

- *Seddon JA, Godfrey-Faussett P, Hesseling AC, Gie RP, Beyers N, Schaaf HS. Management of children exposed to multidrug-resistant Mycobacterium tuberculosis. Lancet Infect Dis. 2012; 12: 469-479*
- *Seddon JA, Godfrey-Faussett P, Hesseling AC, Schaaf HS, Enarson D. Should preventive treatment be provided to child contact of tuberculosis in high burden settings? (submitted)*

For this section of the thesis, the literature surrounding preventive therapy was systematically searched (Table 1) From the articles found, together with a synthesis of relevant themes and topics, a discussion of the relative merits of preventive therapy for contacts of DR-TB is provided.

Preventive therapy

TB control programmes have traditionally focused on case-finding and treatment of infectious patients with TB disease, usually adults. From a public health perspective, this must remain the priority as it will reduce population transmission and consequently the number of new infections. However, to decrease future disease burden and for individual clinical care, these strategies need to be complemented with the identification and treatment of exposed individuals who are at a high risk of becoming infected and then of progression to disease.[123] Those at the highest risk are young children and the immunosuppressed. Few studies have examined the management of children exposed to MDR-TB and in the field of preventive therapy there is no consensus in published articles and expert guidelines.

The rationale for preventive therapy

Preventive therapy regimens have been used since 1951 in an effort to prevent the progression from infection to disease.[124] The WHO advises that those with *M. tuberculosis* infection who are at a high risk of progression, should be given daily isoniazid for at least six

months.[16] In high burden settings high risk is considered children less than five years of age or those HIV-infected in household contact with an infectious TB case.[125] Alternative preventive therapy options that have been proposed include rifampicin or combinations of rifampicin, isoniazid and pyrazinamide.[126-129] A number of studies have demonstrated that when uptake and adherence to preventive therapy is good, there is a reduction in the likelihood of progression from infection to disease. The reduction is in the order of 60% for those without HIV infection[83, 85, 130] increasing to 90% in analysis restricted to patients with good adherence.[131] HIV-infected children, given preventive therapy, had a 72% reduction in disease progression in the era before cART.[84, 132] However in a recent placebo-controlled trial of HIV-infected and HIV-exposed, uninfected infants with no known exposure to a TB source case at the time of enrolment and universal access to cART, 96 weeks of isoniazid did not lead to a reduced incidence of TB disease.[133] WHO has recently proposed an aggressive strategy for HIV-infected patients, where all should be given preventive therapy, irrespective of age, contact with a TB source case, degree of immunosuppression or evidence of infection.[134] Therapy is advised for up to 36 months. Prophylaxis refers to treatment given after exposure to prevent infection while treatment of latent infection implies that infection has been determined. Preventive therapy includes both these situations and will be used throughout the thesis (see definitions section later).[6]

The assessment of child contacts of multidrug-resistant tuberculosis

When presented with the child contact of an MDR-TB source case a number of decisions must be made. The first is to assess whether the child has TB disease. This is not always straightforward but giving preventive therapy to a patient with TB disease is not only inadequate but also runs the risk of promoting further resistance. Once TB disease is excluded, the attending physician must make a series of assessments which, in the absence of reliable diagnostic tests, will always be probability-based judgements. These are demonstrated in Figure 1. Initially the likelihood of infection must be evaluated. If the child is likely to be infected, the risk of disease progression must be determined. If the child is likely to be infected and has a high chance of disease progression then the final assessment is of the drug susceptibility of the infecting strain. Once all these factors are evaluated preventive therapy can be considered. In each individual case, the risks associated with preventive therapy must be carefully weighed against the risk of disease.

Evidence regarding the management of multidrug-resistant tuberculosis contacts

Few studies have examined the management of child contacts of MDR-TB. My own review of the literature identified no randomised controlled trials investigating those exposed to MDR-TB and only three studies that have looked at preventive therapy in contacts of MDR-TB. This was the same result as others who had systematically searched the literature.[135] One study in Cape Town followed 105 children, of which 41 had been given a multi-drug preventive therapy regimen tailored to the drug susceptibility test (DST) pattern of the source case strain.[21] This demonstrated a protective effect of the tailored regimen as only two out of the 41 treated children (5%) developed TB disease compared to 13 out of the 64 (20%) who had been observed carefully without intervention. The second study, in Rio de Janeiro, retrospectively assessed 218 adult and child contacts of MDR-TB source cases of whom 45 had been given isoniazid.[65] This study showed a non-significant trend towards a protective effect of the preventive therapy. Finally, 110 infected adult and child contacts of 19 MDR-TB source cases were given a multi-drug preventive therapy regimen in Chuuk, Federated States of Micronesia. Twelve months of preventive therapy was given under DOT and no patients given preventive therapy developed TB disease.[136] Although no definitive conclusions can be drawn from these studies, they would suggest that it may be beneficial to give some form of preventive therapy to child contacts of MDR-TB.

Recommendations from international organisations and departments of health

International guidelines vary regarding the management of MDR-TB contacts. The British National Institute for Health and Clinical Excellence (NICE) advocates careful follow-up of all contacts of infectious MDR-TB patients with no treatment as no medication has been demonstrated to be effective in preventing progression from infection to disease.[137] The WHO recommends close observation as there are limited data supporting the use of drugs other than isoniazid and rifampicin. It does, however, imply that contacts of MDR-TB source cases may be infected with multiple strains, some of which might be susceptible to isoniazid.[16] The South African Department of Health suggests giving isoniazid to contacts of MDR-TB source cases.[138] One Delphi survey failed to gain consensus, suggesting that it was up to the attending physician to make the decision and advocating the need for further studies.[139]

The Centers for Disease Control and Prevention (CDC), the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) have issued a joint statement suggesting that high-risk contacts of MDR-TB source cases should receive a regimen that includes two

drugs to which the source case's TB strain is susceptible.[93] The American Academy of Paediatrics (AAP) suggest specialist referral.[140] Published expert opinions vary[141-142] as does reported practice.[89, 143-148] Although treating with medications to which the source case's isolate is susceptible is intuitively convincing and biologically plausible, there are limited data to indicate if it is successful in preventing infection or the progression from infection to disease. The adverse effects of such treatments have also not been explored and the costs to the patient and to the health system have not been assessed.

Available medications that might have a place in a preventive treatment strategy for child contacts of multidrug-resistant tuberculosis

Whichever regimen is used, it is important that clinicians are familiar with the medications available, their mechanism of action and possible adverse effects[149]. The drugs in Table 2 have all been suggested as preventive therapy for MDR-TB. Although the individual drugs will be discussed later in the literature review on second-line drugs, they are discussed here in relation to their use in preventive therapy as drug characteristics are central to preventive therapy decision-making. In addition the use of first-line drugs, not covered in the later literature review, are discussed here.

Delivering the correct amount of a drug to a child is problematic, both in terms of being confident of ingestion as well as knowing the correct drug dosage. Traditionally, paediatric dosing was extrapolated from adult pharmacokinetic studies. However, children generally metabolise medications more rapidly than adults with drug concentrations lower than adults given a corresponding dose for weight.[150] This has been demonstrated in a number of recent studies assessing TB drug concentrations in children[105, 107, 110, 112] resulting in a revision to WHO first-line TB drug dosing recommendations in 2009.[113] The caveat to this, however, is that neonates and very young infants, with immature liver and enzyme development, seem to metabolise drugs less rapidly than older children. Care must be taken when prescribing for this sub-population.[108, 151] Determining the optimal drug concentration to aim for in children is also problematic and the pharmacodynamic properties of drugs may not be the same for children and adults. Most of the second-line drugs have been minimally studied in children with TB.

Isoniazid is effective when used alone as preventive therapy for drug-susceptible TB infection because it is highly bactericidal and leads to rapid bacterial clearance.[152] Resistance has not been shown to develop in infected patients treated with this monotherapy when TB disease has been excluded prior to initiation.[153] Its use in contacts of known MDR-TB is questionable,

however, as the contact is likely to be infected with an MDR strain. The majority of isoniazid resistance is coded for on one of two genes and so resistance can be detected using genetic tests. It can be divided into high-level or low-level resistance, the former being coded for on the *kat*G gene and the latter on the *inh*A promoter region.[154]

Pyrazinamide is effective in killing *M. tuberculosis* but only in the acidic environment created by active inflammation responding to rapidly replicating mycobacteria. This situation is not usually found in *M. tuberculosis* infection and so its place in preventive therapy is uncertain.[155] Pyrazinamide-containing preventive therapy regimens have been shown to have significant adverse effects in adults and adherence can be poor.[156-158] However, studies in children being treated for TB disease demonstrate it to be well tolerated and rarely associated with adverse effects.[159-160] Resistance to pyrazinamide is complicated to test as it requires conditions of very low pH which are difficult to replicate *in vitro.* Direct resistance testing, as well as surrogate and genetic techniques, have shown that levels of resistance can be high in strains already resistant to isoniazid and rifampicin.[161-162]

Fluoroquinolones have good activity against *M. tuberculosis*[163] but concerns had previously prevented their use in children due to the effect on the cartilage growth of immature beagles.[164] A growing body of experience has subsequently assessed their use in children and found no evidence to support these concerns.[165] Fluoroquinolones have good bactericidal activity and seem to be well tolerated. Although effective *in vitro,* ciprofloxacin has poor early bactericidal activity; other fluoroquinolones are therefore likely to be more effective.[166] A recent study has demonstrated that although moxifloxacin has the best *in vitro* activity of the tested fluoroquinolones against *M. tuberculosis* in the exponential growth phase, levofloxacin was the most effective against those in the latent phase, suggesting it may be effective when used as preventive therapy.

Ethambutol is effective but concerns regarding its effect on the optic nerve have limited its use in those for whom colour vision and visual acuity was not possible to test, essentially precluding its use from young children. Studies have subsequently shown that when used at modern doses, optic neuritis has rarely been seen.[167] There is emerging evidence, however, that in MDR strains the frequency of ethambutol resistance is very high,[161] suggesting that it may not be an effective choice.

Ethionamide is a similar drug to isoniazid, inhibiting the synthesis of mycolic acid and consequently impairing the formation of the cell wall. If the mycobacteria have a mutation in

the *inh*A promoter region, the mutation that usually leads to low-level isoniazid resistance, then there will also be resistance to ethionamide.[154] However, if there is a *kat*G gene mutation, the mutation that usually leads to high-level isoniazid resistance, there is no increased risk of resistance to ethionamide. Adverse effects are common and include nausea and vomiting. However, these usually lessen with time and can be reduced by initially splitting the daily dose or giving a reduced dose at the start of treatment and increasing over a couple of weeks to the full dose.

Preventive treatment strategies for the management of drug-resistant contacts

Contacts may be given a single drug, a standardised multidrug regimen, where all patients are given the same combination regardless of susceptibility, or an individualised, tailored multidrug regimen determined by the DST of the source case.

Contacts of TB source cases with isolates resistant to only either rifampicin or isoniazid can usually be treated with the other agent alone. As both these drugs have been shown to be effective in reducing the risk of progression from infection to disease in contacts of drug-susceptible disease, either drug should be effective. It must be noted, however, that with the increasing use of line probe assay (LPA) genotypic tests to diagnose MDR-TB a proportion of source cases labelled as having rifampicin-monoresistant (RMR) TB may, in fact, have MDR-TB as the genotypic tests only detect the *kat*G and *inh*A mutations, missing a small but important proportion of isoniazid resistance. Contacts of XDR-TB may be infected with a strain that is resistant to multiple agents. Few treatment options are available and until new agents become available close follow-up remains a key component of management.

Although good evidence exists for the efficacy of isoniazid in preventing disease progression in drug-susceptible infection, there are limited data to support its use alone in those exposed to MDR-TB. A rationale exists for using it alone at high dose as it is well tolerated, adverse effects are rare and it would treat drug-susceptible strains as well as strains with low-level resistance.[90, 92, 168] There have, however, been documented cases of children exposed to MDR-TB progressing to disease whilst on isoniazid preventive therapy given at the previously recommended dose (5mg/kg).[88] Using a fluoroquinolone on its own might also be effective as they are well-tolerated and have both bactericidal and sterilising activity. Fluoroquinolones are, however, used widely to treat non-tuberculosis bacterial infections and there is concern that widening their use still further and using them over long periods of time may promote resistance to this essential class of drug needed to treat MDR-TB. A number of drugs in the

research and development pipeline, TMC-207, OPC-67683, PA-824, may also be suitable for MDR-TB preventive therapy.[169]

A standardised multi-drug regimen will have some operational advantages over a regimen tailored to the DST pattern of the isolate from the source case. It is simpler to implement and healthcare workers can become familiar with a small number of drugs, dosages, adverse effects and interactions. It is only necessary to have a limited DST on the strain of the source case, sufficient to know that they have MDR-TB. Fundamentally, the choice of agents must reflect the DST pattern of the prevailing organisms and so making universal recommendations may not be appropriate. What is suitable for one area may not be for another. Deciding at which level to provide guidance is also difficult, whether it be global, regional, country, district or at hospital level, as is often the case for antibiotic policy. Balancing the tensions between easy-to-follow unambiguous general policies and providing treatment that is most likely to be effective for the target population is a constant public health challenge. Proposed standardised regimens have included combinations of pyrazinamide, ethambutol and a fluoroquinolone.[93, 170]

The advantages of a tailored regimen include an increased likelihood of success and reduced promotion of resistance if a diagnosis of disease were missed at the beginning. However, it requires a health system that is able to perform DST to a variety of first- and second-line medications and requires extensive experience and expertise from front-line medical staff. By giving three or more medications to which the bacilli is susceptible, it could be argued that this is little different from a disease treatment regimen. Some would suggest close observation with rapid identification and treatment if TB disease develops as a more appropriate strategy in a well child.[125, 137]

The Research Excellence to Stop TB Resistance Group (RESIST-TB) met in December 2009 to discuss research into MDR-TB. They produced three clinical trial protocols of which one examined the question of preventive therapy to adult and child contacts of MDR-TB. It was suggested that a trial be conducted comparing isoniazid with either a new drug or one used in combination with existing drugs.[98] It would need to be a large trial as the endpoint of TB disease is relatively infrequent and large numbers of patients would need to be recruited in both arms to demonstrate a difference. The composition of the new regimen is still under discussion.

Other factors which will affect which regimen is employed in a given circumstance will be certain pragmatic considerations such as regulatory approval, often challenging in children, experiences of certain drugs in other diseases, cost and national programme policy. The optimal duration of treatment has not been explored.

Adherence

Almost all studies performed have demonstrated that, in routine clinical care, personal adherence to a course of isoniazid is singularly poor.[171] If the treatment needs to be given to a child who is clinically well and may not be compliant with the parents' wishes, it seems to be even worse.[172-173] In a recent, prospective study in Cape Town, 180 child contacts of drug-susceptible TB were started on isoniazid. Only 20% completed five months or more of preventive therapy and the assessment of adherence consisted of whether they came to the clinic monthly to collect the medications with no record of whether they were actually taken or not. As this is with isoniazid, which has a relatively benign adverse effect profile, there are concerns that with more complex regimens it may be even worse.

However, with education, counselling and peer support, good levels of adherence can be achieved and TB programmes must consider the best ways of promoting good adherence to completion of treatment. Novel techniques are being employed including DOT by family members or non-medical treatment supporters, use of mobile telephone technology, incentives and decentralised care in the community.[174-176] Preventive therapy has rarely been given under DOT and it is usually the responsibility of the parent to administer. The available resources and local prevalence of TB in the community will also affect which model of supervision and support families receive.

The influence of Human Immunodeficiency Virus

As preventive therapy regimens given to those exposed to MDR-TB do not contain rifampicin many of the usual interactions with cART medications are less profound. Limited work has been done on the interaction between second-line TB drugs and cART drugs.[177] It is unclear if therapeutic levels are being achieved, especially in children where less data are available. In addition, chronic diarrhoea, a common consequence of HIV, may affect the absorption of both MDR-TB preventive therapy as well as those of cART.[178]

The other key question is the timing of the initiation of cART and MDR-TB preventive therapy. If the patient is already well established on cART it is probably safe to start preventive therapy immediately without stopping the cART. If the diagnosis of HIV is made whilst the patient is established on MDR-TB preventive therapy then cART should be initiated in accordance with the appropriate guidelines for initiation of cART based on clinical and immunological criteria. The problem arises if the two are diagnosed at the same time. If a patient is simultaneously diagnosed with HIV and TB disease, HIV is immediately classed as WHO clinical stage 3 or 4 which necessitates immediate or imminent cART treatment.[179] Often cART initiation is delayed for a couple of weeks to avoid the worst of the interactions and adverse effects. However, if the patient is diagnosed with HIV and *M. tuberculosis* infection it may be appropriate, dependent on other clinical and immunological circumstances, to start preventive therapy and wait until either the preventive therapy is well established or completed before starting cART. The debate about when to start cART is complex and little evidence is available.[180] However, clinicians are treating HIV infection now at an earlier stage than previously and there is convincing evidence that early initiation of cART is associated with reduced TB incidence and mortality, particularly in children.[181] In fact, providing cART to these children may be more effective than any MDR-TB preventive therapy. Once HIV is diagnosed co-trimoxazole should be started according to appropriate guidelines, irrespective of whether cART or MDR-TB preventive therapy is initiated or not.[182]

Immune Reconstitution Inflammatory Syndrome (IRIS) can be divided into paradoxical and unmasking IRIS and usually manifests in the first few weeks of cART.[183-185] Paradoxical IRIS occurs when currently treated TB disease becomes worse following the initiation of cART. Children with *M. tuberculosis* infection do not fall into this group. Unmasking IRIS, however, must be considered in any HIV-infected MDR-TB contact started on cART. The cART may lead to recognition of previously undetected TB disease as the immune system reconstitutes. Management would be to start full treatment for MDR-TB disease. The role of corticosteroids is still inconclusive but may give some protection in certain situations.[186]

Conclusions

MDR-TB is emerging as a significant challenge to international public health. The disease burden can be reduced by treating exposed patients who are at high risk of becoming infected and of progression to disease. Although the number of contacts will vary between populations, conservative estimates would suggest that in high burden regions, there are at least two contacts that are either less than five years or are HIV-infected for each MDR-TB source case.[21,

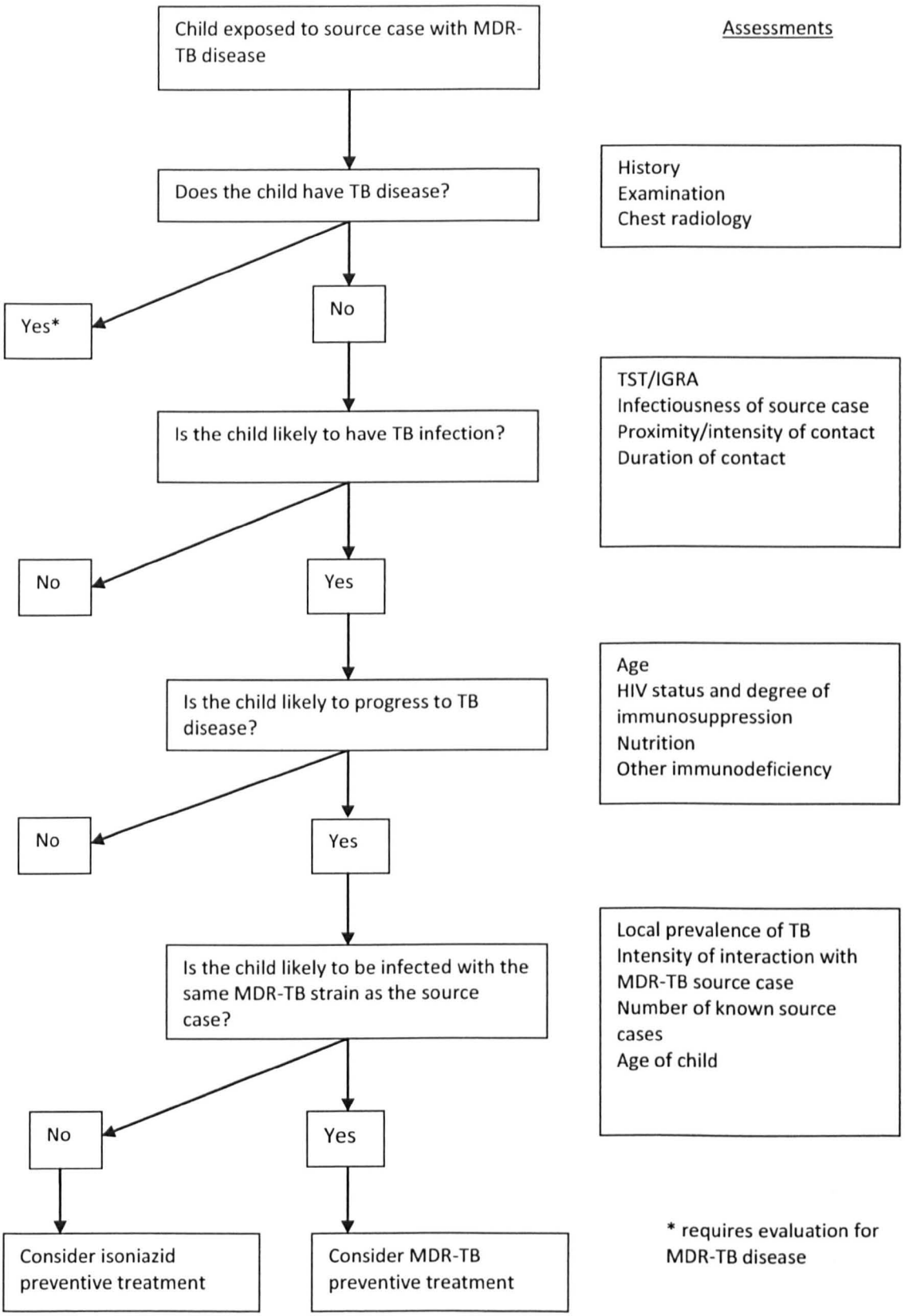
[65] This implies that over a million vulnerable contacts could be considered for preventive therapy each year. From a different perspective, of the half a million people who develop MDR-TB disease each year, a significant number are HIV-infected or less than five years old; for a proportion of these a source case may have been identified and preventive therapy initiated. A study from Peru has demonstrated that of households with a case of MDR- or XDR-TB, nearly a quarter have a contact that develops TB in the subsequent four years.[187] MDR-TB disease is expensive to treat[188-189] and treatment is associated with significant toxicity.[190] Preventing MDR-TB disease may be a practical and cost-effective solution from both the individual and public health perspective. However, treating a well child with potentially toxic drugs also presents a challenge. For each child, the risk of preventive therapy must therefore be weighed against the risk of disease. A very young child, sleeping in the same room as a close family member with sputum smear-positive TB disease, or an HIV-infected child, is likely to benefit more from preventive therapy than an older child in contact with a neighbour with smear-negative disease. Careful follow-up is essential regardless of the treatment decision.

Table 1 - Search strategy for literature review

	Search term	CINAHL	Africa NiPAD	Embase	Medline	WOS	Cochrane	ASP	Global Health
		31/10/09	31/10/09	31/10/09	31/10/09	3/11/09	3/11/09	3/11/09	3/11/09
1	TB	2651	14037	37494	15332	33252	1734	17240	6918
2	Tuberculosis	9253	176033	124042	161113	>100000	2420	22720	51314
3	Mycobacter*	2274	43877	68558	70656	87558	887	14288	63121
4	MDR	327	1756	6874	8547	10667	137	3910	1602
5	XDR	36	216	172	207	336	3	259	121
6	Drug-resist*	9930	19108	62094	150533	>100000	3798	18246	46346
7	Multidrug-resist*	1524	4686	29040	21943	32213	415	6944	4964
8	Prophylaxis	6868	5905	101072	60493	80964	12157	12606	25195
9	Chemoprophylaxis	353	2221	13403	4070	5282	585	745	6854
10	"Preventive therapy"	250	525	1406	1506	1942	196	512	337
11	"Preventive treatment"	279	439	2446	2477	2953	364	787	596
12	Latent	2158	3667	62393	35422	65107	817	15958	10023
13	1 or 2 or 3	10271	178952	167967	193782	>100000	4248	39674	71811
14	4 or 5 or 6 or 7	10737	21283	83626	158906	>100000	4025	23774	47336
15	8 or 9 or 10 or 11 or 12	9666	11890	176094	102141	>100000	13715	30142	38300
16	13 and 14 and 15	71	379	455	411	564	48	92	285

When row 16 for all databases combined in Endnote 2394 references were produced.
When duplicates were removed in Endnote this left 1393 references.
When duplicates were removed manually 1048 references were left.
Elimination following review of titles and abstracts left 173 of which 146 full text references (84%) were retrieved
Of these 146 articles, 55 were found to be useful to the literature review

Figure 1 - Decision algorithm for assessing child contacts of MDR-TB cases



MDR-TB – multidrug-resistant tuberculosis; TST – tuberculin skin test; IGRA – interferon-gamma release assay; HIV – human immunodeficiency virus

Table 2 - Drugs suggested in a preventive treatment regimen for MDR-TB

Medication	Drug Characteristics	Dosage advised	Formulations	Adverse effects***	Comments
Isoniazid	Bactericidal Rapidly absorbed and distributes widely Inhibition of cell wall synthesis via inhibition of mycolic acid	15-20mg/kg once daily	Tablets (100mg & 300mg) Syrup (10mg/ml)	Peripheral neuropathy Hepatitis Hypersensitivity	Give pyridoxine if malnourished or HIV-infected Stop all hepatotoxic drugs if evidence of hepatitis Absorption greatest if taken on an empty stomach but acceptable levels with food
Pyrazinamide	Bacteriostatic (bactericidal to rapidly growing mycobacteria) Well absorbed and distributes widely Inhibition of Fatty Acid Synthase I Active only at low pH	30-40mg/kg once daily	Tablets (500mg)	Hepatitis Hyperuricaemia Arthralgia Myalgia Rash Photosensitivity	Stop all hepatotoxic drugs if evidence of hepatitis Reduce dose if evidence of any joint pain Absorption unaffected by food but adverse effects may be reduced if taken with foods
Ethambutol	Bacteriostatic (bactericidal at high dose) Well absorbed and distributes widely (poor into the CSF) Inhibition of cell wall synthesis	10-25mg/kg once daily	Tablets (100mg & 400mg)	Optic neuritis Peripheral neuropathy Hypersensitivity	Assess colour vision and visual fields (dependant on child, possible from ~ five years) Can be taken with or without food
Ethionamide or prothionamide	Bacteriostatic Inhibits cell wall synthesis via inhibition of mycolic acid Well absorbed and distributes widely	15-20mg/kg once daily	Tablets (250mg)	Hepatitis Hypothyroidism Hypersensitivity Metallic taste Hypoglycaemia	Stop all hepatotoxic drugs if evidence of hepatitis If gastrointestinal disturbance severe, aim to give the full dose once daily within two weeks but consider initially: • giving separately from the other medicines (others in the morning, ethionamide in the evening), • splitting the daily dose • giving a smaller dose and building up over time Monitor thyroid function every two months Absorption unaffected by food
Ciprofloxacin	Bactericidal (poor early bactericidal activity) Well absorbed and distributes widely Inhibition of DNA gyrase	15-20mg/kg twice a day	Tablets (250mg & 500mg) Syrup (50mg/ml & 100mg/ml)	Insomnia Arthralgia Restlessness Confusion Headache	Ciprofloxacin should not be used unless no other fluoroquinolone is available Can be taken with or without food Patients should be advised to drink plenty of fluids to avoid excessive urine alkalinity
Ofloxacin	Bactericidal Well absorbed and distributes widely Inhibition of DNA gyrase	15-20mg/kg once daily	Tablets (200mg & 400mg)	As for ciprofloxacin	Can be taken with or without food Patients should be advised to drink plenty of fluids to avoid excessive urine alkalinity
Levofloxacin	As for ofloxacin	10mg/kg daily (twice daily for children <5 years)	Tablets (250mg & 500mg) Syrup (25mg/ml)	As for ciprofloxacin	Can be taken with or without food Patients should be advised to drink plenty of fluids to avoid excessive urine alkalinity Possible photosensitivity

*All listed drugs have been associated with gastrointestinal disturbance

Literature review 2: management of drug-resistant tuberculosis disease in children

Concepts from the following topic have been written as articles/guidelines:

- *Ettehad D, Schaaf HS, Seddon JA, Cooke GS, Ford N. Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2012; 12: 449-456*
- *Seddon JA, Furin JJ, Gale M, Del Castillo Barrientos H, Amanullah F, Hurtado R, Ford N, Starke J, Schaaf HS. Caring for children with drug-resistant tuberculosis: practice-based recommendations. Am J Resp Crit Care Med 2012; 186: 953-964*
- *Schaaf HS, Seddon JA. Epidemiology and management of childhood multidrug-resistant tuberculosis. Clinical Practice (in press)*
- *Furin JJ, Seddon JA, Perez-Velez C. Management of drug resistant tuberculosis in children: a field guide*

For this section of the thesis, the literature surrounding the treatment of children with DR-TB disease was searched. A systematic review was carried out, in which I assisted with the data collection and writing. However, as I was not the principle author, I have not included this review in the thesis but refer to it in the text. However, in addition to the systematic review, I have also been involved in a number of other reviews and for these have searched the literature thoroughly. A list of the studies that have described the management of MDR-TB in children is shown in Table 3. As with the chapter above, rather than simply presenting a list of the articles that have been found, I present an evidence-based discussion of the management of DR-TB in children.

Treatment initiation

The diagnosis of DR-TB in children is either confirmed or presumed. Confirmed disease occurs when *M. tuber*culosis is isolated from the child with either phenotypic or genotypic resistance. In published studies of children with MDR-TB the proportion culture-confirmed ranges from 30%[144] to 100%.[58, 191-196] Although a number of these investigations excluded presumed cases, it is clear from studies of drug-susceptible paediatric TB, that confirmation is usually achieved in only about 20% of cases with clinical evidence of disease.[5] This suggests a significant proportion of children treated for DR-TB should be presumptively diagnosed. A presumptive diagnosis of DR-TB can be made on clinical symptoms, signs and radiology, in combination with

risk factors for drug resistance, such as contact with a confirmed or presumed DR-TB source[16, 197] or the failure to respond to a first-line regimen. The operational definition of failure is challenging and includes ongoing microbiological positivity, non-resolving symptoms or signs of TB, persistent or deteriorating radiology and poor weight gain or weight loss.[197] Although the time course is different for each child, all should be improving by two months, if therapy is effective.

Children with presumed DR-TB should be started on effective therapy as soon as possible to avoid progression to severe disease, worse clinical outcome and ongoing transmission. However, empiric therapy for DR-TB may needlessly expose a child to toxic medications. Extensive efforts should, therefore, be made to confirm the diagnosis with intensive sampling from the child. Dependent on age of the child and health care resources, attempts can be made to obtain sputum samples, gastric aspirates, induced sputum samples, biologic fluid samples, nasopharyngeal aspirates, lymph node aspiration biopsy or tissue biopsy.[5, 198-201] With extensive sampling the proportion of children with a confirmed diagnosis can rise to greater than 50%.[99] Invasive methods, such as broncho-alveolar lavage, bronchoscopic biopsy or open lung biopsy may be in the child's best interest if a confirmed diagnosis can be made.[202] All isolates confirmed as resistant to rifampicin should be sent for full second-line DST assessment.

Regimen design and treatment duration

The WHO has placed the drugs used in the treatment of DR-TB in five groups;[197] drug characteristics will be discussed in the next section. Group 1 drugs are considered first-line with the remainder second-line. Few of the second-line drugs are produced in paediatric formulations, and the pharmacokinetics of most are incompletely studied in young children.[203] This means that optimal dosing is unknown for many of these medications and that tablets must be broken or cut, potentially leading to inaccurate dosages and blood concentrations that are sub-therapeutic or toxic. The taste of the medications is often unpalatable and a number of the drugs can cause vomiting and diarrhea.[203] This may not only affect the amount of drug absorbed but also deter adherence. Daily injectable drugs are usually given for the first few months of treatment[58, 191-192, 204-205] and the pill burden can be vast; the child may require multiple TB medications, cART, antibiotics, as well as vitamin and calorie supplements. The number of tablets can be divided and spread over the course of the day which may improve tolerability but can make DOT challenging. Drugs can be mixed with different foods or drinks

and, in some situations, nasogastric or percutaneous endoscopic gastrostomy feeding may be appropriate. A programmatic dosing table is demonstrated in Table 4.

Guidelines suggest that the decision on which drugs to include in a DR-TB treatment regimen should be guided by the DST of the child's isolate. If this is not available, regimen composition should be guided by the DST pattern of the presumed source case.[16, 140-141, 197, 206] If DR-TB treatment is given for failure of a first-line regimen, the child should be assumed to have TB that is resistant to rifampin and isoniazid. For children with confirmed MDR-TB, or those with a clear MDR-TB source case, there is no role for rifampicin. However, if the child is either failing first-line therapy or there are multiple source cases, it may be appropriate to include rifampicin for the first six months to treat any potential drug-susceptible organisms. However, the drug-drug interactions seen with rifampicin must be considered, especially in those HIV-infected,[207] and there is some evidence that the fluoroquinolones are less effective in rifampicin-containing regimens.[208-209]

When designing a regimen to treat children with MDR-TB, the target should be to use at least four drugs which are likely to have activity against the infecting organism (Figure 1).[16, 140, 142] As they are effective drugs, with few adverse effects,[210] any first-line drugs to which the organism has not been shown to be resistant should still be used. Even though the organism is resistant to isoniazid, it is sometimes used at a high dose, in case of low-level resistance.[91] High level resistance to isoniazid is usually caused by mutations in the *katG* gene, while low level resistance that may be overcome with higher doses (15-20 mg/kg/day) is usually caused by mutations in the *inhA* promoter region. *InhA* mutations usually confer resistance to ethionamide.[90] With increasing use of genotypic diagnostics, the implications of different mutations will become increasingly important.[92, 211-212]

The next step is to add a second-line injectable drug from group two and a fluoroquinolone from group three.[142] The later generation fluoroquinolones (levofloxacin and moxifloxacin) are more effective than earlier generation (ofloxacin) *in vitro,*[213-215] but are poorly studied in children. Further drugs from group four are then added. Either ethionamide or prothionamide should be used (if no *inhA* mutation is documented) as their metabolic pathways are similar and cross-resistance is total.[214] The same is true for cycloserine and terizidone and only one of these two should be used.[214] *Para*-aminosalicylic acid (PAS) can be added if there are not sufficient effective drugs at this stage but due to gastrointestinal intolerance, the other drugs from group four are usually used in preference. Finally, agents from group five can be added if required. Drugs from group five are described as having relatively weak or uncertain activity

against *M. tuberculosis*.[16, 197] However, both clofazimine and linezolid have, in recent studies, demonstrated promising efficacy and can be considered useful drug options.[216-217] Novel agents such as delamanid,[218] PA-824[219] and bedaquiline[220] are in advanced stages of clinical trials. However, as no child-friendly formulations have been produced or any paediatric pharmacokinetic studies conducted, it will be a number of years before these drugs are available for use in children.

The decision on number of drugs and duration of therapy is dependent on both extent of disease and degree of drug resistance, as well as penetration to different body sites and treatment response. For children with cavitary or widespread disease, with resistance to only rifampicin and isoniazid, treatment is usually given for 18 months from the time of sampling of the first negative culture. Good outcomes have been reported in children treated with regimens of this duration, even with extensive disease.[58, 144, 192, 205] Treatment normally includes an injectable drug for the first four to six months; given daily. Limited evidence currently exists regarding the efficacy, and reduced toxicity, of giving three times a week.[140] The systematic review of MDR-TB treatment in children suggests that in those studies where injectable drug use was more common, treatment outcomes were better.[221] WHO has recently recommended that injectable drugs should be given for eight months as longer durations are associated with better outcomes in adults.[100] For older children with extensive disease this may be appropriate, but for most children four to six months is likely to be sufficient.[221] For children with limited, paucibacillary disease, such as isolated intra- or extra-thoracic lymph node involvement and with susceptibility to the second-line drugs, it may be possible to treat the child for 12-15 months in total, dependent on response. It may be possible in such situations to give a shorter duration of the injectable medication or omit the injectable medication altogether and treat only with oral drugs. Although evidence for such shorter regimens is lacking and these regimens are of unproven efficacy, good treatment outcomes have been seen in some studies.[58, 205, 222] If the isolate is XDR or pre-XDR, (see Table 9 for definitions) treatment relies on less effective drugs and in reported studies of children with extensive resistance, more drugs have been used and the treatment given for a minimum of 24 months in total.[192, 196] In the treatment of XDR-TB, consideration can be given to the inclusion of streptomycin, as cross-resistance between second-line injectables is incomplete.[223]

There is limited available evidence to inform the management of TB in children caused by isolates resistant to isoniazid alone. A single study of children with isoniazid mono-resistant (HMR) TB describes good treatment outcomes using three to four drugs.[224] Guidelines suggest treatment for six to twelve months with rifampicin, pyrazinamide and ethambutol.[16, 140, 142] In

cases of extensive disease or for TBM, a fluoroquinolone and one other drug can be added. A study assessing the treatment of 18 children with RMR-TB demonstrate good outcomes when treated with four months of amikacin and a total of 18 months using a further 4-5 drugs.[225] Guidelines suggest that RMR-TB can be treated with isoniazid, pyrazinamide, ethambutol and a fluoroquinolone for 12-15 months.[16] In cases of extensive disease an injectable agent can be employed for the first few months, a further drug can be added and treatment extended to 18 months. If genotypic tests are employed to perform DST, most national programs advise treating RMR-TB with an MDR-TB regimen as these tests do not identify all of the mutations conferring isoniazid resistance.[226]

In addition to TB drugs, guidelines suggest children with TB should be given pyridoxine if they are HIV-infected, malnourished, breast-fed or are being given terizidone, cycloserine or high-dose isoniazid,[16] as pyridoxine deficiency is common.[227] Most experts put all children being treated for DR-TB on multivitamin supplements. Steroids have been demonstrated to improve outcome in children with TBM[228-230] and are additionally advised for airway obstruction and pericardial TB.[16, 140] Nutritional and metabolic requirements should be assessed as these children are commonly malnourished [191-192, 204] and have often been in a catabolic state prior to the diagnosis of DR-TB. They may also have high calorific requirements due to the ongoing tissue damage, repair and inflammation.

Other adjunctive treatments include bronchoscopy and surgery. In cases of intra-thoracic lymph node disease, with external pressure on the airways leading to compression and respiratory compromise, assessment by bronchoscopy is advised.[231-232] In cases of extensive resistance, where the disease is localized to one anatomical lobe or part of the lung, surgical resection may still have a place. If there is extensive destruction and fibrosis, it may be difficult for some drugs to penetrate into lesions with poor vascularization. Enucleation of the nodes may be required bronchoscopically or surgically, both to relieve the pressure on the airway and also to de-bulk the lymph node lesion.[231, 233]

Management of co-morbid conditions

Co-morbid medical conditions can increase the risk of TB and affect treatment outcomes. Examples include HIV infection,[63] diabetes, [234] and malnutrition.[43] Rates of HIV infection in paediatric MDR-TB cohorts range from 0%[144, 222] to 54%.[191] All children treated for DR-TB should be offered testing for HIV infection following counselling and consent from parents/guardians or, if old enough, the child. Important practical considerations in the co-

treatment of TB and HIV infection include the timing of initiation of cART, IRIS, drug-drug interactions[97, 177], and overlapping toxicities of cART and TB therapy.[203] Generally, it is recommended that children with DR-TB and HIV infection be started on cART within two weeks of initiating TB therapy.[15, 197, 235] This will decrease the likelihood of adverse drug reactions while allowing rapid initiation of immunorestorative therapy. The management of TBM in this situation is complex and requires further investigation.[236] IRIS occurs within the first few weeks of ART when a resurgent immune system begins to recognize *M. tuberculosis* antigens[183-185] and, when severe, may respond well to corticosteroids. Differentiating IRIS from treatment failure can be challenging but decreasing HIV viral load and improving CD4 count should point to IRIS. Little data exist on the interactions between cART and second-line TB therapy in adults[97, 177] with even less for children.[203] In general, stavudine should be avoided, and concomitant use of tenofovir and an injectable requires regular testing of renal function and electrolytes.

For children with DR-TB and diabetes, more frequent glucose monitoring may be indicated as both TB disease and some TB drugs (rifampicin, ethionamide, PAS and fluoroquinolones) can disrupt glycemic control. Malnourished children should be treated according to established protocols[237] and malnutrition should be prevented by the provision of nutritional support to children and their families.

Morbidity associated with drug-resistant tuberculosis

Chronic pulmonary disease may exist concurrent with pulmonary DR-TB or can occur later due to chronic lung inflammation and tissue damage. Peak flow testing or more extensive spirometry should be carried out with appropriate infection control precautions if the child is old enough to co-operate. Breathing exercises and physiotherapy are advised to improve function and as there is frequently a reversible component, a trial of bronchodilators is often merited.

Little is published regarding osteo-articular DR-TB in children. The few case series of spinal disease describe relatively good treatment outcomes.[238-239] It is advised that children should be followed by orthopaedic surgeons as deformities can deteriorate with the growth of the child. Spinal lesions particularly need to be monitored for many years as spinal growth can exaggerate any deformity, with the potential to compress the spinal cord and cause neurological damage. In settings where there are no orthopaedic specialists, nurses and community members can assist with limb and spine splinting and with physiotherapy. Reports

of DR-TBM in children describe very poor outcomes.[193, 240] TBM can cause devastating neurological damage and affected children ideally should have access to intensive physiotherapy and occupational therapy during and after their illness. Developmental assessments and level of functioning should be determined at the end of therapy and children should be followed up to monitor progress and to provide support. The care of severely disabled children is challenging and parents should be supported with access to care services as well as assistance in funding applications for resources to which they are entitled.

Although most of the adverse events of the drugs reverse on termination of therapy, the effects on hearing[241] and vision are often permanent. These can have a significant impact on the child's development and quality of life.[242-243] Hearing loss in adults treated for MDR-TB is common[244] but in children is poorly described. One study assessing hearing loss documented ototoxicity in 7% of children treated for MDR-TB.[204] Another study found hearing loss in 25% of children.[245] Adverse effects on vision and hearing should be quantified and appropriate aids that will improve function should be given to the child. The child may need physical intervention, such as hearing aids, or they may need extra school support or financial assistance. A final area of morbidity that is seldom addressed is the psychological aspect of both the condition and its treatment.[246] Children receive treatment for extended periods and TB is stigmatizing in some contexts. It may be necessary for the child to be admitted to hospital initially but for the majority, ambulatory treatment should be the norm, sparing the child separation from friends, families and communities.

Treatment monitoring

Children should be monitored for three reasons: to determine response to therapy; to identify adverse events early; and to promote adherence. A suggested monitoring schedule, which can be adapted to local conditions and resources, is demonstrated in Table 5. Response to therapy includes clinical, microbiological and radiological monitoring.[16] It is advised that children are clinically assessed on a regular basis to identify symptoms or signs that might signal response, including activity levels, respiratory function and neurological development.[16] Height and weight should be measured regularly and plotted on an appropriate percentile chart.[247] For children with pulmonary disease, respiratory samples should be collected. For older children, able to expectorate, the adult schedule is suggested, with monthly sampling.[197] For younger children, with an initial positive smear or culture result, samples can initially be taken monthly. After culture conversion this can be carried out every two to three months. Significant rates of 'cure' rather than simply 'treatment completed' have been reported in children treated for

MDR-TB, implying that ongoing microbiological testing is possible, even in young children.[58, 192, 195, 204, 222] For those with negative smear and culture samples at treatment initiation, samples are obtained if the clinical or radiological situation changes. All samples should be sent for culture and DST, in addition to smear microscopy. Finally, regular radiological monitoring with CR is advised for children with pulmonary disease[16] with additional radiology if clinically indicated. It can be useful to have a CR at the end of therapy to provide a baseline for follow-up. Although CR improvement is an important indicator of successful treatment response, complete resolution may not occur and a normal CR is not required to complete therapy. In the majority of reported cases, however, significant CR resolution at the end of therapy was observed.[58]

Adverse events

In children treated for MDR-TB, toxicity is common, occurring in up to 40% of cases.[221] Significant adverse events, however, and ones that necessitated stopping or changing treatment, were less common. The toxicity of the first- and second-line TB drugs has been well described in other reviews and is summarized in Table 3.[140, 203, 210] The toxicity of the second-line drugs in children will be described later in the thesis. This section therefore focuses on the monitoring and management of adverse events, specifically in children. Due to renal, thyroid, auditory and visual adverse events possible with second-line TB drugs, it is advised that prior to initiating therapy, children should have their hearing and vision tested as well as their renal and thyroid function. Children old enough to co-operate (usually from about five years) can be assessed using Ishihara charts and by pure tone audiometry (PTA).[248] Oto-acoustic emissions (OAE) can be used to test the hearing in younger children but visual testing is challenging for this age group. Clinicians should, however, be reassured that the incidence of ocular toxicity is very rare (0.05%) when ethambutol is given at the recommended dosage.[249]

Children should be assessed clinically for adverse events on a regular basis by their healthcare provider and on a daily basis by DOT supporter and/or caregivers following training in the recognition of signs and symptoms of adverse events. Thyroid function should be checked regularly if on a potentially thyrotoxic drug. Renal function and hearing should also be tested while taking an injectable drug. There is no need to monitor full blood count or liver function routinely. Transient elevations in transaminase levels are common at the start of TB therapy and are rarely associated with significant adverse events.[210] Due to the increased risk of myelosuppression, a regular full blood count is advised if the child is receiving linezolid.[250] Drugs to alleviate adverse events, such as analgesics, anti-emetics, anti-pruritics and drugs to

manage diarrhoea, are likely to improve adherence if provided free of charge. TB drugs should also always be provided without cost to the family. A suggested monitoring schedule is shown in Table 5 with the management of adverse events described in Table 6.

Promoting adherence

DOT is a key component of successful treatment and the use of community health workers (CHW) or DOT supporters can be valuable for promoting adherence and identifying adverse events early.[174, 251] DOT is a comprehensive package of support and assistance, rather than a paternalistic observation of ingestion.[252] Although young children, in effect, always receive their treatment under DOT, in a programmatic sense DOT implies treatment given under the supervision of someone outside the family. DOT should be made as easy as possible; CHWs and DOT supporters can be employed to give the medications at a convenient location such as at home or at a nearby clinic.[174] Long waiting times, peer pressure, unsympathetic staff and stigmatization at health facilities can deter attendance at clinic and impair overall adherence. If children are old enough to understand, it is important to invest time and effort in educating them about the disease and allow them to take responsibility for their illness and their treatment. Adolescents can be at high risk of severe disease and adherence can be challenging with associated poor treatment outcomes.[197, 253] If the child is not old enough, the parents must be prepared appropriately. The child and family should be warned about the possibility of all adverse events and what to do if they occur.[16] These adverse events should be managed proactively and promptly. Creative mechanisms should be employed to encourage adherence, with reward systems appropriate to the child's age; mobile telephone technology has been used successfully in adults and could play an important role in the adolescent age group.[175]

Infection control

Children traditionally have been considered to pose a low infection control risk as they generally have paucibacillary disease and limited tussive force. However, as the diagnosis of DR-TB is frequently delayed in children,[58, 204, 221] those with diagnosed DR-TB tend to be older than those with drug-susceptible disease [254-255] and have more severe pathology. In one paediatric MDR-TB cohort, over 60% of children were sputum smear-positive.[192] Infection control should therefore form a vital part of any management strategy.[256]

Children are a significant transmission risk if they have sputum smear-positive disease and a moderate risk whilst they still have sputum culture-positive disease. While smear-positive they

should sleep in a room separate from others. Those culture-positive should not sleep in the same room as the most vulnerable such as those HIV-infected or the very young. If the climate allows, children should be encouraged to spend as much of their time outside as possible. Play, eating and schooling areas should attempt to facilitate this. When outside, it is reasonable to allow children to play and eat without a mask. Where it is not possible to spend long periods of time outside, windows should be kept open, passive air extraction systems put in place and areas with sufficient resources should consider active air flow management systems. Those without pulmonary disease are unlikely to pose an infection risk unless there is pus discharging, uncovered from a body site.

Staff should protect themselves when interacting with infectious children. If the child is sputum smear-positive, staff should wear a fit-tested respirator with a filter efficiency of 95% or greater (e.g., N95, N99, N100). More comprehensive guidance on infection control measures to employ in healthcare facilities has been documented by the WHO and the CDC.[257-258]

Multidisciplinary care

Multidisciplinary care is a cornerstone in the successful management of children with DR-TB. In addition, the child and caregiver should be engaged as active members of the health care team. Input from pharmacists can be invaluable in providing appropriate medications, formulations and advice concerning interactions and pharmacokinetics. Support from a dietician is important in monitoring and planning calorie intake and the correct balance of nutrients, vitamins and minerals. Physiotherapy and occupational therapy are of benefit not only for those with neurodevelopment involvement but also for those with respiratory and musculo-skeletal deficit. Social services should assess home circumstances and support the caregiver to look after a child who may have complex medical needs and must take multiple medications. They must also assist the family in securing any funding or grants that they are eligible for to assist in the process of home-based care. In cases of neglect, abuse or drug and alcohol use, child placement with alternative caregivers may be necessary. In areas of limited resources, many of these key tasks can be carried out by CHWs. Ongoing education is important and when no longer infectious, children should be encouraged to return to school.

Table 3 - Studies describing drug-resistant tuberculosis treatment in children

First author	Year of study	Location	Number of children included	Number culture-confirmed	Treatment success (%)	Adverse events
Seddon[192]	2003-2008	Cape Town, South Africa	111	111	88 (79)	NS
Fairlie[191]	2008	Johannesburg, South Africa	13	13	7 (54)	2
Leimane[222]	1998-2006	Latvia	76	NS	70 (92)	26
Feja[144]	1995-2003	New York, USA	20	6	16 (80)	4
Mendez Echevarria[205]	1994-2005	Madrid, Spain	8	5	8 (100)	4
Granich[259]	1994-2003	California, USA	10	NS	9 (90)	NS
Drobac[204]	1999-2003	Lima, Peru	38	28	36 (95)	16
Schaaf[58]	1998-2001	Cape Town, South Africa	39	39	21 (54)	20
Padayatchi[193]	1992-2003	Durban, South Africa	8	8	1 (13)	NS
Schluger[194]	1983-1993	New York, USA	2	2	2 (100)	NS
Suessmuth[195]	2005	Hannover, Germany	1	1	1 (100)	NA
Pinon[260]	2010*	Turin, Italy	2	NS	1 (50)	0
Kjöllerström[196]	2011*	Lisbon, Portugal	4	4	4 (100)	3

*year of publication as year of study unclear; NS: not stated

Table 4 - A proposed dosing table for the drugs used in the treatment of drug-resistant tuberculosis in children

		Isoniazid	**Pyrazinamide**	**Ethambutol**		**Ofloxacin**		**Levofloxacin**	**Moxifloxacin**	**Terizidone**	**Ethionamide**	**PAS**
	Dosing range (mg/kg)	15-20	30-40	20-25		15-20		7.5-10	7.5-10	15-20	15-20	150
Weight (kg)	Tablet size (mg)	100	500	400	100	200	400	250	400	250	250	4000
3-4.9		50 (1/2 tab)	125 (1/4 tab)	100 (1/4 tab)	100 (1 tab)	100 (1/2 tab)	100 (1/4 tab)	*	*	62.5 (1/4 cap)	62.5 (1/4 tab)	500 (1/8 sach)
5-6.9		100 (1 tab)	250 (1/2 tab)	100 (1/4 tab)	150 (1½ tab)	100 (1/2 tab)	100 (1/4 tab)	62.5 (1/4 tab)	*	125 (1/2 cap)	125 (1/2 tab)	1000 (1/4 sach)
7-9.9		150 (1 ½ tab)	250 (1/2 tab)	200 (1/2 tab)	200 (2 tabs)	150 (3/4 tab)	200 (1/2 tab)	125 (1/2 tab)	*	187.5 (3/4 cap)	187.5 (3/4 tab)	1500 (3/8 sach)
10-13.9		200 (2 tabs)	500 (1 tab)	300 (3/4 tab)	300 (3 tabs)	200 (1 tab)	200 (1/2 tab	125 (1/2 tab)	100 (1/4 tab)	250 (1 cap)	250 (1 tab)	2000 (1/2 sach)
14-19.9		300 (3 tabs)	500 (1 tab)	400 (1 tab)	400 (4 tabs)	300 (1 ½ tab)	300 (3/4 tab)	187.5 (3/4 tab)	200 (1/2 tab)	375 (1 ½ caps)	375 (1 ½ tab)	3000 (3/4 sach)
20-29.9		400 (4 tabs)	750 (1 ½ tab)	600 (1 ½ tab)	600 (6 tabs)	400 (2 tabs)	400 (1 tab)	250 (1 tab)	200 (1/2 tab)	500 (2 caps)	500 (2 tabs)	4000 (1 sach)
30-39.9		400 (4 tabs)	1000 (2 tabs)	800 (2 tabs)	800 (8 tabs)	600 (3 tabs)	600 (1 ½ tab)	312.5 (1 ¼ tabs)	300 (3/4 tab)	625 (2 ½ caps)	625 (2 ½ tabs)	6000 (1 ½ sach)
>40		400 (4 tabs)	1500 (3 tabs)	1200 (3 tabs)	1200 (12 tabs)	800 (4 tabs)	800 (2 tabs)	375 (1 ½ tabs)	400 (1 tab)	750 (3 caps)	750 (3 tabs)	8000 (2 sach)

*Unable to create an appropriate fraction of a tablet for a child of this weight

Table 5 - A proposed monitoring schedule to determine response and detect adverse events when treating drug-resistant tuberculosis in children

All children	**Baseline**	**Month**										**Ongoing**
		1	**2**	**3**	**4**	**5**	**6**	**9**	**12**	**15**	**18**	
HIV status	•											
Toxicity (symptoms, signs)	•	•	•	•	•	•	•	•	•	•	•	•
Height and weight	•	•	•	•	•	•	•	•	•	•	•	•
Audiology[1]	•	•	•	•	•	•	•					
Colour vision testing[2]	•	•	•	•	•	•	•	•	•	•	•	•
CR[3]	•			•			•					
TB culture and DST[4]	•	•	•	•	•	•	•					
Creatinine and potassium[1]	•	•	•	•	•	•	•					
TSH, T_4[5]	•			•			•	•	•	•	•	•
Haematology (FBC with differential)[6]	•	•	•		•		•	•	•	•	•	•
HIV-infected												
LFTs, Cholesterol	•						•			•		•
CD4 count and viral load	•						•			•		•

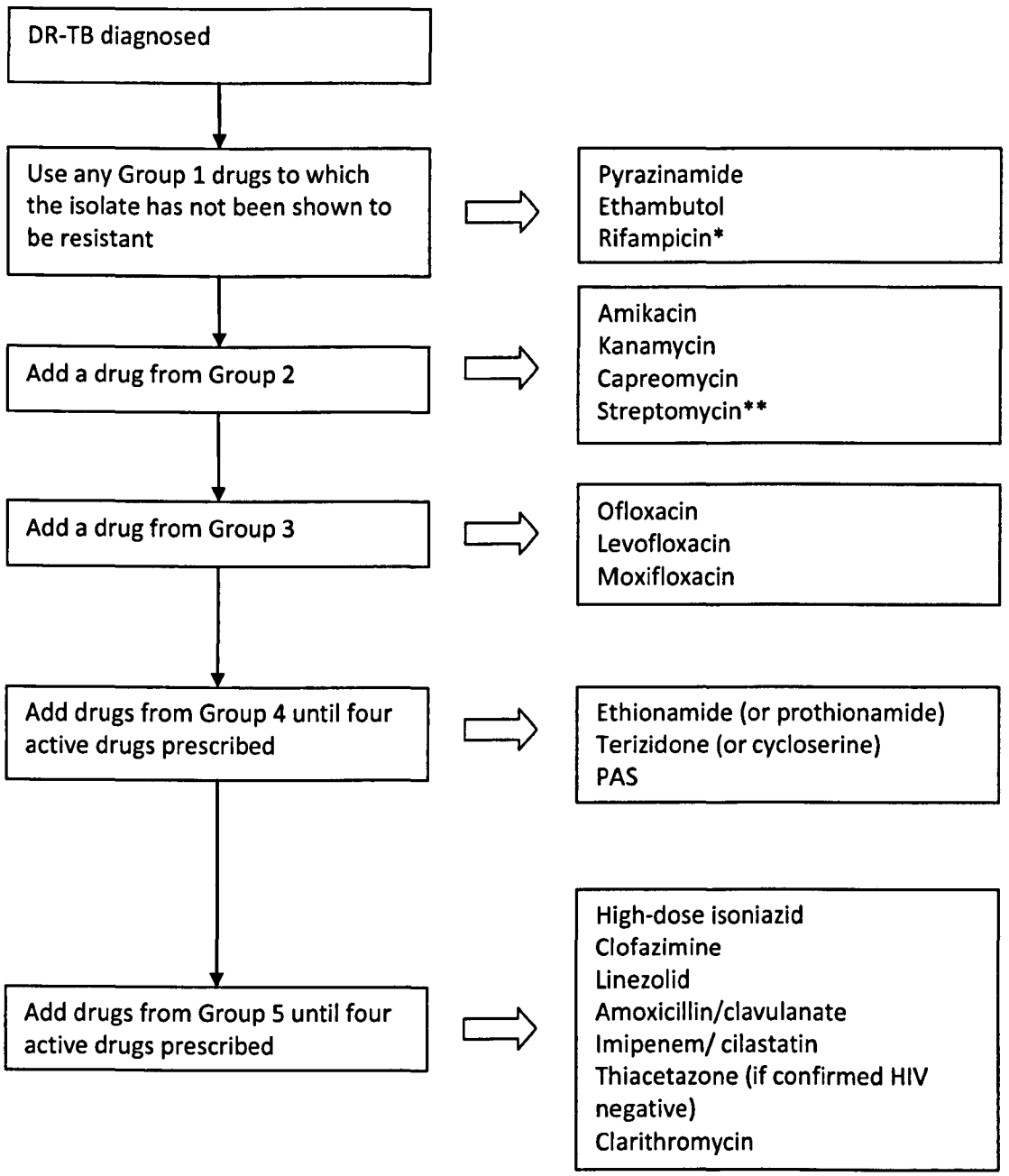
HIV: Human Immunodeficiency Virus; CR: chest radiograph; TB: tuberculosis; DST: drug susceptibility test; TSH: thyroid stimulating hormone; FBC: full blood count; LFT: liver function tests;
[1]Monthly whilst on an injectable and at six months following termination of injectable
[2]If on ethambutol
[3]If any pulmonary involvement or at any point if clinically indicated. To be repeated at the end of treatment
[4]Monthly if old enough to expectorate. If unable to expectorate and initially smear or culture positive, monthly until culture-converted then three monthly. If initially smear and culture negative, to perform if clinically indicated. For extra-pulmonary TB, samples can be taken when clinically indicated
[5]if on ethionamide, prothionamide or PAS
[6]if on linezolid or HIV-infected

Table 6 - **The management of adverse events in the treatment of drug-resistant tuberculosis in children**[140-142, 197, 203, 261]

Adverse event	TB drugs possibly responsible	cART drugs possibly responsible	Monitoring	Management
Hearing loss[241]	Amikacin, kanamycin, capreomycin		PTA or OAE assessed and classified using ASHA guidelines[248]	If any hearing loss is detected strong consideration should be given to stopping/switching the injectable drug
Renal impairment[241]	Amikacin, kanamycin, capreomycin,	Tenofovir	Blood testing	1. Evidence of mildly elevated creatinine should prompt re-testing 2. Markedly elevated creatinine or potassium should lead to the cessation of all nephrotoxic drugs
Visual impairment[167]	Ethambutol		Clinical or Ishihara Chart	Any deterioration in visual fields or colour vision should lead to stopping/switching the ethambutol
Hypothyroidism[262]	Ethionamide, prothionamide, PAS		Blood testing	1. If T4 is low, continue medications and supplement with 0.05mg thryoxine supplement daily 2. Continue to monitor T4 and consider increasing supplementation to 0.1mg daily
Hepatitis[210]	Rifampicin, isoniazid, pyrazinamide, ethionamide, prothionamide	Nevirapine, efavirenz, PIs	Clinically and blood testing	1. Clinical suspicion of hepatitis (vomiting not directly associated with medications, abdominal pain or jaundice) should lead to immediate cessation of all hepatotoxic drugs 2. Investigation into non-drug aetiologies (hepatic viruses etc.) should take place 3. Treatment should continue with medications that are less hepatotoxic (ethambutol, injectables, fluoroquinolones, terizidone/cycloserine and PAS). 4. The hepatotoxic TB drugs can be re-introduced one-by-one every two days 5. Given that the child is on treatment for DR-TB the relative merits of re-introducing isoniazid, rifampicin and pyrazinamide should be considered.
Rash	All TB drugs	Nevirapine, efavirenz	Clinical	1. Mild reactions – symptomatic relief 2. Stevens Johnson reactions - immediate cessation of all drugs (including all TB and HIV medications) until the symptoms have resolved. 3. Sequential re-introduction can then occur. Re-start the TB medications one by one every two days and monitor response. If the child was on cART, once TB treatment is re-established all cART medications should be restarted at the same time to prevent the development of resistance. 4. Once TB and cART drugs are established other agents can be added. Co-trimoxazole is an important, but rare, cause of severe skin reactions.

Vomiting	Ethionamide, prothionamide, PAS, ethambutol	Zidovudine, PIs	Clinical	If nausea and vomiting compromise drug delivery, it may be prudent to split the dose of ethionamide/prothionamide or give it at a separate time from the other drugs
Diarrhoea	PAS, ethionamide, prothionamide	Zidovudine, PIs	Clinical	1. PAS is usually given twice a day but if diarrhoea is severe, the dosage can be reduced or the drug given in smaller quantities more frequently 2. If diarrhoea is profuse, regular monitoring of hydration status and serum potassium should be conducted 3. CHWs or DOT supporters can be trained to provide oral rehydration solutions for those with vomiting or diarrhoea.
Peripheral neuropathy[263]	Isoniazid,	Stavudine, didanosine	Clinical	1. Mild reactions - increase the dose of pyridoxine or reducing the dose of the offending TB drug 2. If severe or persisting in spite of above, the TB drug should be stopped.
Neuropsychiatric effects[264]	Terizidone, cycloserine, isoniazid, fluoroquinolones	Efavirenz	Clinical	1. As a first step, it is important to verify that the child has been prescribed and is receiving the correct dose as over-dosing can be associated with adverse events 2. The next step is to reduce the dosage of the drug felt most likely to be responsible and monitor the effect. 3. If this does not help then the drug should be stopped. 4. If no resolution then the drug should be re-introduced and the next most likely drug reduced in dose and then, if necessary, stopped.
Joint problems[265]	Pyrazinamide, fluoroquinolones		Clinical	1. Analgesia 2. Reducing dose or stop one of potentially offending drugs
Metabolic problems	Linezolid	Stavudine, didanosine, zidovudine	Clinical and blood tests	Lactic acidosis is life-threatening and if determined, all potentially implicated drugs should be stopped
Bone marrow suppression[266]	Linezolid	Zidovudine	Clinical and blood tests	The responsible drug should be switched or stopped

Figure 2 - An algorithm to aid in the construction of a drug-resistant tuberculosis treatment regimen for children



*Consider including rifampicin for six months if the child is treated for failure of first-line therapy or if there are multiple potential source cases

**Consider streptomycin if the isolate is found to be resistant to amikacin, kanamycin or capreomycin but is demonstrated to be susceptible to streptomycin

Literature review 3: second-line tuberculosis drugs in children

Concepts from the following topic have been written as articles:

- *Seddon JA, Hesseling AC, Marais BJ, McIlleron H, Peloquin CA, Donald PR, Schaaf HS. Paediatric use of second-line anti-tuberculosis agents: A review. Tuberculosis (Edinb) 2012; 92: 9-17*
- *Schaaf HS, Seddon JA, Caminero JA. Second-line antituberclosis drugs: current knowledge and controversies. Prog Respir Res 2011; 40: 81-95.*
- *Seddon JA, Schaaf HS, Hesseling AC. Retooling existing tuberculosis drugs for children. Clin Infect Dis 2012 (in press)*

In this section, the literature surrounding the use of second-line TB drugs in children is reviewed. As with the two previous sections, the literature is presented as a discussion of the drugs and their use. The review starts by reviewing the properties of the drugs, moves to the toxicity of the drugs and concludes with a discussion of the interaction with cART medications. To search this literature systematically, a systematic review for each of the individual drugs would have needed to have been completed. This would have been a vast project and so although what follows is not a systematic review, it is comprehensive. Multiple data sources were accessed and references cross-checked to identify relevant articles. A summary of dosages and adverse effects is shown in Table 7.

Characteristics of the second-line drugs in children

Injectable medications used in the treatment of drug-resistant TB include the aminoglycosides, amikacin and kanamycin, as well as the cyclic polypeptide, capreomycin. Streptomycin, another aminoglycoside, was previously used widely in re-treatment TB cases in combination with first-line medications and this has led to high levels of resistance to streptomycin in strains already resistant to rifampicin and isoniazid. Hence, it is rarely used in the treatment of MDR-TB. However, streptomycin can be used in the treatment of XDR-TB, if the organism is found to be susceptible, as there is limited cross-resistance with the other injectable medications. High levels of cross-resistance between amikacin and kanamycin mean that if a strain is found to be resistant to one, the other is very unlikely to be of use.[267] For children, amikacin is usually given in preference to kanamycin as it has a lower minimum inhibitory concentration (MIC) and the available ampoule sizes are smaller, preventing wastage. Amikacin and kanamycin are generally preferred to capreomycin as the first choice injectable

for MDR-TB in children with capreomycin reserved, in most programmes, for the treatment of XDR-TB. However, there is evidence that if a strain is resistant to an aminoglycoside it will already be resistant to capreomycin.[268-269] Alternatively, if resistant to capreomycin there is a chance that it will still be susceptible to amikacin or kanamycin. The amikacin MIC for *M. tuberculosis* (strain type H37Rv) is 0.5-1.0μg/ml[214, 270-271] which compares to 2-4μg/ml for both kanamycin and capreomycin.[214, 270-271] Here, MIC in liquid broth culture refers to the concentration at which the drug inhibits mycobacterial growth as compared to a culture containing a 1:100 dilution of mycobacteria (i.e. 99% inhibition). Pharmacokinetic profiles have been studied in children receiving short-courses of aminoglycosides for bacterial infections given intravenously (IV)[272] but not in prospective studies of children on prolonged courses of treatment, where it is typically given intramuscularly (IM). Half-lives ($t_{1/2}$) of 2.5-3.5 hours are reported for amikacin and kanamycin given IV.[273] As the maximum serum concentration (C_{max}) is dose-dependent consideration should be given to therapeutic drug monitoring at the start of therapy to establish the ideal dose for each child.[214] Time to maximum serum concentration (t_{max}) is at the end of the infusion for IV injections and is estimated to be between 30 and 60 minutes for IM injections. Elimination is by urinary excretion and doses should be reduced in patients with renal impairment. Guidelines recommend that the dose of amikacin in children should be from 15 to 22.5mg/kg daily[16, 197] and kanamycin or capreomycin from 15 to 30mg/kg. Oral absorption is very poor and so administration for all three agents is only possible via IM or IV injection.

The fluoroquinolones have a central role in the management of MDR-TB in children. Resistance to early generation fluoroquinolones (ofloxacin) may not necessarily imply resistance to later generations (moxifloxacin or levofloxacin).[274] The MICs and mutant prevention concentrations (MPCs) of the fluoroquinolones follow a sequential progression with lower concentrations required to prevent growth in the higher generation fluoroquinolones.[215] MICs, on plates of 7H11 media, of 0.5, 0.71, 0.35, 0.177 and 0.125μg/ml were reported for ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin and gatifloxacin respectively, using a definition of the lowest concentration required to inhibit any growth within four weeks.[213-214] Few studies have assessed the pharmacokinetics of the fluoroquinolones in young children; the available data is largely from studies in older children with cystic fibrosis.[275-277] None have been conducted in children treated for MDR-TB.[278] Caparelli et al[279] studied children aged six months to sixteen years after single gatifloxacin doses of 5, 10 and 15mg/kg bodyweight (maximum 600mg). Drug clearance was more rapid than in adults and children required a higher mg/kg dosage to achieve similar blood concentrations. This was confirmed in a study by Chien et al[280] who studied 85 children, also aged six months to sixteen years, given IV or oral levofloxacin. IV and

oral dosing led to comparable blood concentrations. They concluded that children younger than five years of age clear levofloxacin almost twice as fast as adults and consequently are exposed to approximately one half of the dose. They recommend that children older than five years receive 10mg/kg daily but children less than five should be given 10mg/kg twice daily. Early bactericidal activity (EBA) studies showed that ofloxacin,[152] levofloxacin, moxifloxacin and gatifloxacin[281] all have activity close to that of isoniazid and activity exceeding that of isoniazid after several days of treatment. Whether this later effect relates to sterilizing activity is uncertain. Although ciprofloxacin has a low MIC, it is not recommended in the treatment of MDR-TB due to its poor EBA.[166]

Thioamides include ethionamide and prothionamide; as the mechanism of action for the two is similar and cross-resistance is complete only one of the two should be used. The thioamides share a number of biochemical pathways with isoniazid in their activation and, dependent on mutation, can show cross-resistance.[91, 154] Ethionamide has a narrow therapeutic margin between efficacy and toxicity with a MIC in broth (99% growth inhibition) of 0.25-0.5μg/ml.[271] In adults, absorption from the intestinal tract is almost complete and is little affected by food or antacids.[282] Protein binding is 30%[283] and ethionamide distributes with ease throughout the body, including to the cerebrospinal fluid (CSF). In adults, peak plasma concentrations occur approximately two hours post dose and C_{max} has been found to be between 1.9 to 2.5μg/ml following an oral dose of 500mg.[282, 284-285] For adults, increasing the dosage above 750mg results in severe intolerance and so for clinical purposes, the recommended peak serum concentration for susceptible strains of *M. tuberculosis* is 2.5μg/ml. Studies in children are limited. Published data include an isolated case report and a study evaluating CSF levels of ethionamide in children with TBM.[284, 286] Recently, a study from South Africa has demonstrated that dosages of 15-20mg/kg achieve adequate serum concentrations in children.[287] Younger children (≤2 years of age) eliminated the drug more rapidly than older children and HIV co-infection was associated with lower concentrations.

Cycloserine is an analogue of D-alanine, is bacterostatic and acts by inhibition of peptidoglycan synthesis. The alternative drug, terizidone, comprises two molecules of cycloserine attached to a molecule of terephtalaldehyde. The MIC for terizidone is very variable.[288] Cycloserine has a MIC in broth (99% growth inhibition) between 25-75μg/ml.[214, 271] Cycloserine is completely and rapidly absorbed after oral administration with a t_{max} of 2-4 hrs.[288-289] Distribution is widespread, including to the CSF. Although unaffected by orange juice or antacids, absorption is significantly delayed when taken with a high fat meal.[290] There are no pharmacokinetic data to guide paediatric dosing in different age groups for either drug.

PAS is produced in two formulations: free acid PAS (enteric-coated slow-release granules) and sodium salt PAS (granules and tablets). The mechanism of action is unclear but may be related to thymidylate synthesis or disruption of acquisition of iron. MIC for drug-susceptible strains in broth is <1μg/ml[291] (using the Alamar blue colourimetric method) and slightly higher (4-8μg/ml) for some MDR-TB strains.[214] PAS is 50-60% protein bound and $t_{1/2}$ of the free drug is 45-60 minutes. Absorption is increased with food[214] and CSF penetration is poor. Since PAS has no post-antibiotic effect, it is recommended that twice daily dosing is used to constantly keep its concentration above MIC.[292] Treatment with either formulation results in similar blood concentrations. Despite being the oldest TB drug, only one small English language study of four children has reported paediatric pharmacokinetic data.[293] Children were given 300mg/kg/day, in five divided doses of 60mg/kg. T_{max} was at 60 minutes with C_{max} between 6.25μg/ml and 12μg/ml. CSF peak concentrations were generally greater than 1μg/ml.

The group five medications have either uncertain efficacy against *M. tuberculosis* or an uncertain place in the treatment of MDR-TB. Clofazimine is an old drug, discovered in 1954. Used extensively to treat *Mycobacterium leprae*, it has only recently been used in the treatment of *M. tuberculosis* disease. The mechanism of action is unknown but it has an MIC in broth of ≤1μg/ml (99% growth inhibition)[271, 294] and may have a synergistic effect when used in combination with amikacin.[295] Oral absorption is 45-62% and is increased with a high fat meal.[296] Serum concentrations are often very low[214, 296] but as the drug tends to concentrate inside macrophages it may be more effective at killing intracellular organisms than the concentrations in serum would suggest. A recent study from Bangladesh demonstrated that adults with MDR-TB benefit from the addition of clofazimine to their treatment regimens.[217] No pharmacokinetic studies have been conducted in children. Linezolid is an oxazolidinone, a new class of antibiotic with a novel mechanism of action. Cross-resistance is therefore unlikely but it does appear that the MIC is increased in strains already resistant to other first-line drugs.[214] The pharmacokinetics of linezolid has been studied in children of various ages[297-298] and children have more rapid clearance and shorter $t_{1/2}$ than adults, indicating a need for more frequent dosing. However, the optimal dosing frequency in children with TB has not been established. It is well absorbed after oral administration and distributes widely, including good CSF penetration.[299] EBA in an adult study was similar for once or twice daily dosing with 600mg[300]and the limited clinical experience in children on treatment for XDR-TB has seen good outcomes with twice daily dosing in younger children and once daily in those older.[301-302] Thiacetazone was previously used extensively to treat TB and only fell out of favour with severe Stevens-Johnson reactions seen in association with HIV. The MIC (complete inhibition)

is 0.4-1.0μg/ml[303] and cross-resistance with ethionamide is 29-79%. In adults, it is well absorbed after oral administration with C_{max} 1.59μg/ml, t_{max} 3.3 hours and $t_{1/2}$ 15-16 hours.[304] There are no published pharmacokinetic studies in children. The final drugs in class five are the beta-lactams and the macrolide clarithromycin. Amoxicillin and the carbapenems (imipenem and meropenem) have some activity against *M. tuberculosis*, but MICs are not achievable in serum. When combined with clavulanic acid, however, the MIC is lower and becomes possible to achieve in serum.[305-307] The addition of ethambutol seems to provide a synergistic effect, even if given at sub-inhibitory concentrations.[308] Amoxicillin and clavulanic acid are rapidly absorbed orally but the carbapenems must be given parentrally. Meropenem has good CSF penetration and has also been shown to be active against 'persistent' strains grown in anaerobic media.[305] Clarithromycin has been used extensively to treat bacterial infections, non-tuberculous mycobacteria as well as *M. lepra*. Although MICs are high using agar 7H10 at 99% inhibition of growth (4 to ≥16μg/ml)[309-310] it may have a bi-directional synergistic role with some of the first-line drugs – improving the efficacy of the first-line drugs as well as the first-line drugs reducing its MIC.[311-312] Moreover, it works mainly intracellularly and so this high MIC may not accurately reflect its bactericidal activity. Studies in children (aged six months to ten years) have shown that it is well absorbed orally and reaches C_{max} (3.59μg/ml) after about 3 hours. High doses are tolerated and food seems to increase bioavailability.[313]

Safety and toxicity

Monitoring and describing adverse effects in children is challenging; young children cannot articulate pain, nausea, vertigo, peripheral neuropathy, anxiety or confusion. Rashes are common due to a variety of aetiologies and the testing of hearing and vision is more difficult than in adults. However, it is particularly important to detect adverse effects as, in addition to life-threatening and unpleasant effects, growth and neuro-cognitive development may be affected. Children treated for MDR-TB are usually on multiple medications and determining the drug responsible for an adverse effect can be difficult. This is of concern as HIV frequently complicates MDR-TB and overlapping drug toxicity should be considered.[97, 177]

In the treatment of MDR-TB any first-line drugs to which the organism is still susceptible are used. The adverse effects of the first-line medications have been well described and children seem to develop adverse effects less frequently than adults.[16, 314-317] Isoniazid can cause peripheral neuropathy,[263] while pyrazinamide and isoniazid can lead to hepatitis. All can cause rash, gastrointestinal upset and arthritis.[16, 261, 317] Isoniazid used at high dose has not been well studied and adverse effects may be more pronounced than with the traditional dose. Although

the incidence of ethambutol-related optic neuritis is much lower in children than in adults, concerns remain regarding toxicity. [167, 249]

The aminoglycosides and polypeptides can cause peripheral neuropathy, hypersensitivity and rash, but the main toxicities of concern are nephrotoxicity, ototoxicity and vestibular derangement. Fatal renal failure and electrolyte imbalances, particularly hypokalaemia, have been reported in adults treated with capreomycin.[318] Hearing loss is irreversible, usually developing first in the high frequencies and then progressing to the speech recognition frequencies. If high frequency loss is detected early and the drug can be stopped without compromising the child's health, communication may be preserved. Therefore, unless monitored regularly hearing loss is only detected once communication problems develop. No studies have assessed toxicity using these agents in children with TB. Studies in neonates[319] and children with cystic fibrosis[320] demonstrate limited toxicity[63] but assessment of hearing loss in children receiving longer courses of aminoglycosides following liver transplantation, as occurs in MDR-TB treatment, found hearing loss in 15 of 66 children evaluated, using a 35dB loss at one frequency to define hearing loss. [321] Adult studies of MDR-TB treatment demonstrate high rates of hearing loss, vestibular dysfunction and renal impairment, the latter two often reversible.[322-324] In adults the cumulative dose is the greatest indicator of ototoxicity with a mean onset time of nine weeks following treatment initiation.[273] Certain familial mitochondrial mutations predispose patients to hearing loss[325-328] and aspirin may offer some protection. These mutations and their relationship with hearing loss have not been studied in children, however.

The fluoroquinolones were shown in the 1970s to cause cartilage damage in the joints of juvenile beagles[164] and although multiple studies and reviews have subsequently demonstrated safe use in children,[165, 329-335] concerns remain. They can also cause psychological/neurological disorders, sleep problems, gastrointestinal upset and peripheral neuropathy. The newer fluoroquinolones seem to be associated with fewer adverse effects than the older medications,[265] but caution must be exercised with moxifloxacin due to possible QT interval prolongation.[336] When used in the treatment of MDR-TB, they are generally well tolerated in both adults and in children with few significant adverse effects.[330] However, a large number of adverse effects have been documented in adults receiving a fluoroquinolone and pyrazinamide for preventive therapy.[146, 337] The reason for this is not clear.

Few studies have assessed the adverse effects of the thioamides on children. Both ethionamide and prothionamide are commonly associated with adverse effects[338-340] and can

cause profound gastrointestinal upset; severe nausea and vomiting can compromise adherence for both adults and children. The severity of symptoms usually subsides with time but symptoms can be reduced by initially splitting the daily dose or introducing the drug gradually with escalation of the dose over time. The full dose, given once daily, should, however, be aimed for within a few weeks of starting treatment. The thioamides show structural similarities to the potent thyrostatic drug methimazole, which seems to inhibit thyroid hormone synthesis by inhibition of organification.[341] As a result, hypothyroidism can occur.[262, 342-346] Pellagra-like rash[347], hepatitis[340, 348-354] and hypoglycaemia[355] have also been documented as well as rare central nervous system adverse effects including seizures, encephalopathy and acute psychosis.[347] Prothionamide seems to be marginally better tolerated in adults.[356]

Cycloserine and terizidone have been poorly studied in children. In adults, cycloserine has been widely implicated in neuropsychiatric adverse effects such as anxiety, depression, confusion, psychosis, irritability, tremor, convulsions and aggression.[264, 357-359] It has also been associated with hypersensitivity reactions in those with HIV[360] and with an episode of encephalitis.[361] From the very limited data that are available, terizidone seems to be better tolerated. Emerging data suggest that terizidone has fewer adverse effects (1%) than cycloserine (11%).[362]

The newer granular formulation of PAS is well tolerated and easily administered to children. PAS can cause hypothyroidism,[262, 363-364] an effect which may be potentiated by the concomitant use of ethionamide.[262] Gastrointestinal problems,[365-366] hepatitis,[367] thrombocytopenia,[368-370] hypoglycaemia,[371] vasculitis, arthralgia, eosinophilia, malabsorption [372-373] and a lymphoma-like syndrome (lymphadenopathy, rash and hepatomegaly)[374-377] are other potential adverse effects. Hypersensitivity reactions, characterised by fever, conjunctivitis and rash, may occur in up to 5-10% of patients on PAS, usually within the first couple of months.[366-367, 375-376] It may be possible to desensitise those with hypersensitivity to PAS by starting with a low dose and slowly increasing.[378] However this is not recommended.[377]

Toxicity of the group five drugs is considerable but adverse effects are less common in children compared to adults. There is much experience in the use of clofazimine as it has been given extensively in the treatment of leprosy. It commonly causes gastrointestinal symptoms such as diarrhoea, nausea, vomiting and abdominal pain. The majority of patients develop a red-brown hyperpigmentation of the skin and conjunctiva which is reversible but may take many months to revert. A recent leprosy trial in India and China included 422 children less than 15 years of

age. Clofazimine was very well tolerated and few drug reactions were noted. Skin discolouration was usually short-lived and felt to be acceptable to patients.[379] Adverse effects in children on short courses of linezolid are rare but include headache and gastrointestinal disturbance.[266, 298, 380-382] With prolonged use in adults withdrawal of the drug is frequently required due to myelosuppression (including pancytopenia) and peripheral and optic neuropathy; lactic acidosis has also been reported.[383-389] Reports of linezolid use in children with MDR-TB have found it to be well tolerated.[260, 302, 383-384] Thiacetazone was used widely to treat TB prior to the advent of HIV. Severe, life-threatening Stevens Johnson reactions were associated with thiacetazone use in HIV-infected adults[390-391] and children.[392] Although it is contraindicated only in HIV-infected individuals, it is now rarely available in most countries. Other adverse effects include gastrointestinal disturbances, skin reactions, hepatotoxicity, haemolytic anaemia and agranulocytosis.[393] The most common adverse effects of the beta-lactams are gastrointestinal and hypersensitivity reactions. Occasionally liver and renal derangement can occur. The macrolides can cause gastrointestinal disturbances, hepatotoxicity, prolonged QT syndrome and rash.

Effect of Human Immunodeficiency Virus co-infection and interaction with antiretroviral therapy

Co-infection with both TB and HIV is common in areas where both diseases are widespread.[394-395] Rapid initiation of cART in children with MDR-TB is critical due to the advanced spectrum of TB disease observed in this paediatric subpopulation.[192] The drug interactions between cART and first-line TB drugs have been extensively reviewed.[396-397] Rifampicin reduces the concentrations of many concomitantly administered drugs including the key antiretroviral non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Data describing the pharmacokinetic interactions between cART and the second-line TB drugs are incomplete and the metabolic pathways of some of the drugs are poorly characterized. Hence, unanticipated interactions might occur. However, the potential for clinically important changes in cART or TB drug concentrations is less for most second-line TB regimens compared to the rifampicin-containing first-line regimens. cART and second-line TB drugs have many adverse effects in common. High rates of neuropathy, hypokalaemia, hypothyroidism and marked renal impairment have been reported in adult populations with MDR-TB and a high proportion of HIV infected patients. [398-399] Drug interactions between TB drugs and cART are therefore important to consider.[97, 177] However, the risks attributable to the TB and cART drug combinations versus those due to potential confounding factors such as the extent of immune

suppression, co-morbidities (e.g. chronic viral hepatitis, or diabetes), concomitant medication or toxins and nutritional status, are uncertain. Table 8 summarises possible interactions and adverse effects that may be exacerbated. Few studies have assessed second-line drugs in combination with cART in adults and no studies have done so in children.

Table 7 - A summary of the dose and adverse effects of the second-line drugs used in the treatment of drug resistant tuberculosis

Drug	Dose recommended	Formulation size	Main adverse effects
Kanamycin	15-30mg/kg once daily	1g vial	Ototoxicity, nephrotoxicity
Amikacin	15-25mg/kg once daily	100mg, 250mg, 500mg and 1g vials	As for kanamycin
Capreomycin	15-30mg/kg once daily	1g vial	As for kanamycin
Ofloxacin	15-20mg/kg once daily	200mg, 400mg	Sleep disturbance, gastrointestinal disturbance, arthritis, peripheral neuropathy,
Levofloxacin	10mg/kg once daily (twice daily for <5 years)	250mg, 500mg	As for ofloxacin
Moxifloxacin	7.5-10mg/kg once daily	400mg	As for ofloxacin, prolonged QT syndrome
Ethionamide/Prothionamide	15-20mg/kg once daily	125mg and 250mg tablets	GI disturbance, metallic taste, hypothyroidism
Cycloserine/Terizidone	15-20mg/kg once daily	250mg capsules	Neurological and psychological effects
PAS	150mg/kg granules daily in two or three divided doses	Sachets of 4g	GI intolerance, hypothyroidism, hepatitis
Clofazimine	5mg/kg once daily	50mg, 100mg tablets/capsules	Skin discoloration
Linezolid	10mg/kg twice daily (once daily for >10 years)	600mg tablets and syrup	Diarrhoea, headache, nausea, myelosuppression, neurotoxicity, lactic acidosis and pancreatitis
Amoxicillin/clavulanate, Imipenem, Meropenem	As for bacterial infections	Amoxicillin/clavulanate – various formulations Meropenem – 500mg and 1g vials Imipenem – 250mg and 500mg vials	Gastrointestinal intolerance, hypersensitivity reactions, seizures, liver and renal dysfunction
Thiacetazone	5-8mg/kg once daily	150mg tablets	Stevens Johnson Syndrome in HIV-infected patients, gastrointestinal intolerance, hepatitis, skin reactions
Clarithromycin	7.5- 15mg/kg twice daily	500mg tablets	GI intolerance, rash, hepatitis, prolonged QT syndrome, ventricular arrhythmias
High dose isoniazid	15-20mg/kg once daily	100mg tablets	Hepatitis, peripheral neuropathy

Table 8 - Potential interactions and combined toxicity between the second-line tuberculosis drugs and antiretroviral treatment[95,96]

Drug	Pharmacokinetic interactions	Increased risk of adverse effects
Injectables	Unlikely	Nephrotoxicity with tenofovir*
Fluoroquinolones	Moxifloxacin concentration may be reduced by ritonavir Moxifloxacin concentration may be increased by unboosted atazanavir* Buffered didanosine may reduce oral absorption of all fluoroquinolones	Psychiatric symptoms with efavirenz Hepatitis with nevirapine, efavirenz or protease inhibitors Prolongation QT interval with protease inhibitors and efavirenz
Ethionamide/Prothionamide	Unknown	Peripheral neuropathy with stavudine or didanosine Psychiatric symptoms with efavirenz Hepatitis with nevirapine, efavirenz or protease inhibitors Gastrointestinal intolerance with zidovudine or protease inhibitors
Cycloserine/Terizidone	Renally cleared so interactions unlikely Nephrotoxicity caused by tenofovir* could affect serum concentrations	Peripheral neuropathy with stavudine or didanosine Psychiatric symptoms with efavirenz Stevens Johnson Syndrome with nevirapine and efavirenz
PAS	Unlikely	Hepatitis with nevirapine, efavirenz or protease inhibitors Gastrointestinal intolerance with zidovudine or protease inhibitors
Clofazimine	May increase etravirine* and protease inhibitor concentrations	Gastrointestinal intolerance with zidovudine or protease inhibitors
Linezolid	Unlikely	Peripheral neuropathy with stavudine or didanosine Gastrointestinal intolerance with zidovudine or protease inhibitors Lactic acidosis with stavudine, didanosine or zidovudine Bone marrow toxicity with zidovudine
Amoxicillin/Imipenem/ Meropenem with clavulanic acid	Unlikely	Nephrotoxicity with tenofovir*
Thiacetazone	Not advised in HIV-infected patients due to risk of Stevens-Johnson Syndrome	Not advised in HIV-infected patients due to risk of Stevens-Johnson Syndrome
Clarithromycin	Concentrations increased by ritonavir Concentrations reduced by efavirenz and nevirapine Clarithromycin reduces zidovudine concentrations	Combination with non-nucleoside reverse transcriptase inhibitors not recommended due to increased concentrations of the 14-hydroxy metabolite which is associated with rashes

* Currently not advised for use in children

Standardised definitions for research

Concepts from the following topic have been written as an article:

- *Seddon JA, Schaaf HS, Furin JJ, Marais BJ, Tebruegge M, Detjen A, Hesseling AC, Perez-Velez CM, Shah S, Adams LV, Starke JR, Becerra MC, Swaminathan S. Consensus statement on research definitions for drug-resistant tuberculosis in children. (submitted)*

In this chapter, I set out the terms and definitions that I am to use throughout the thesis. This process of formulating these definitions was achieved in collaboration with 'The Sentinel Project on Pediatric Drug-Resistant Tuberculosis' – a group of researchers, healthcare providers and advocates committed to preventing child deaths from DR-TB.[400] I took the lead in formulating and writing a consensus statement of experts to consolidate and clarify the definitions used in research into paediatric DR-TB. The proposed definitions were revised through meetings, conference calls and written feedback in order to achieve clarity and consensus. As well as allowing me to be consistent throughout my own work for this thesis, the definitions provide a tool for others to use when carrying out research into paediatric DR-TB. The few studies that have described children with DR-TB have used inconsistent definitions, making standardization and synthesis of data challenging. The current programmatic WHO definitions used to describe adults with DR-TB and children with drug-susceptible TB are inadequate for research studies of children with DR-TB. More rigorous definitions are required for use in research recording the epidemiology of exposure, infection and disease, as well as research into diagnosis, treatment, prevention and outcome. Definitions need to be relevant for both prospective studies, where comprehensive data can be collected, and for retrospective studies. The distinction between definitions used in clinical management, programmatic reporting and research studies is complex; many research studies document clinical management or report programmatic data. Whilst the following definitions will hopefully strengthen programmatic reporting, these proposed definitions are intended for use in the research setting, rather than for clinical decision-making.

Terminology and measures of exposure

To facilitate comparisons between different studies it is vital that key terms be standardized. Table 9 provides a summary of the suggested consensus definitions regarding epidemiological terms, disease classification, type of treatment, and categories of drug resistance. Exposure is

a continuum, with no documented exposure at one extreme and extensive exposure at the other. Although any exposure to a DR-TB source case could potentially result in a child becoming infected, in reality this exposure must reach a *significant* threshold for the child to be deemed a contact. This necessitates the use of a binary definition. The issue is complex and incorporates elements of the infectiousness of the source case, the proximity and intensity of interaction between source case and contact, the daily duration of exposure, the length of exposure over time, as well as environmental factors such as air exchange. [31, 401] Different definitions will provide different degrees of sensitivity and specificity and it is important that definitions are consistent and well described. Recent interactions are more likely to result in disease in the child compared with interactions that took place more than a year ago.[61, 83, 402-403]

A 'DR-TB contact' should be defined as a child exposed to an infectious DR-TB source case who, in the last twelve months, had either slept in the same household or had daily interaction with the child.[404] If possible, enough data points are collected to provide an exposure 'score' (Table 9) as this concept provides a more precise and comprehensive description of the likely infection risk and correlates well with tests of *M. tuberculosis* infection.[401]

In the same way that exposure is a gradient, so too is the spectrum from exposure through infection to disease.[19] Despite this continuum, it is necessary to assign children into distinct categories for research studies. The terminology used in the literature for children who demonstrate immunological evidence of infection with *M. tuberculosis,* in the absence of clinical symptoms, is confusing. Latent TB infection, latent TB, *M. tuberculosis* infection and TB infection have all been employed. The word "tuberculosis" implies a disease state and therefore it was felt that TB infection should not be used for a well child. For children who have been recently infected by *M. tuberculosis*, the use of the word latency is incongruous as it implies an established immunological equilibrium, which may not have been achieved. A child with a positive immunological test (e.g. TST or IGRA) should be classified as having "*M. tuberculosis* infection" to cover both recent and latent infection. This is consistent with other consensus definitions.[405] In order for a child to be classified as having 'DR *M. tuberculosis* infection', the child must have a positive immunological test result as well as being a DR-TB contact. The terminology used for children with clinical, radiological or microbiological pathology is similarly inconsistent across the published literature. 'Active disease' is a term used widely to denote an ill child, but 'inactive disease' is not a useful concept. For consistency the term 'TB disease' should be used.

Terms used for the treatment given to those with DR-TB disease include 'curative treatment', 'disease treatment', anti-TB treatment, and 'TB treatment'. To avoid ambiguity the term 'DR-TB treatment' should be used. In the existing literature there is also inconsistency surrounding the terminology used to describe the treatment given to children without DR-TB disease. Primary prophylaxis refers to treatment given to a child before any known exposure to an infectious TB case. Post-exposure prophylaxis, window prophylaxis or preventive therapy refers to treatment given to a child after documented TB exposure. Treatment of TB infection and treatment of latent infection are both used to refer to drugs given following a positive immunological test result indicating infection. Secondary prophylaxis refers to treatment given to a child after a course of TB treatment. For consistency the use of the summative term 'DR-TB preventive therapy' can be used to cover all of these circumstances.

Definitions of drug resistance and testing methodology

Although drug resistance is generally divided into the discrete categories of mono-, poly-, MDR- or XDR-TB,[197] (Table 9) it is more useful to view drug resistance as a continuum. For research into paediatric DR-TB, it is important to describe the precise DST pattern. It is also important to record the DST pattern of the likely source case(s), rather than their DST category, when the child has been diagnosed presumptively.

Due to the wide variety of testing methodologies available to determine drug resistance, at a minimum, researchers should clearly state the laboratory techniques employed in determining drug resistance. It should be documented to which drugs DST was performed and which techniques were used for each of the drugs. If DST is determined by phenotypic testing, the Clinical and Laboratory Standards Institute standards should be employed.[406] It is anticipated that more DST will be carried out using genotypic methods in the future. A number of genotypic tests exist using nucleic acid amplification to determine DST. Some assays only determine whether the organism belongs to the *M. tuberculosis* complex and whether mutations in the *rpoB* gene are present (associated with rifampicin resistance in >95% cases). The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is one such test, which is currently being rolled out widely.[407] If this test is employed and the *rpoB* mutation result is positive, the sample should be recorded as having resistance to rifampicin, as this test cannot confirm or refute MDR. The frequency of RMR strains is increasing in some settings,[408] and samples found to be rifampicin-resistant should therefore not be assumed to be MDR. Conversely, HMR-TB is common in many regions; if a sample is found not to have an *rpoB* gene mutation, it should not be assumed to be fully drug-susceptible. Consequently, it is important to follow up results

determined from samples tested with the Xpert MTB/RIF assay with a cultured sample that can have DST determined to isoniazid.

The genotypic testing of resistance to isoniazid usually involves testing for mutations in the *inhA* promoter region and the *katG* gene.[226] A molecular line probe assay (e.g. GenoType® MTBDR*plus*; Hain Lifescience, Nehren, Germany) is frequently used for this purpose. As well as recording the presence of genotypic resistance to isoniazid, it is desirable to also record the mutation conferring resistance, as this has clinical and epidemiological significance.[92] Other molecular tests are under development and in the future, genotypic testing to the second-line drugs is likely to become more widespread, as drug resistance to these agents is associated with known gene mutations.[212]

Previous episodes and treatment

A distinction should be made between a previous episode of disease and any previous treatment given (Table 10). Prior studies have employed a six-month symptom-free period following the completion of at least one month of previous treatment as a pragmatic differentiator of disease episodes .[192] For a child newly diagnosed with DR-TB disease, it is important to distinguish between: (a) a child who was previously treated with first-line therapy for TB disease, had a favourable treatment outcome and has subsequently been re-infected with a DR-TB strain; (b) a child who was infected with a DR-TB strain and treated with first-line drugs before the diagnosis of DR-TB was made; and (c) a child who was infected with a DS-TB strain with resistance developing during first-line treatment. The first two types of drug resistance are referred to as transmitted or primary resistance, while the third is termed acquired resistance. Although clinically it is sensible to suspect the development of resistance in a child if treatment has been poorly adhered to and/or incorrectly prescribed/supplied, for this conclusion to be reached in a research context, it is necessary to have had an initial drug-susceptible sample. Most children with DR-TB disease, however, have transmitted resistance.[254]

To document treatment delay, a standard definition of when the DR-TB episode began should be used to determine the interval from the assumed start of the disease episode to the start of DR-TB treatment. Published studies have defined a DR-TB episode as beginning (in the event that DR-TB was subsequently confirmed) at either the child's initial documented presentation to the healthcare system, when a specimen was obtained that eventually confirmed DR-TB, or

alternatively, when the child commenced TB treatment for the current episode, based on whichever was the first documented event.[192]

Certainty of diagnosis of disease

When treating children for DR-TB disease, the decision is binary – the child is treated or not. For the clinician this diagnosis is either confirmed or presumed. This may be sufficient for clinical management and for recording and reporting purposes. For research purposes, however, it is important to document the degree of certainty for both the diagnosis of TB and the diagnosis of drug resistance. For the diagnosis of TB disease in children, the WHO first proposed categories of suspect, probable and confirmed TB for reporting and for research.[409] This classification has recently been refined by a National Institute of Health (NIH) expert panel, focusing specifically on intra-thoracic disease.[405] (see Table 10). For extra-thoracic TB a similar system should be adopted; one has been proposed for TBM.[410] A definition of 'confirmed DR-TB disease' requires clinical evidence of TB disease together with the detection of *M. tuberculosis* from a specimen collected from the child with resistance demonstrated. All samples from children should be subjected to culture and DST. A definition of 'probable DR-TB disease' should be used when a diagnosis of probable TB disease has been made and the child is a DR-TB contact. Cases should be classified as 'possible DR-TB disease' if a diagnosis of probable TB disease has been made and either the child fails adherent first-line TB treatment or has been exposed to a source case with risk factors for drug resistance (failed therapy, death or default with no known DST).

Site of disease and disease severity

Site and severity of disease can have an impact on the choice and duration of treatment as well as treatment outcome. Disease severity, for example, has been shown to correlate with bacterial yield in children and culture conversion.[99, 192, 411] TB programs usually report disease site using ICD-10 codes,[412] and these codes should be used for reporting disease site in children with DR-TB. Defining the severity of disease in children is challenging and existing approaches are limited. Radiological findings can be used to describe the spectrum of intra-thoracic disease and can be an indicator of severity.[413] A recently-proposed classification system divides different types of both intra- and extra-thoracic childhood TB into severe and non-severe disease based on known host-pathogen interaction and pathophysiology of disease.[411] Where possible, this classification should be employed for research purposes.

Adverse events

Second-line TB drugs are associated with increased risk of adverse events.[414] For research, it is important to determine the type of adverse event, the severity, the relationship to the medications being given, any action taken and any associated risk factors.[146] The Division of Microbiology and Infectious Diseases (DMID) within the US National Institute of Allergy and Infectious Diseases (NIAID) has published tables to allow the grading of adverse events.[415] These tables are specific for children and should be used for research on paediatric DR-TB. However, a number of adverse events that are frequently encountered in the treatment of children with DR-TB disease and DR *M. tuberculosis* infection are not adequately covered in this classification system.[204] These include thyroid dysfunction, hearing loss, arthralgia and arthritis. Proposed criteria for grading these adverse events are included in Table 11.

It is important to note the action taken when an adverse event occurs.[416] For each adverse event, data should be collected documenting whether any action was taken and if so, what type. Where possible other factors possibly associated with the adverse event should be recorded. These include co-morbidities such as HIV infection, diabetes, and asthma, as well as the nutritional status and the type and severity of TB disease.

Disease outcome

Adult guidelines typically use microbiological parameters to determine response to treatment. The outcome definitions currently recommended by WHO for adults with DR-TB disease, were first proposed by an expert consensus group for use in the analysis of retrospective data. Cure was defined as 'five consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment.'[197, 417] For children with drug-susceptible TB disease, cure has been defined as a child who is 'sputum smear-negative in the last month of treatment and at least one previous occasion.'[16] Neither of these definitions are appropriate for children with DR-TB disease. Instead, 'cure' should be the completion of treatment, with simultaneous evidence of clinical and radiological improvement, in conjunction with three or more negative sputum cultures in the last 12 months of treatment (in the absence of subsequent positive results). As only a relatively small proportion of children will have a confirmed diagnosis at the beginning of their treatment,[5-6, 418] and as microbiological investigations are frequently not repeated during follow-up, the majority of children will not fulfil the definition for cure. 'Probable cure' is defined as the presence of the same constellation, but without the

microbiological component. The proposed definitions for treatment outcome are summarized in Table 12.

Treatment response can be divided into microbiological, radiological and clinical responses. A key component of clinical response is nutritional status, with poor status a risk for both the development of TB disease as well as poor treatment outcome.[419-422] Nutritional variables that require monitoring, at a minimum, include height and weight. These parameters should be assessed at treatment initiation and then monthly, and should be plotted on standardized charts. An improvement in nutritional status should be included among the criteria used to define 'probable cure'. Radiological improvement encompasses partial or complete resolution of chest radiographic features. However, it is important to consider that some children with HIV infection who are started on cART may experience a radiological deterioration despite clinical improvement due to IRIS.[185, 423-424] Nevertheless, this phenomenon is unlikely to influence classification of final disease outcome, as IRIS typically presents early in the treatment course and resolves before final outcome is determined.

Other treatment outcomes that should be included are primary death and primary default. These occur if a child is diagnosed with DR-TB disease but dies, refuses treatment or is lost to follow up before DR-TB treatment is initiated. Finally, treatment failure is defined as at least six months of adherent therapy on an appropriate DR-TB regimen with evidence of microbiological, clinical or radiological deterioration in the absence of IRIS.

Table 9 - Proposed terminology for drug-resistant tuberculosis in children and the assessment of drug-resistant tuberculosis exposure

	Recommended Term	**Definitions**
Epidemiological terms	DR-TB index case	The first identified, confirmed DR-TB case in a social group (e.g. a household) during an investigation or outbreak (which may be the child)
	DR-TB source case	An infectious (sputum-smear microscopy and/or culture positive) DR-TB case who could have infected the contact
	DR-TB exposure	Ten points to be used for exposure score[401] • Is the source case the child's mother? • Is the source case the child's primary caregiver? • Does the source case sleep in the same bed as the child? • Does the source case sleep in the same room as the child? • Dose the source case live in the same household as the child?* • Dose the source case see the child every day?* • Is the source case coughing? • Does the source case have pulmonary TB? • Is the source case sputum-smear microscopy positive? • Is there more than one source case in the child's household?
Infection and disease	DR *M. tuberculosis* infection	A positive immunological test of infection including TST or IGRA in combination with being a DR-TB contact
	DR-TB disease	Clinical, radiological or microbiological pathology
Type of treatment	DR-TB treatment	The treatment of DR-TB disease
	DR-TB preventive therapy	Includes primary (pre-exposure) prophylaxis, post-exposure prophylaxis (including window prophylaxis), secondary prophylaxis, and treatment of TB infection
Drug resistance categories	Mono-resistant	Resistance to one TB drug
	Poly-resistant	Resistance to two or more TB drugs other than both rifampicin and isoniazid
	Multidrug-resistant	Resistant to at least both rifampicin and isoniazid
	Pre-extensively drug resistant	MDR-TB with resistance to either a fluoroquinolone or an injectable second-line TB drug but not both
	Extensively drug resistant	MDR-TB with resistance to both a fluoroquinolone and an injectable second-line TB drug

DR: drug-resistant; TB: tuberculosis; *M. tb: Mycobacterium tuberculosis;* IGRA: interferon-gamma release assay; TST: tuberculin skin test; MDR: multidrug-resistant
*Either of these two components will classify the child as being a DR-TB contact if occurring in the preceding twelve months

Table 10 - Classification of previous disease episodes, diagnostic certainty and description of drug-resistant tuberculosis disease in children

	Recommended Term	Definitions
Previous episodes and treatment	Previous TB disease episode	Treatment taken for at least one month, after which there was a reported symptom-free period of ≥ 6 months before the start of the current DR-TB disease episode
	DR-TB disease episode	If DR-TB disease is subsequently confirmed, the episode began when the child is first documented to have presented to the health care system, when the specimen was obtained that eventually confirmed DR-TB disease, or when the child commenced any TB treatment, whichever is the first available documented event
	Previously treated with first-line TB drugs	Treatment for one month or more with WHO group one drugs
	Previously treated with second-line TB drugs	Treatment for one month or more with any WHO group two to five drugs
Certainty of diagnosis of TB disease[405]	Confirmed TB disease	At least one of the signs and symptoms suggestive of TB disease* and microbiological confirmation of *M. tuberculosis*
	Probable TB disease	At least one of the signs and symptoms suggestive of TB disease* and the CR is consistent with intra-thoracic TB disease** and presence of one of the following: a) a positive clinical response to TB treatment, b) documented exposure to a source case with TB disease or c) immunological evidence of TB infection
	Possible TB disease	At least one of the signs and symptoms suggestive of TB disease* and either a) a clinical response to TB treatment, documented exposure to a source case with TB disease or immunological evidence of TB infection or b) CR consistent with intra-thoracic TB disease**
Certainty of diagnosis of DR-TB disease	Confirmed DR-TB disease	At least one of the signs and symptoms suggestive of TB disease* and detection of *M. tuberculosis* from the child with demonstration of genotypic or phenotypic resistance
	Probable DR-TB disease	DR-TB contact and diagnosis of probable TB disease
	Possible DR-TB disease	Diagnosis of probable TB disease together with either failure of first-line TB treatment or contact of a source case with TB disease and risk factors for drug resistance (failed, irregular or chronic therapy, death, or default)
Site of TB and disease severity	ICD-10 code	Code to be recorded
	Severe or non-severe	Severity to be recorded[411]

WHO: World Health Organization; TB: tuberculosis; DR: drug-resistant; CR: chest radiograph; *M. tuberculosis: Mycobacterium tuberculosis*

*Persistent cough, weight loss or failure to thrive, persistent unexplained fever, persistent unexplained lethargy or reduced playfulness or additional signs in the neonate (these signs and symptoms are defined in detail in referenced article)

** For extra-thoracic TB disease alternative appropriate radiological imaging should be substituted

Table 11 - Classification of adverse events in children with drug-resistant tuberculosis

	Recommended Term	Definitions
Adverse drug events	Clinical	DMID grading scale 0-4[415]
	Laboratory	DMID grading scale 0-4[415]
	Arthralgia/arthritis	Not covered but parallels with DMID • Grade 0 – No pain • Grade 1 – Pain, but no interference with function or movement • Grade 2 – Moderate pain affecting function, but able to carry out normal activities • Grade 3 – Severe pain limiting activities • Grade 4 – Disabling pain and unable to carry out normal activities
	Thyroid function	Abnormal considered if TSH raised above and T4 below the threshold of normal, using the reference ranges that have been specified by the laboratory with consideration of the analyzer used and the age of the child
	Hearing	American Speech and Hearing Association (ASHA) criteria for hearing loss[248, 425-426] using pure tone audiometry. Hearing loss defined as a change from baseline of: • 20dB decrease at any one frequency *or* • 10dB decrease at any two adjacent frequencies *or* • Loss of response at three consecutive test frequencies where responses were previously obtained.

DMID: Division of Microbiology and Infectious Diseases; TSH: thyroid stimulating hormone

Table 12 - Classification of treatment outcome in children with drug-resistant tuberculosis

	Recommended Term	**Definitions**
Treatment Outcome	Cure	Treatment completed, clinical and radiological improvement together with three or more negative sputum cultures in the last twelve months of treatment with no subsequent positive culture
	Probable cure	Treatment completed with clinical and radiological improvement
	Treatment completed	Completion of prescribed treatment
	Default	Treatment interruption for two months or more
	Primary default	Never started on DR-TB treatment
	Death	Death for any reason while on DR-TB treatment
	Primary death	Death prior to starting DR-TB treatment
	Treatment failure	Ongoing sputum culture positivity, or clinical or radiological deterioration after more than six months of the child receiving an appropriate DR-TB regimen (with adherence > 80%)

TB: tuberculosis; DR: drug-resistant

Original research

Study 1: the evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa

The following study has been published as an article:

- *Seddon JA, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. Int J Tuberc Lung Dis 2012; 16: 928-33*

The first original research article in the thesis documents the burden of TB drug resistance in the context that the research is to be carried out. A database of all children with culture-confirmed TB at TCH is collected prospectively, including clinical characteristics as well as details of the DST of the strain isolated. This has been undertaken since 2003 and provides surveillance and detection of trends over time. For this study, I gathered the data for a two year period (previous reports have described two year periods), analysed it and compared it with previous surveillance periods. As this surveillance period included the point at which molecular LPAs were introduced into the Western Cape, we took the opportunity to compare the LPA results with conventional DST techniques in number of isolates.

Introduction

Children with DR-TB usually have transmitted resistance, whereby the child is infected by an organism with established resistance.[21, 36-37, 80] This contrasts to adults where drug resistance is a result of both transmission and acquisition, the latter due to a susceptible organism developing resistance because of inadequate treatment.[427] Children rarely have acquired resistance as paediatric TB is usually paucibacillary; with small organism loads it is unlikely that resistant mutants will arise and be selected. This is supported by studies comparing the genetic DNA fingerprint (restriction fragment length polymorphism; RFLP), as well as the DST pattern of organisms from children with drug-resistant TB together with the likely source case.[64] Usually both the RFLP and DST in such cases have been concordant, implying transmitted resistance from adults to children.[68] Since paediatric MDR-TB cases represent recent infection

with a DR strain, DST patterns in children, particularly those amongst young children, provide important information regarding current transmission patterns in a community or setting, facilitating individual case management, surveillance and public health planning.

Traditionally DST has been determined by phenotypic methods whereby bacilli are grown in the presence of an antibiotic. If more than a certain percentage (usually 1% or more) of bacilli grow in comparison to a control without antibiotic, the bacilli are classified as being resistant. These tests are reliable but are expensive, time-consuming and operator-dependent. More recently, nucleic acid amplification tests (NAATs) have been developed to identify genetic mutations that are commonly associated with antibiotic resistance. The great majority (>95%) of rifampicin-resistant strains possess mutations in the *rpoB* gene. Most, but not all, strains that are resistant to isoniazid possess mutations in either the *inhA* promoter region or the *katG* gene. Since August 2008, the National Health Laboratory System in South Africa has used a NAAT, or LPA, to detect the presence of *M. tuberculosis* complex and mutations in *rpoB*, *inhA* and *katG*. Since not all isoniazid resistance mutations are detected, it is unclear what proportion of isoniazid-resistant strains has been missed since the introduction of LPA testing. This could potentially lead to either a sample being labelled as drug-susceptible when it is, in fact, HMR, or misclassification of RMR when it is, in fact, MDR.

Previous surveillance studies from Cape Town described the proportion of children with DR-TB during different periods: 1994-1998,[428] 2003-2005[255] and 2005-2007, all using phenotypic DST.[254] In the current study the prevalence of drug resistance amongst children with culture-confirmed TB is determined from 2007 to 2009 and their clinical characteristics described including HIV co-infection. In addition, the results of this surveillance period are compared to previous studies to determine changes and trends over time. In order to document currently prevailing DST patterns, the children from this and previous study periods have been stratified into those less than and older than five years.[429] Finally, the genotypic and phenotypic DST for isoniazid is compared on mycobacterial isolates since the introduction of the LPA.

Methods

All children less than 13 years old with culture-confirmed TB, routinely tested at TCH from 1 March 2007 through 28 February 2009 were included.

Mycobacterial culture and drug susceptibility testing

Samples were first decontaminated and then cultured using the MGIT 960 system (Becton Dickinson, Sparks, MD, USA). The presence of *M. tuberculosis* was confirmed by polymerase chain reaction (PCR) amplification.[430] DST was performed on at least one isolate from each child with culture-confirmed TB. DSTs were performed for isoniazid and rifampicin, and if MDR, testing was completed for ethambutol and the second-line drugs amikacin, ethionamide and ofloxacin. For the first 18 months of the study, from March 2007 through July 2008, only conventional phenotypic DST was undertaken for isoniazid and rifampicin using the Bactec 460TB system (Becton Dickinson, Sparks, MD, USA), according to international criteria.[431] Isoniazid was tested at a concentration of 0.1μg/ml, rifampicin at 2.0 μg/ml and ethambutol at 7.5μg/ml. The susceptibility of a strain was judged by comparing growth of organisms in drug containing versus non-drug containing media; resistance was defined as 1% or more bacterial growth in the drug containing media.

During the latter six months of the study, routine genotypic testing was implemented by the local reference laboratory using LPA (GenoType® MTBDR*plus*; Hain Lifescience, Nehren, Germany), according to the manufacturer's instructions.[432] All samples that underwent genotypic testing were then re-evaluated by conventional techniques for isoniazid DST to determine the concordance between the two testing strategies. If samples lost viability, gave inconclusive results or were contaminated, a second attempt was made to culture them. DST to second-line agents was performed individually by the indirect proportional method on Middlebrook 7H10 agar using critical concentrations of amikacin 40μg/ml, ofloxacin 2μg/ml and ethionamide 10μg/ml.

Clinical data and patient management

Once a specimen was found to be positive for *M. tuberculosis*, laboratory details were recorded regarding the date of sampling, specimen site and DST. Clinical case notes and laboratory data were reviewed and demographic and clinical data extracted. Chest radiographs were read by a single expert reader using a standardised approach.[413] Children were treated according to national and international guidelines[16, 102, 433] and if not already known, HIV testing was undertaken following informed consent from the parent or legal guardian with pre- and post-test counselling using ELISA or DNA PCR testing, according to national protocol.

Different DST patterns are presented as percentages of the total number of samples (one per child) that had DST with 95% confidence intervals calculated. The significance of change over time for a DST pattern was calculated using a test of trend for the odds ratio of that DST

pattern. The significance of differences between the DST patterns for younger and older children in the most recent surveillance period was calculated using the χ^2 test or Fishers exact test.

Results

Two-hundred and ninety-four children were diagnosed with culture-confirmed TB in the period under review; demographic data are provided in Table 13. DST results were available in 292 (99.3%): 45 (15.4%) had isoniazid and/or rifampicin resistance, 41 (14.0%) were isoniazid-resistant including 26 (8.9%) that had MDR-TB. Table 14 compares findings from the current survey with those of previous surveillance periods. Any resistance to rifampicin increased between 1994 and 2009 ($p<0.001$) as did RMR- ($p=0.009$) and MDR-TB ($p<0.001$). Although resistance to either isoniazid and/or rifampicin ($p=0.001$) and any resistance to isoniazid ($p=0.006$) also increased (Table 14), these changes were not significant if comparison analysis was restricted to the period 2003-2009 ($p=0.35$ and $p=0.65$ respectively). However, trends in any resistance to rifampicin ($p=0.03$) and RMR-TB ($p=0.04$) remained significant in analysis restricted to this period, with a trend in MDR-TB of borderline significance ($p=0.09$). The DST patterns for children less than five years and those older were not significantly different (isoniazid and/or rifampicin resistance: $p=0.86$; any isoniazid resistance: $p=0.85$; HMR-TB: $p=0.14$; any rifampicin resistance: $p=0.39$; RMR-TB: $p=1.0$; MDR-TB: $p=0.37$)

The prevalence of HIV infection remained stable amongst those tested over the last six years (see Table 14; $p=0.80$). Ethambutol resistance was present in 12/24 (50.0%) of MDR-TB cases tested in the present survey. Two isolates were resistant to ofloxacin, one to amikacin and one to ethionamide; none had XDR-TB.

Of the 73 samples that initially underwent DST through LPA in the central reference laboratory, four could not be found, 14 lost viability on two attempts to culture them and one sample gave an inconclusive result. Fifty four isolates were located and successfully cultured to yield a conclusive conventional DST result; seven had initially demonstrated isoniazid resistance on genotypic DST; all of these were also resistant on phenotypic DST. Of the 47 classified as being isoniazid susceptible on genotypic DST, 46 were found to also be susceptible on phenotypic DST. If phenotypic testing was used as the reference standard, LPA testing yielded a sensitivity of 87.5% and specificity of 100%.

Discussion

The overall proportion of drug resistance has remained relatively unchanged amongst children with culture-confirmed TB in the Western Cape Province of South Africa over the last few years; however, rifampicin resistance is increasing. From an epidemiological perspective, the greatest change is that less HMR-TB is compensated for by more MDR-TB, possibly signifying additional acquisition of rifampicin resistance among adult HMR-TB cases. Of great concern is that for those children with *M. tuber*culosis resistant to isoniazid and rifampicin, half were also resistant to ethambutol which has serious implications for the clinical management of MDR-TB in both children and adults in this setting. DST to pyrazinamide was not routinely undertaken as testing is complicated to perform and requires acidic conditions which inhibit mycobacterial growth. However, other studies from the Western Cape have demonstrated high levels of resistance to ethambutol and pyrazinamide in strains already MDR.[161, 434-435] The implication of these findings is that, in our context, both ethambutol and pyrazinamide should not be assumed to be effective drugs in the treatment of MDR-TB, further restricting the choice of suitable drugs.

The use of LPA reduces the turnaround time from specimen production to result.[432] For acid-fast bacilli smear-positive samples LPAs can be performed directly on clinical specimens whereas smear-negative samples must be first cultured prior to genotypic analysis. The majority of paediatric samples are paucibacillary and are cultured routinely prior to LPA. However, time is still saved using LPA as even following culture, phenotypic DST requires further processing. A concern with using LPA, however, is that a significant proportion of isoniazid-resistant strains are missed resulting in misclassification of DST status and inappropriate management. We found that the sensitivity and specificity of LPA was high. Only one of 47 isolates was classified as susceptible on genotypic DST but found to be resistant on phenotypic DST. All cases identified as isoniazid-resistant on genotypic DST were also confirmed to be resistant on phenotypic testing. Of note is that four cases of BCG disease (one disseminated) were missed using genotypic testing as the LPA does not identify isolates as *M. bovis* BCG or detect the presence of isoniazid-resistance, since this is not associated with *katG* gene or *inhA* promoter region mutations.[436-437] If phenotypic isoniazid resistance is detected in a sample from an infant, BCG disease should always be a consideration especially in an immune compromised child; this opportunity is lost with the use of genotypic testing only.

A limitation of this study is that children included may not be representative of all children with TB in the setting. First, children with culture-confirmed TB tend to have more extensive

disease than those with a presumptive diagnosis without bacteriological confirmation. It is also possible that some children with DR-TB had been treated previously with inadequate first-line therapy, leading to more advanced disease, in turn leading to a higher probability of culture-confirmation. Second, this is a hospital based study and the spectrum of disease seen and the drug resistance profile may be different compared to community cohorts. The latter phenomenon has not, however, been observed in a previous study from our group.[255] Third, TCH is a regional referral hospital for children with DR-TB and so higher proportion of TB cases may therefore have DR-TB compared to other hospital settings. Finally, child contacts of MDR-TB source cases (referred to TCH) are likely to be investigated more rigorously through repeat mycobacterial sampling than contacts of drug-susceptible source cases, possibly leading to a higher likelihood of culture-confirmation in those with DR rather than drug-susceptible disease. Although these factors may have contributed to our findings, all have been consistent over the previous study periods and so comparisons and trends over time are therefore likely to be valid.

Table 13 - Patient characteristics and disease spectrum in children with drug-resistant and drug-susceptible tuberculosis

Characteristic	With any drug-resistance (%)	Fully drug-susceptible or unknown* (%)	Total (%)
Number of cases	45 (100)	249 (100)	294 (100)
Known contact with TB source case	32 (71.1)	134 (53.8)	166 (56.5)
Tuberculin skin test positive (>10mm if HIV-uninfected and >5mm if HIV-infected)	24/36 (66.7)	118/171 (69.0)	142/207 (68.6)
Weight <3rd percentile for age	23 (51.1)	102 (41.0)	125 (42.5)
Severe malnutrition (marasmus/kwashiorkor)	5 (11.1)	44 (17.7)	49 (16.7)
Pulmonary TB** (All)	41 (91.1)	204 (81.9)	245 (83.3)
Extrapulmonary TB (All)	20 (44.4)	142 (57.0)	162 (55.1)
Both Pulmonary & Extrapulmonary TB	16 (35.6)	99 (39.8)	115 (39.1)
Types of extrapulmonary TB (some had more than one type)	20 (100)	142 (100)	162 (100)
TBM (miliary TB in 7)	5 (25.0)	29 (20.4)	34 (21.0)
Miliary TB (TBM in 7)	4 (20.0)	22 (15.5)	26 (16.0)
Abdominal TB	3 (15.0)	31 (21.8)	34 (21.0)
Peripheral lymphadenopathy	7 (35.0)	70 (49.3)	77 (47.5)
Pleural effusion (large or loculated)	1 (5.0)	15 (10.6)	16 (9.9)
Pericardial effusion	2 (10.0)	3 (2.1)	5 (3.1)
Osteoarticular TB	2 (10.0)	17 (12.0)	19 (11.7)
Ear or mastoid TB	3 (15.0)	9 (6.3)	12 (7.4)
Skin involvement	1 (5.0)	5 (3.5)	6 (3.7)
Children with chest radiographs (some had more than one finding)	45 (100)	234 (100)	279 (100)
Ghon focus	2 (4.4)	4 (1.7)	16 (5.8)
Hilar/mediastinal lymphadenopathy	21 (46.7)	113 (48.3)	34 (12.3)
Large airway compression	13 (28.9)	50 (21.4)	63 (22.8)
Collapse lobe/segment	4 (8.9)	13 (5.6)	16 (5.8)
Hyperinflation lobe/segment	2 (4.4)	10 (4.3)	12 (4.3)
Pleural effusion – all	6 (13.3)	25 (10.7)	31 (11.2)
Miliary opacification (not LIP)	4 (8.9)	22 (9.4)	26 (9.4)
Alveolar opacification lobe/segment	29 (64.4)	116 (49.6)	145 (52.5)
Cavities	13 (28.9)	34 (14.5)	47 (17.0)
Bronchopneumonic opacification	5 (11.1)	22 (9.4)	27 (9.8)
Calcification	1 (2.2)	4 (1.7)	5 (1.8)
Normal CXR	7 (15.6)	43 (18.4)	50 (18.1)

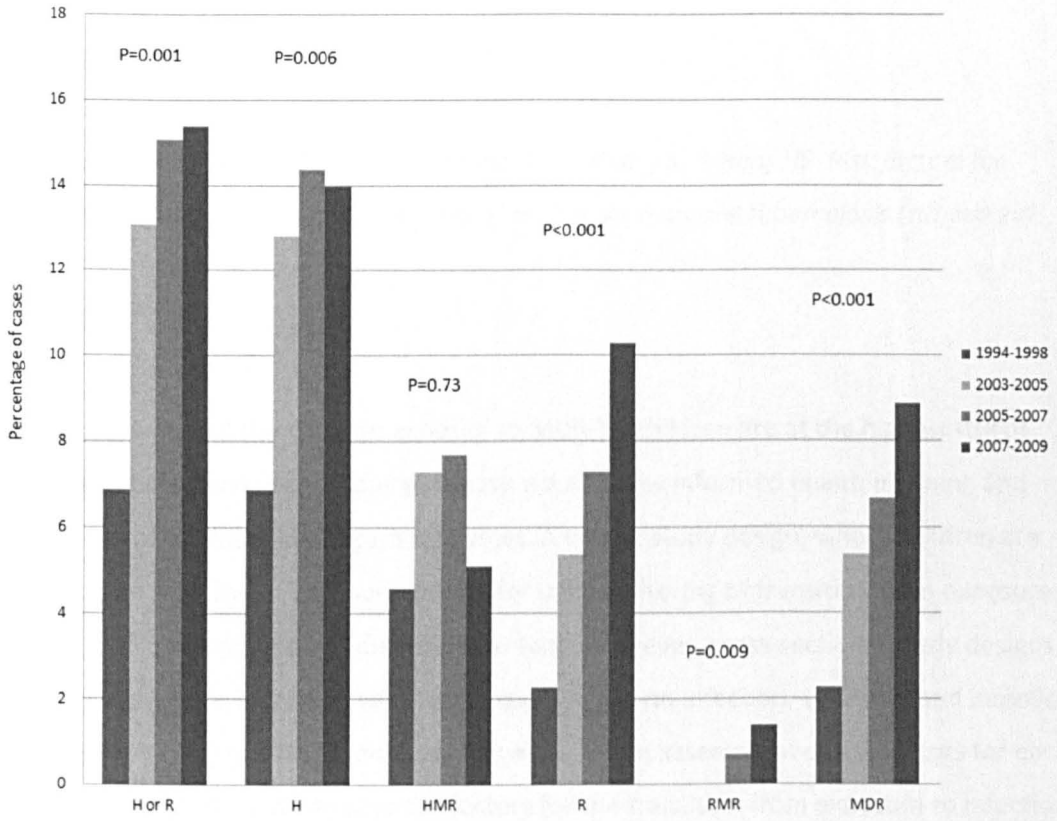
* Only two cases had unknown drug susceptibility test pattern
** Pulmonary TB included hilar and mediastinal lymphadenopathy
TB - Tuberculosis
TBM – Tuberculous Meningitis
LIP – Lymphocytic Interstitial Pneumonitis

Table 14 - Comparison of drug susceptibility test results for children with culture-confirmed tuberculosis over four surveillance periods (1994-2009)

	1994-1998*	2003-2005*	2005-2007*	2007-2009*
Number of cases	338	323	291	294
Median Age (years)	2.6	2.5	2.75	2.13
Boys	193 (57.1)	173 (53.6)	154 (52.9)	156 (53.1)
Previous TB treatment	32 (9.5)	59 (18.3)	65 (22.3)	50 (17.0)
HIV test done	166 (49.1)	243 (75.2)	174 (59.8)	217 (73.8)
HIV-infected	13 (7.8)	64 (26.3)	49 (28.2)	63 (29.0)
DST undertaken	306 (90.5)	313 (96.9)	285 (97.9)	292 (99.3)
Any resistance to isoniazid or rifampicin[1]	21 (6.9; 4.5-10.3)	41 (13.1; 9.8-17.3)	43 (15.1; 11.4-19.7)	45 (15.4; 11.7-20.0)
Any isoniazid resistance[2]	21 (6.9; 4.5-10.3)	40 (12.8; 9.5-16.9)	41 (14.4; 10.8-18.9)	41 (14.0; 10.5-18.5)
Isoniazid mono-resistance[3]	14 (4.6; 2.8-7.5)	23 (7.3; 5.0-10.8)	22 (7.7; 5.2-11.4)	15 (5.1; 3.1-8.3)
Any rifampicin resistance[4]	7 (2.3; 1.1-4.7)	17 (5.4; 3.4-8.5)	21 (7.3; 4.9-11.0)	30 (10.3; 7.3-14.3)
Rifampicin mono-resistance[5]	0 (0; 0-1.2)	0 (0; 0-1.2)	2 (0.7; 0.2-2.5)	4 (1.4; 0.5-3.5)
Multidrug-resistance[6]	7 (2.3; 1.1-4.7)	17 (5.4; 3.4-8.5)	19 (6.7; 4.3-10.2)	26 (8.9; 6.2-12.7)
Number of children 0-5 years	241	230	187	212
DST undertaken	218 (90.5)	223 (97.0)	184 (98.4)	210 (99.1)
Any resistance to isoniazid or rifampicin	16 (7.3; 4.6-11.6)	27 (12.1; 8.5-17.1)	25 (13.6; 9.4-19.3)	32 (15.2; 11.0-20.7)
Any isoniazid resistance	16 (7.3; 4.6-11.6)	27 (12.1; 8.5-17.1)	25 (13.6; 9.4-19.3)	29 (13.8; 9.8-19.1)
Isoniazid mono-resistance	9 (4.1; 2.2-7.7)	16 (7.2; 4.5-11.3)	14 (7.6; 4.6-12.4)	8 (3.8; 2.0-7.3)
Any rifampicin resistance	7 (3.2; 1.6-6.5)	11 (4.9; 2.8-8.6)	11 (6.0; 3.4-10.4)	24 (11.4; 7.8-16.5)
Rifampicin mono-resistance	0	0	0	3 (1.4; 0.5-4.1)
Multidrug-resistance	7 (2.3; 1.6-6.5)	11 (4.9; 2.8-8.6)	11 (6.0; 3.4-10.4)	21 (10.0; 6.7-14.8)
Number of children 5-13 years	97	93	104	82
DST undertaken	88 (90.7)	90 (96.8)	101 (97.1)	82 (100)
Any resistance to isoniazid or rifampicin	5 (5.7; 2.5-12.6)	13 (14.4; 8.7-23.2)	18 (17.8; 11.6-26.4)	13 (15.9; 9.5-25.3)
Any isoniazid resistance	5 (5.7; 2.5-12.6)	13 (14.4; 8.7-23.2)	16 (15.8; 10.0-24.2)	12 (14.6; 8.6-23.9)
Isoniazid mono-resistance	5 (5.7; 2.5-12.6)	7 (7.8; 3.9-15.2)	7 (6.9; 3.4-13.6)	7 (8.5; 4.3-16.6)
Any rifampicin resistance	0	6 (6.7; 3.1-13.8)	11 (10.9; 6.2-18.5)	6 (7.3; 3.5-15.1)
Rifampicin mono-resistance	0	0	2 (2.0; 0.6-6.9)	1 (1.2; 0.3-6.5)
Multidrug-resistance	0	6 (6.7; 3.1-13.8)	9 (8.9; 4.8-16.1)	5 (6.1; 2.7-13.5)

Human immunodeficiency virus – HIV; Drug susceptibility test – DST
*Percentages and 95% confidence intervals in parentheses
Trend in odds ratios over successive surveillance periods: [1]p=0.001; [2]p=0.006; [3]p=0.73; [4]p<0.0001; [5]p=0.009; [6]p0.0005
"Isoniazid mono-resistance" may be poly-resistance in some cases

Figure 3 - Trends in drug resistance for children with culture-confirmed tuberculosis over four surveillance periods (1994-2009)



H or R – resistance to either isoniazid or rifampicin; H – any resistance to isoniazid; HMR – isoniazid mono-resistance; R – any resistance to rifampicin; RMR – rifampicin mono-resistance; MDR – multidrug-resistance
P values are for test of trend in odds ratio for each type of drug susceptibility category

Study 2: risk factors for infection and disease in child contacts of drug-resistant tuberculosis

The following study has been prepared as an article:

- *Seddon JA, Godfrey-Faussett P, Hesseling AC, Fielding K, Schaaf HS. Risk factors for infection and disease in child contacts of multidrug-resistant tuberculosis (submitted)*

Introduction

Determining which of the children exposed to MDR-TB children are at the highest risk of becoming infected and of developing disease would allow informed health planning and targeted use of available healthcare resources. A cohort study design, where children are followed from the time of exposure, allows for the monitoring of transition from exposure to infection, and from infection to disease, over time. However, cross-sectional study designs, whereby children are identified who have exposure but no infection, exposure and infection and those who present with TB disease, also allow for an assessment of risk factors for each state. This study aims to determine risk factors for the transition from exposure to infection, and from infection to disease in child MDR-contacts, using a cross-sectional study design.

Methods

Patient population

All children evaluated at TCH or community outreach specialist paediatric TB clinics during May 2010 through April 2011, were eligible if they were less than five years old, had been in significant contact with an infectious (sputum smear or culture positive) pulmonary MDR-TB source case within the preceding six months and had an available TST result. Significant exposure was defined as living with or having regular daily interaction with the MDR-TB source case. Children were recruited following written, informed consent from the parent/caregiver.

Data collection and classification

This study employed a cross-sectional design. Following informed consent, families were interviewed and data collected regarding the demographic profile and clinical condition of the child. Information on the source case was collected both from the attending families and subsequently from the provincial TB register. The nature and intensity of the interaction

between the source case and child, as reported by the parent/caregiver was documented as well as the duration of exposure. Children were classified as uninfected or infected, and if infected, as having TB disease or not. While the study aimed to assess risk factors for prevalent TB disease, confirmation of diagnosis depended on radiological and bacteriological investigations, in some instances requiring a number of weeks for liquid culture results. Standard research definitions were applied to classify TB disease;[405] children with either confirmed and probable disease were included. Infection was classified as having a positive TST; infection was also assumed in the presence of TB disease. A transverse TST diameter of ≥10mm was considered positive in HIV-negative and ≥5mm in HIV-positive children.

Statistical analysis

Data were double-entered into a database and checked for entry errors. Logistic regression was used to assess risk factors for (i) *M. tuberculosis* infection and (ii) TB disease (among those with infection). Results are reported as unadjusted and adjusted odds ratios (OR), 95% confidence intervals (CIs) and p-values, calculated using the likelihood ratio test (LRT). For ordinal variables a test for trend and departures from linearity using the LRT was conducted. For models assessing risk factors for infection, exposures were included if they demonstrated a relationship with infection in univariable analysis based on $p<0.05$. If two exposures were thought to be co-linear they were not included together in the same model. For models assessing risk factors for disease, variables were only adjusted for the age of the child, given the small number of children with disease. Ages for the child and source case were re-coded as ordinal variables, using age bands. Relationships between exposures were assessed using the χ^2 test. All statistical analyses were conducted using Stata software (version 11; Stata Corp, College Station, TX).

Results

Description of cohort

Over the twelve-month study period 377 children were referred to the specialist clinic services as child contacts of MDR-TB source cases. A number of children did not meet the eligibility criteria for the study: the source case did not have MDR-TB (n=27), the child was older than five years (n=56), the intensity of contact was not judged to be significant by the clinical team (n=11) or a TST result was not available in the presence of an asymptomatic child (n=2). Of 281 children eligible for the study, 228 (81%) were recruited. The remaining 53 children were not brought to the clinic by a parent or legal guardian who could provide informed consent (n=31), the parents did not consent to the study (n=3) or the families left the clinic before the research

team could speak to them (n=19). These children all received routine standard of care. The median age of children recruited was 30 months (inter-quartile range [IQR]: 13-43 months); of 217 children tested for HIV, 8 (3.7%) were positive. Of the 228 children, 102 (44.7%) were classified as *M. tuberculosis*-infected. Of the 102 infected, 15 (14.7%) also had TB disease.

Risk factors for infection

In adjusted analysis, increasing age of the child was associated with increasing odds of infection (adjusted odds ratio (AOR) for one year increase in age: 1.43; 95%CI: 1.13-1.91; p=0.002); children of coloured ethnicity (compared to children of Xhosa ethnicity) were also more likely to be infected (AOR: 2.51; 95%CI: 1.22-5.17; p=0.01). Children with a previous treatment history were more likely to be infected in univariable analysis, with the effect reduced after adjustment (Table 15). Increasing age of the source case was associated with a reduced odds of infection (AOR: 0.67 for an approximate 10 year increase in age; 95%CI: 0.45-1.00; p=0.05; Table 16) and reported alcohol use by source case was associated with increased odds of infection (AOR: 2.59; 95%CI: 1.29-5.22; p=0.007). Before adjustment there was a strong relationship between HIV status in the source case and *M. tuberculosis* infection in the child, with HIV positivity in the source case associated with lower odds of infection. In multivariable analysis this association was reduced (AOR 0.48, 95% CI 0.22-1.04; p=0.04).

Risk factors for prevalent tuberculosis disease

Younger age of the child was associated with increased odds of disease (p-value for test of trend; p=0.01) as was HIV-positive status in the child (AOR: 25.3; 95%CI: 1.63-393; p=0.01), HIV positivity of the source case (AOR: 4.07; 95%CI: 1.19-13.8; p=0.03), increasing number of rooms in the house (AOR: 1.39; 1.02-1.91; p=0.04; Table 17) and alcohol use by the source case (AOR 2.90; 95% CI 0.90-9.31; p=0.07; Table 18). Male gender (AOR: 0.29; 95%CI: 0.08-1.00; p=0.04) was associated with reduced odds of disease. Characteristics of prevalent TB cases are presented in Table 19.

Relationships between exposures

HIV positivity in the source case was associated with HIV-positive status in the child (p=0.019), but also with ethnicity (p<0.001), and with the age of the source case (p-value for test of trend=0.003). Ethnicity of the child was associated with type of residence (p<0.001), type of water source (p<0.001) and type of toileting (p<0.001). The age of the source case was associated with sputum smear status (p=0.001), with older source cases less likely to have sputum smear-positive TB.

Discussion

This is the first study to assess risk factors for *M. tuberculosis* infection and disease in children exposed to an adult with MDR-TB. Consistent with data from the natural history of drug-susceptible TB in children, this study demonstrates that as children become older, they are more likely to be infected but once infected, that younger children are more likely to develop disease. HIV-positive children, while having no additional risk of infection, have a substantially increased risk for disease following infection. Alcohol use in the source case appears to be a risk factor for infection in the child and possibly for disease. The relationship between ethnicity, HIV positivity, age and sputum-smear status of the source case is complex. In the study, older source cases were more likely to be HIV-positive and ethnicity was strongly associated with HIV positivity. Other studies have demonstrated that HIV-positive adults with pulmonary TB are less frequently smear-positive than HIV-negative adults.[438] The increased risk of TB disease in child contacts of HIV-positive source cases may be due to biological (e.g. increased risk of HIV positivity) and epidemiological factors in HIV-affected households.

Previous studies have demonstrated that as children get older they are more likely to become infected, likely due to increasing duration of exposure to more potential source cases and more interaction with the community in addition to household exposure.[50] The relationship between alcohol use in the source case and both infection and disease in the child requires further study. It may be that alcohol is a surrogate for other socioeconomic factors but alcohol was not associated with ethnicity or any of the other socioeconomic exposures recorded in our study. It may be that source cases that drink are in some way more infectious than those who do not drink or that their behavior is more likely to lead to infection (e.g. prolonged exposure due to diagnostic and treatment delay) in the child. Adherence to treatment may be affected as might health-seeking behavior. Alcohol is a demonstrated risk factor for TB disease in adults with drug-susceptible[114] and MDR-TB.[439] That HIV positivity [63] and younger age[61] of the child is associated with increased risk of progression to disease following infection is well described in the drug-susceptible TB literature and it has also been demonstrated to be true for children exposed to MDR-TB. The proportion of children with disease is consistent with other household contact studies from this setting.[440] The association between gender of the child and disease also requires further examination. It is possible that girls are more likely to progress to disease or this association may reflect some sociological or cultural attitude to

child-rearing or health-seeking behavior. Houses with more rooms may be a surrogate for socioeconomic status, TB transmission, lifestyle, behavior or nutrition. It may be that more families live in houses with more rooms, whereas buildings with fewer rooms only house one family.

A limitation of the study is the relatively small number of children included, and the small number of children with disease. This may have concealed associations that may have been evident if larger numbers had been included. In previous drug-susceptible childhood contact studies, measures of intensity (e.g. proximity of sleeping) and duration of exposure demonstrated a graded relationship with risk of infection in the child, as did the infectiousness of the source case.[50] These relationships were not seen in this study. This study also did not compare children exposed to MDR-TB with children exposed to drug-susceptible TB to determine if systematic differences between the two populations exist (e.g. in child and source case demographics or risk factors for infection and disease). It is possible that MDR-TB in the source case, where typically long exposure durations are seen due to previous failed first-line treatments, may potentially obscure the relationship between risk factors and infection. It would also have been useful to compare MDR-TB-exposed children with community controls without a known source case to document the background (i.e. community, presumably drug-susceptible) infection rate. Finally, the definition of infection in this study was one TST measurement undertaken at the initial evaluation. Not only is TST an imperfect measure of infection, but TST retesting was not retested at follow-up visits in this cross-sectional study.

Table 15 - Risk factors for infection in child contacts of multidrug-resistant tuberculosis: child and household characteristics (n=228)

Variable		Total (n=228)	Infected (row %) (n=102)	Unadjusted OR (95% CI)	p-value	AOR (95%CI)[1]	p-value[1]
Age of child (n=224)	<1 year	53	15 (28.3)	1	0.05 0.007[2]	1.43 (1.13-1.91)	0.002[2]
	1-2 years	36	15 (41.7)	1.81 (0.74-4.42)			
	2-3 years	51	25 (49.0)	2.44 (1.08-5.48)			
	3-4 years	48	27 (56.3)	3.26 (1.43-7.44)			
	4-5 years	36	18 (50.0)	2.53 (1.05-6.14)			
Gender (n=227)	Female	109	46 (42.2)	1	0.43		
	Male	118	56 (47.5)	1.24 (0.73-2.09)			
Ethnicity	Xhosa	101	30 (29.7)	1	<0.001	1 2.51 (1.22-5.17)	0.01
	Colored	125	70 (56.0)	3.01 (1.69-5.36)			
Previous TB treatment	No	207	86 (41.5)	1	0.002	1 2.76 (0.84-9.12)	0.08
	Yes	21	16 (76.2)	4.50 (1.59-12.8)			
HIV status (n=217)	Negative	209	96 (45.9)	1.0	0.64		
	Positive	8	3 (37.5)	0.71 (0.16-3.03)			
Weight-for-age z-score (n=218)	More than -1	138	60 (43.5)	1	0.14		
	-1 to -2	47	26 (55.3)	1.61 (0.83-3.13)			
	Less than -2	33	11 (33.3)	0.65 (0.29-1.44)			
BCG scar visible (n=222)	No	40	14 (35.0)	1	0.14		
	Yes	182	87 (47.8)	1.70 (0.83-3.47)			
Type of residence (n=217)	Tin shack	33	8 (24.2)	1	0.007		
	Brick House	168	79 (47.0)	2.77 (1.18-6.50)			
	Wendy House	16	11 (68.8)	6.87 (1.83-25.8)			
Number of rooms in house	1-2	61	28 (45.9)	1	0.58		
	3-4	102	42 (41.2)	0.83 (0.44-1.56)			
	>4	65	32 (49.2)	1.14 (0.57-2.30)			
Number of people living in house	≤5 people	109	47 (43.1)	1	0.64		
	>5 people	119	55 (46.2)	1.13 (0.67-1.91)			
Density of people living in house	≤2 people per room	153	72 (47.1)	1	0.31		
	>2 people per room	75	30 (40.0)	0.75 (0.43-1.31)			
Water source (n=225)	Piped water in residence	176	84 (47.7)	1	0.06		
	Piped water from public source	49	16 (32.7)	0.53 (0.27-1.03)			
Toilet	Flush toilet in house	163	79 (48.5)	1	0.07		
	Other	65	23 (35.4)	0.58 (0.32-1.05)			

[1]Adjusted for ethnicity, age of child, previous TB in the child, age of source case, alcohol use in source case and HIV status of source case , [2]Test for trend, AOR: adjusted odds ratio; CI: confidence interval

Table 16 - Risk factors for infection in child contacts of multidrug-resistant tuberculosis: source case and exposure characteristics (n=228)

Variable		Total (n=228)	Infected (row %) (n=102)	OR (95% CI)	p-value	AOR (95%CI)[1]	p-value[1]
Age of source case (n=219)	16-25	55	31 (56.4)	1	0.06 0.02[2]	0.67 (0.45-1.00)	0.05[2]
	26-35	86	40 (46.5)	0.67 (0.34-1.33)			
	>35	78	28 (35.9)	0.43 (0.21-0.88)			
Gender of source case (n=226)	Female	138	62 (44.9)	1	0.93		
	Male	88	39 (43.3)	0.98 (0.57-1.67)			
Smoking status of source case (n=225)	Non-smoker	130	52 (40.0)	1	0.09		
	Smoker	95	49 (51.6)	1.60 (0.94-2.73)			
Alcohol use by source case (n=225)[3]	Never drinks	166	65 (39.2)	1	0.004	1 2.59 (1.29-5.22)	0.007
	Drinks alcohol	59	36 (61.0)	2.43 (1.32-4.47)			
Smear result of source case (n=224)	Negative	28	9 (32.1)	1	0.17		
	Positive	196	90 (45.9)	1.79 (0.77-4.16)			
Smear grade of source case (n=217)	Negative	28	9 (32.1)	1	0.13		
	Scanty	18	8 (44.4)	1.69 (0.50-5.73)			
	1+	28	15 (53.6)	2.44 (0.82-7.22)			
	2+	107	44 (41.1)	1.47 (0.61-3.56)			
	3+	36	22 (61.1)	3.31 (1.17-9.37)			
HIV status of source case (n=224)	Negative	150	79 (52.7)	1	<0.001	1 0.48 (0.22-1.04)	0.06
	Positive	74	20 (27.0)	0.33 (0.18-0.61)			
CD4 count of source case if HIV-positive (n=71)	<200	33	7 (21.2)	1	0.46		
	>200	38	11 (28.9)	1.51 (0.50-4.56)			
Relationship of source case to child	Parents	100	38 (38.0)	1	0.34		
	Grandparent	31	15 (48.4)	1.53 (0.68-3.45)			
	Uncle or aunt	66	33 (50.0)	1.63 (0.87-3.06)			
	Other	31	16 (51.6)	1.74 (0.77-3.92)			
Duration of exposure between source case and child	Less than a month	30	13 (43.3)	1	0.62		
	One month to six months	92	38 (41.3)	0.92 (0.40-2.11)			
	More than six months	106	51 (48.1)	1.21 (0.54-2.74)			
Primary caregiver to child	Index case	55	21 (38.2)	1	0.26		
	Not index case	173	81 (46.8)	1.43 (0.77-2.65)			
Frequency of contact between source case and child	Daily	213	93 (43.7)	1	0.23		
	Less frequently	15	9 (60.0)	1.94 (0.67-5.63)			
Intensity of contact between child and source case	Sleeps in the same bed	57	25 (43.9)	1	0.67		
	Sleeps in the same room	34	13 (38.2)	0.79 (0.33-1.89)			
	Sleeps in the same house	101	45 (44.6)	1.03 (0.53-1.98)			
	Sleeps in a different house	36	19 (52.8)	1.43 (0.62-3.31)			

[1]Adjusted for ethnicity, age of child, previous TB in the child, age of source case, alcohol use in source case and HIV status of source case, [2]Test of trend, [3]Regular alcohol use in source case as reported by the parent or legal guardian of the child, AOR adjusted odds ratio; CI confidence interval

Table 17 - Risk factors for disease in child contacts of multidrug-resistant tuberculosis: child and household characteristics (n=102)

Variable		Total (n=102)	Disease (row %) (n=15)	OR (95% CI)	p-value	Age-adjusted OR (95%CI)	p-value
Age (n=100)	<1 year	15	4 (26.7)	1	0.28 0.01[1]	n/a	n/a
	1-2 years	15	3 (20.0)	0.69 (0.12-3.79)			
	2-3 years	25	6 (24.0)	0.87 (0.20-3.77)			
	3-4 years	27	2 (7.4)	0.22 (0.03-1.38)			
	4-5 years	18	0 (0)	-			
Gender	Female	46	11 (23.9)	1	0.02	1 0.29 (0.08-1.00)	0.04
	Male	56	4 (7.1)	0.24 (0.72-0.83)			
Ethnicity	Xhosa	30	7 (23.3)	1	0.13		
	Colored	70	8 (11.4)	0.42 (0.14-1.33)			
Previous TB treatment	No	86	14 (16.3)	1	0.32		
	Yes	16	1 (6.3)	0.34 (0.04-2.81)			
HIV status (n=99)	Negative	96	13 (13.5)	1	0.04	1 25.3 (1.63-393)	0.01
	Positive	3	2 (66.7)	12.8 (1.07-151.1)			
Weight-for-age z-score (n=97)	More than -1	60	10 (16.7)	1	0.19		
	-1 to -2	26	1 (3.8)	0.20 (0.02-1.65)			
	Less than -2	11	1 (9.1)	0.50 (0.06-4.45)			
BCG scar visible (n=101)	No	14	2 (14.3)	1	0.95		
	Yes	87	13 (14.9)	1.05 (0.21-5.27)			
Type of residence (n=98)	Tin shack	8	1 (12.5)	1	0.84		
	Brick House	79	12 (15.2)	1.25 (0.14-11.1)			
	Wendy House	11	1 (9.1)	0.70 (0.04-13.2)			
Number of rooms in house	1-2	28	2 (7.1)		0.04 0.02[1]	1.39 (1.02-1.91)	0.04[1]
	3-4	42	4 (9.5)	1.37 (0.23-8.03)			
	>4	32	9 (28.1)	5.09 (1.00-26.0)			
Number of people living in house	≤5 people	47	5 (10.6)	1	0.29		
	>5 people	55	10 (18.2)	1.87 (0.59-5.91)			
Density of people living in house	≤2 people per room	72	12 (16.7)	1	0.39		
	>2 people per room	30	3 (10)	0.56 (0.14-2.16)			
Water source (n=100)	House tap	84	12 (14.3)	1	0.65		
	No house tap	16	3 (18.8)	1.38 (0.34-5.60)			
Toilet	Flush toilet in house	79	12 (15.2)	1	0.80		
	Other	23	3 (13.0)	0.84 (0.21-3.26)			

[1]Test of trend, OR: odds ratio; CI: confidence interval

Table 18 - Risk factors for disease in child contacts of multidrug-resistant tuberculosis: source case and exposure characteristics (n=102)

Variable		Total (n=102)	Disease (row %) (n=15)	OR (95% CI)	p-value	Age-adjusted OR (95%CI)	p-value
Age of source case (n=99)	16-25	31	4 (12.9)	1	0.81		
	26-35	40	5 (12.5)	0.96 (0.24-3.94)			
	>35	28	5 (17.9)	1.47 (0.35-6.12)			
Gender of source case (n=101)	Female	62	11 (17.7)	1	0.31		
	Male	39	4 (10.3)	0.53 (0.16-1.80)			
Smoking status of source case (n=101)	Non-smoker	52	8 (15.4)	1	0.88		
	Smoker	49	7 (14.3)	0.92 (0.31-2.75)			
Alcohol use by source case (n=101)[1]	Never drinks	65	6 (9.2)	1	0.04	1 2.90 (0.90-9.31)	0.07
	Drinks alcohol	36	9 (25.0)	3.28 (1.06-10.1)			
Smear result of source case (n=99)	Negative	9	0 (0)	-	0.35		
	Positive	90	15 (16.7)	-			
Smear grade of source case (n=98)	Negative	9	0 (0)	-	0.05		
	Scanty	8	0 (0)	-			
	1+	15	5 (33.3)	-			
	2+	44	9 (20.5)	-			
	3+	22	1 (4.5)	-			
HIV status of source case (n=99)	Negative	79	8 (10.1)	1	0.009	1 4.07 (1.19-13.8)	0.03
	Positive	20	7 (35.0)	4.78 (1.45-15.5)			
CD4 count of source case if HIV-positive (n=18)	<200	7	3 (42.9)	1	0.78		
	>200	11	4 (36.4)	0.76 (0.11-5.28)			
Relationship of source case to child	Parents	38	5 (13.2)	1	0.62		
	Grandparent	15	4 (26.7)	2.4 (0.55-10.6)			
	Uncle or aunt	33	4 (12.1)	0.91 (0.22-3.71)			
	Other	16	2 (12.5)	0.94 (0.16-5.45)			
Duration of exposure between source case and child	Less than a month	13	3 (23.1)	1	0.35		
	One month to six months	38	7 (18.4)	0.75 (0.16-3.47)			
	More than six months	51	5 (9.8)	0.36 (0.07-1.77)			
Primary caregiver to child	Index case	21	2 (9.5)	1	0.46		
	Not index case	81	13 (16.0)	1.82 (0.38-8.76)			
Frequency of contact between source case and child	Daily	93	12 (12.9)	1	0.12		
	Less frequently	9	3 (33.3)	3.38 (0.74-15.3)			
Intensity of contact between child and source case	Sleeps in the same bed	25	3 (12.0)	1	0.47		
	Sleeps in the same room	13	1 (7.7)	0.61 (0.06-6.54)			
	Sleeps in the same house	45	6 (13.3)	1.13 (0.26-4.96)			
	Sleeps in a different house	19	5 (26.3)	2.62 (0.54-12.7)			

[1]Regular alcohol use in source case as reported by the parent or legal guardian of the child, OR; odds ratio; CI: confidence interval

Table 19 - Characteristics of children presenting with prevalent TB disease following exposure to adult with multidrug-resistant tuberculosis (n=15)

Age in months	Gender	HIV status	Source case(s)	DST[1]	TB diagnosis
3	Girl	Negative	Father	MDR	Confirmed
6	Boy	Negative	Aunt	MDR	Confirmed
12	Girl	Negative	Grandmother	Pre-XDR	Probable
12	Girl	Negative	Great uncle	MDR	Probable
13	Boy	Negative	Sister	XDR	Probable
13	Girl	Positive	Mother	MDR	Probable
16	Girl	Negative	Father	Pre-XDR	Probable
27	Boy	Negative	Aunt	MDR	Confirmed
29	Girl	Negative	Uncle	MDR	Probable
31	Girl	Negative	Aunt	MDR	Probable
32	Girl	Negative	Aunt	MDR	Probable
35	Boy	Negative	Mother	MDR	Probable
36	Girl	Negative	Aunt	XDR	Probable
39	Girl	Negative	Mother and aunt	XDR	Probable
43	Girl	Positive	Mother	MDR	Probable

DST: drug susceptibility test; MDR: multidrug-resistant; XDR: extensively drug-resistant; pre-XDR: MDR with additional resistance to either a fluoroquinolone or an injectable medication
[1]DST of the isolate from the child where the diagnosis was confirmed and from the source case when probable

Study 3: drug-resistant tuberculosis in children is caused by transmission and amplification of resistance within families

The following study has been published as an article:

- *Seddon JA, Warren R, Enarson DA, Beyers N, Schaaf HS. Drug-Resistant tuberculosis transmission and resistance amplification within families. Emerg Infect Dis 2012; 18:1342-5*

The first study in the thesis documented the burden of drug resistance in the context of the research. The second explored the epidemiological risk factors for infection and disease in child contacts of DR-TB. This study and the next examine the transmission of DR M. tuberculosis strains from adults to children within families. The first of these two describes the clinical and molecular investigations of two families following the identification of children with drug-resistant TB.

The study

This investigation was carried out in a suburban community of Cape Town, South Africa where the TB incidence was 978/100,000 population in 2009.[441] Since 1994, microbiological samples from all patients treated for TB in this area have been collected routinely. Between 2008 and 2010 two children from these communities were diagnosed with MDR-TB.

Information was obtained from several sources to document the sequence of events culminating in the child developing MDR-TB. A home visit was carried out and the family was interviewed following written informed consent. Family members were included if they either lived with or spent significant periods of time with the child.[442] Information on TB diagnoses, treatment and outcome was obtained at interview. If a family member was identified as having had TB, significant family contacts of that person were then also included. Searches were made at the local clinic, the academic hospitals and the regional TB hospital responsible for drug-resistant TB management, for case notes of any of those included. Additionally, the local clinic TB register was consulted.

Samples were identified from patients in the two social networks and isolates were genotyped by spoligotyping[443] and IS*6110* DNA fingerprinting.[444] Strains were identified according to distinct IS*6110* banding patterns using Gelcompar II (Applied Maths, Sint-Martens-Latem, Belgium) or by their characteristic spoligotype pattern.[445] Mutations conferring resistance to

isoniazid, rifampicin, ethambutol, pyrazinamide, ofloxacin and amikacin were determined by DNA sequencing of the *inhA* promoter, *katG*, *rpoB*, *embB*, *pncA*, *gyrA* and *rrs* genes, respectively.[211]

Case one

A 19-month-old girl (A3) was diagnosed with TB in March 2008 following a six month course of isoniazid preventive therapy. She presented with two weeks of cough, respiratory distress and fever. She had significant contact with a pre-XDR-TB patient (MDR-TB resistant to either a fluoroquinolone or a second-line injectable drug) and so was treated with capreomycin, ethionamide, ethambutol, PAS, terizidone, clarithromycin and high-dose isoniazid. Gastric aspirate samples were sent, from which *M. tuberculosis* was cultured, resistant to rifampicin, isoniazid and ofloxacin, susceptible to amikacin and ethionamide. She was treated for 18 months from the time of her first negative culture, the first six months including the injectable medication. She was cured.

Family one

Eighteen people were found in the family (Figure 4). The husband of an aunt (A2) had known DR-TB. He cared for the girl on a daily basis. He had been treated initially for drug-susceptible TB, changed to MDR-TB therapy when resistance to rifampicin and isoniazid was determined and then to XDR-TB treatment when resistance to second-line drugs was discovered. He subsequently died. His mother (A1) had repeatedly defaulted treatment and was finally diagnosed with DR-TB in 1998. She refused further treatment until her death in 2003. The clinical chronology is shown in Figure 5 with molecular details for the samples located shown in Table 20.

Case two

A 13-year-old girl (B5) was identified in April 2009 as a contact of multiple family members with XDR-TB. She was asymptomatic but had an abnormal chest radiograph. She was started on capreomycin, ethionamide, pyrazinamide, terizidone, PAS, co-amoxicillin/clavulanic acid, clarithromycin, linezolid and high-dose isoniazid. *M. tuberculosis* was cultured from a sputum sample, resistant to isoniazid, rifampicin, ethambutol, ofloxacin and amikacin. The capreomycin was given for six months and she was treated for a total of 18 months. She was cured.

Family two

The family is demonstrated in Figure 4. The eldest brother (B1) had been in prison and developed TB soon after release in 1998. He was started on first-line treatment and died soon afterwards. His sister (B2), mother (B3) and brother (B4) then developed TB. All were started initially on first-line therapy, converted onto MDR-TB and, for the brother, XDR-TB treatment regimens when resistance profiles became available. All three died. A chronology is shown in Figure 5 with molecular details for the samples demonstrated in Table 20.

Discussion

In family one, the uncle's mother (A1) had pre-XDR-TB and probably transmitted it to her son (A2). He likely transmitted it to his niece (A3). All three had identical strains. In family two, it is unknown whether the oldest brother (B1) had DR-TB. His sister (B2) had pre-XDR-TB but then in sequence her mother (B3), brother (B4) and sister (B5) developed XDR-TB, of a strain identical to hers. This investigation, therefore, demonstrates the potential for resistance to be both transmitted and amplified within families.

Other than the two child index cases (A3 & B5), all were initially started on first-line therapy and treated until DST results became available, often despite a known drug-resistant contact. Local policy is to diagnose TB solely from sputum smear in new patients who have no risk factors for drug resistance. Re-treatment patients and those at risk of resistance have DST done to rifampicin and isoniazid. If MDR-TB is diagnosed, DST to second-line drugs is then performed. Giving inadequate regimens not only leads to more advanced disease until effective treatment is initiated but also risks amplifying resistance.[446-447] For a patient with TB symptoms, in contact with drug-resistant TB, it is important to obtain microbiological samples and then start treating according to the DST of the source case. If a less resistant organism is grown, treatment can be changed. In the context of multiple possible TB source cases, deciding on treatment is challenging. Consideration must be given to the infectiousness of potential source cases as well as the intensity, frequency and duration of exposures. Local policy is to carry out household contact tracing for drug-resistant TB patients. Whilst in reality this occurs infrequently, we demonstrate the importance of careful investigation of contacts to identify those who may have sub-clinical disease who could be treated early. Given the social interactions, chronology and mycobacterial results, it is highly likely that the transmission sequence occurred as described. However, in both clusters, the strain identified is one that is

predominant locally. We must, therefore, be aware that this is a potential confounder to the transmission lines suggested.

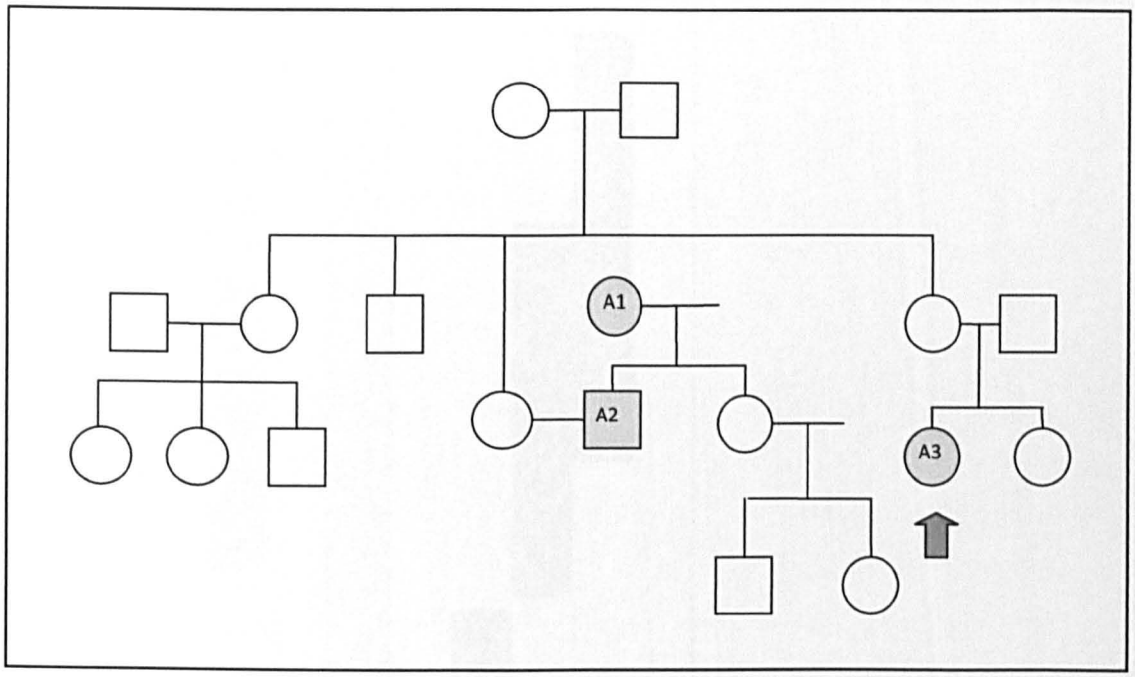
Table 20 - Gene sequencing, IS*6110* DNA fingerprinting and genotype for isolates from members of the two families

Family One		*rpoB*	*inhA*	*katG*	*embB*	*gyrA*	*pncA*	*rrs* 1401	IS*6110* Cluster number	Genotype
		R	H	H	E	F	Z	A		
Uncle's mother	A1	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	WT	213	Beijing
Uncle	A2	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	WT	213	Beijing
Index child	A3	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	WT	**	Beijing
Family Two										
Oldest brother*	B1									
Sister	B2	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	WT	213	Beijing
Mother	B3	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	ACG1401GCG	213	Beijing
Other brother	B4	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	ACG1401GCG	213	Beijing
Index child	B5	TCG531TTG	WT	AGC315ACC	ATG306ATA	GCG90GTG	ACA160GCA & ACC100ATC	ACG1401GCG	**	

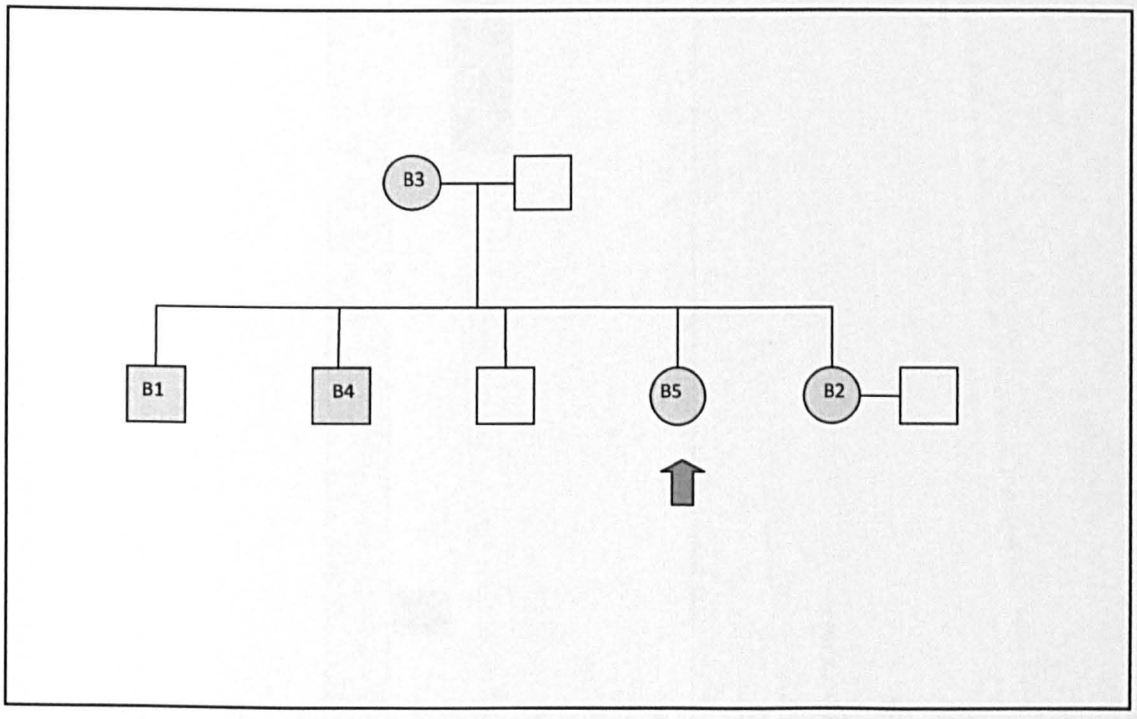
R – rifampin; H – isoniazid; E – ethambutol; F – fluoroquinolones; Z – pyrazinamide; A – aminoglycosides, WT – wild type
The earliest sample available for each patient is shown; in all incidences where more than one sample was available for a patient, all samples demonstrated identical gene sequence and strain type results
*Developed TB and died prior to systematic sample collection and storage. No culture or drug susceptibility testing requested on sample
**Only spoligotype performed as isolates repeatedly lost viability on culture

Figure 4 - Families investigated following the diagnosis of two children with multidrug-resistant tuberculosis

Family One



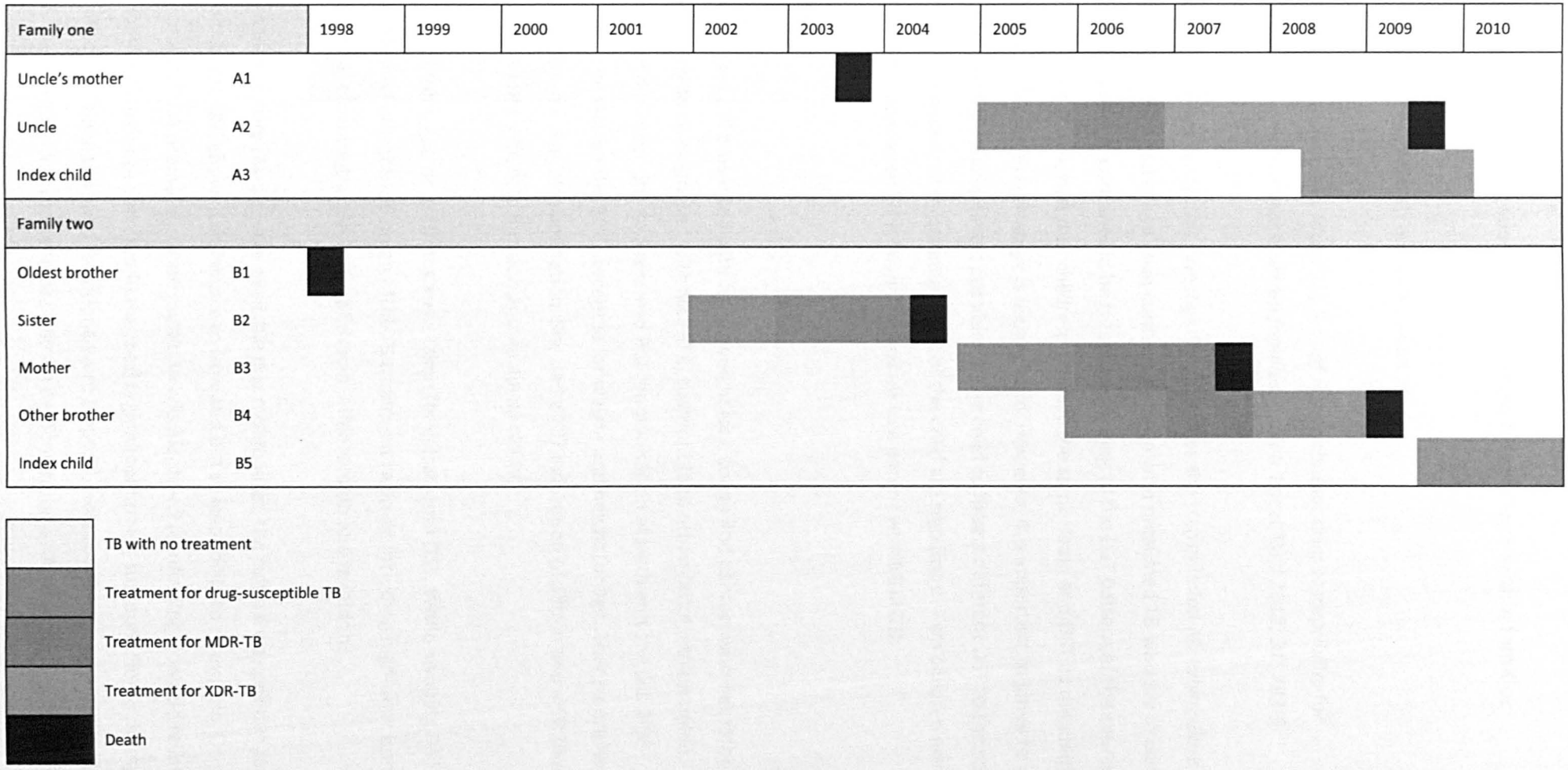
Family Two



Person identified as having tuberculosis

Identified child index case

Figure 5 - Chronology of tuberculosis treatment and outcomes for the two families



Study 4: discordant drug susceptibility for *M. tuberculosis* within families

The following study has been published as an article:

- *Seddon JA, Jordaan AM, Victor TC, Schaaf HS. Discordant drug susceptibility for Mycobacterium tuberculosis within families. Pediatr Infect Dis J 2012; 31: 783-5*

As with the previous study, this investigation examines the transmission *M. tuberculosis* between adult source case and child contact. Children with presumed TB who are in contact with a MDR-TB source case should be treated according to the DST pattern of the source case's isolate.[16, 197] As it is assumed that children will have the same strain and DST as the identified source case,[64] this general strategy is usually valid. However, it is important to strive to obtain a microbiological diagnosis as it is possible for the child to have a different DST to the source case. The implications for the management of the child are significant. Two children are described who developed TB following exposure to a parent with MDR-TB.

Case one

A 50-month-old girl presented with fever, weight loss, cough and contact with her father who had previously been diagnosed with MDR-TB, susceptible to ethambutol, ethionamide, ofloxacin and amikacin. She had received BCG immunisation at birth and she was HIV-uninfected. She was on the 50th percentile for weight and height for age. There were no abnormal signs on clinical examination. She had a TST induration of 18mm and a CR that showed left upper lobe opacification and an apical cavity.

Two early morning gastric aspirates were taken for culture and DST. While awaiting DST results, the child was started on an MDR-TB treatment regimen including high-dose isoniazid, pyrazinamide, ethambutol, amikacin, ofloxacin, ethionamide and terizidone.

When the laboratory results were available one month later, the culture was positive for *M. tuberculosis* and DST showed resistance to isoniazid but susceptibility to rifampicin. On gene sequencing an *inhA* promoter region mutation was identified (confirming isoniazid resistance), while no mutations were detected in the *rpoB* region (confirming susceptibility to rifampicin). The amikacin, ethionamide and terizidone were stopped, isoniazid continued and rifampicin started. Gene sequencing of the father's strain demonstrated a TAC mutation at the 516

location of the *rpoB* gene, confirming that the father was infected with a rifampicin-resistant strain. The spoligotypes for the girl and her father are shown in Figure 6. The girl responded well clinically and radiologically.

Case two

A 26-month-old boy presented with a two month history of enlarged bilateral cervical lymph nodes. His mother had been diagnosed with MDR-TB three months earlier with a strain susceptible to ethambutol, ethionamide, ofloxacin and amikacin. She had been three-plus sputum smear-positive for acid-fast bacilli and had been started on an MDR-TB treatment regimen.

The clinical examination of the child was unremarkable other than enlarged cervical lymph nodes bilaterally. He was on the 50th percentile weight for age, had a BCG scar, was HIV-uninfected and had an ulcerating 25mm TST induration. His chest radiograph showed bilateral hilar lymphadenopathy. He had gastric aspirate and lymph node fine needle aspiration biopsy (FNAB) samples taken for culture and DST. Due to the clinical presentation, radiology and the history of contact with an MDR-TB source case, he was started on a MDR-TB regimen of ethambutol, pyrazinamide, amikacin, ofloxacin, ethionamide, terizidone and high-dose isoniazid. As part of contact investigations, the child's asymptomatic father was screened with sputum culture and was shown to have MDR-TB, resistant to isoniazid and rifampicin but susceptible to the other medications tested. He was started on MDR-TB treatment.

Six weeks later two independent DST results from the child's gastric aspirate and also the FNAB showed *M. tuberculosis,* resistant to isoniazid but susceptible to rifampicin. Genotypic results showed a *katG* gene mutation, confirming isoniazid resistance. The treatment was continued until full gene sequencing demonstrated that there was no *rpoB* gene mutation (confirming susceptibility to rifampicin). At this point the amikacin, terizidone and isoniazid were stopped and rifampicin was started. Gene sequencing for both parents' isolates showed a TTG mutation at the 531 location of the *rpoB* gene (rifampicin resistance). The spoligotypes for the child, his mother and his father are shown in Figure 6. The boy responded well clinically and radiologically.

Discussion

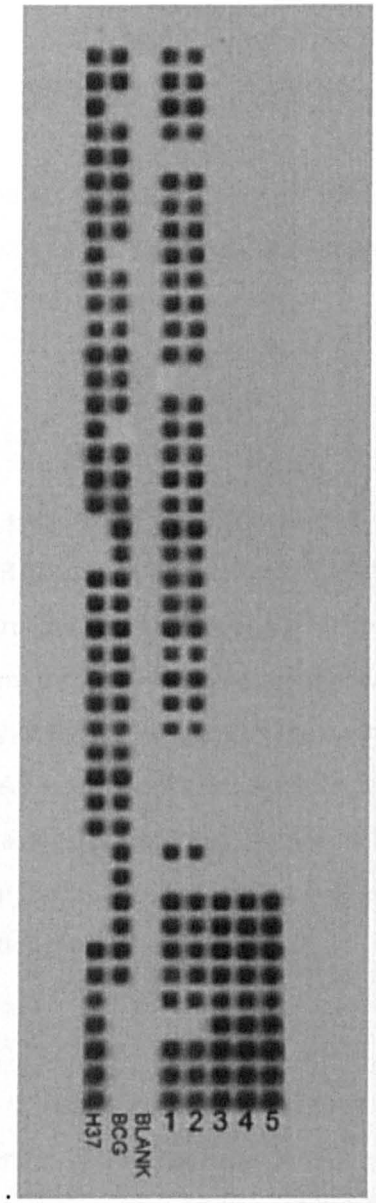
Although it is appropriate to treat children according to the DST pattern of the likely source case when a microbiological sample in the child is not obtained, these cases highlight the importance of striving for a microbiological diagnosis in all patients. The sequence of events and transmission circumstances for these children are not completely clear. A number of possible scenarios could have taken place. First, there may have been a laboratory error in either the DST of the parents or that of the children. Without the gene sequencing and spoligotyping this has to be considered but with these results it seems unlikely. Second, the children could have been infected from a source case other than their parents. In high burden settings it is possible for children to develop disease caused by a strain transmitted from someone other than the identified source case.[66, 69] In the first case the discriminating power of the spoligotype is good and it is unlikely that the child contracted TB from source cases outside the social group. It is possible, however, that a third party transmitted TB first to the child, whilst isoniazid-resistant, and later to the father after developing MDR. In the second case, the strain type is from the Beijing family, which is common in the Western Cape Province of South Africa. This strain could have been transmitted from a number of different source cases but given the close proximity of the child to the parents and the lack of other source cases, it is less likely. Another possibility is that in the parent(s) both isoniazid-resistant and MDR strains co-existed (multiple strain infection) with the MDR strain isolated from the adult but the isoniazid-resistant strain transmitted to the child.[71-72] Finally, and probably most likely, one of the parents may have had HMR-TB and transmitted the mycobacterium to the child prior to developing rifampicin resistance.

Testing the infecting organism for the presence of the mutations that are usually associated with drug resistance (genotypic testing) has advantages over conventional (phenotypic) testing in which the organism is grown in the presence of antibiotic. It is more rapid, cheaper and is less labour intensive. For *M. tuberculosis* the *rpoB* gene is almost always (>95%) associated with rifampicin resistance. Isoniazid resistance, however, is associated with a number of mutations, the most common of which are in the *inhA* promoter region and *katG* gene. If a *katG* mutation is present the strain usually has high-level resistance to isoniazid, whereas if an *inhA* promoter region mutation is present the strain usually has low-level resistance. In the case of *inhA* promoter region mutations, isoniazid, given at high dose, may be of use.[90] As isoniazid and ethionamide share similar biochemical pathways, if an *inhA* promoter region mutation is present the strain is likely to be ethionamide-resistant.[91, 448] With the roll-out of genotypic testing (both the line probe assays[449] and the GeneXpert MTB/RIF assay [Cepheid][407]

have been endorsed by the WHO) it is important that treating clinicians are aware of the implications, strengths and weaknesses of these new tests.

The main reason to strive to obtain a microbiological diagnosis in MDR-TB contacts is the difference in management of MDR, HMR, RMR and drug-susceptible strains. Drug-susceptible strains are treated with a highly effective six-month first-line regimen which is well tolerated and associated with good outcomes. The treatment for HMR disease involves treatment with rifampicin, ethambutol and pyrazinamide (adding a fluoroquinolone in extensive disease if identified early) taken for nine months.[197] MDR-TB strains (and RMR cases if tested by genotypic-based DST only) are treated with a second-line injectable medication for six months and treatment is for eighteen months from the time of the first negative culture. Drugs used for the full duration of treatment include high-dose isoniazid (if an *inhA* promoter region mutation is present) ethionamide (if a *katG* gene mutation is present), pyrazinamide, ethambutol, a fluoroquinolone and terizidone (or cycloserine). The adverse effects of the additional medications, together with the extended treatment duration make treating for MDR or RMR-TB a serious proposition.

Figure 6 - The spoligotypes of the children and parents for the two cases with positive controls, negative controls and BCG shown alongside



1 – Case One – Child
2 – Case One – Father
3 – Case Two – Child
4 – Case Two – Father
5 – Case Two – Mother

Study 5: decentralised care for the management of child contacts of multidrug-resistant tuberculosis

The following study has been accepted as an article:

- *Seddon JA, Hesseling AC, Dunbar R, Cox H, Hughes J, Fielding K, Godfrey-Faussett P Schaaf HS. Decentralised care for child contacts of multidrug-resistant tuberculosis. Pub Health Action 2012; 2: 66-70*

Introduction

This study and the next explore the operational and programmatic identification and referral of child contacts of MDR-TB source cases. The first of these two examines the number of MDR-TB child contacts that are identified and brought for specialist assessment, under routine, programmatic conditions. It then links these children with adults that are registered for MDR-TB treatment in the City of Cape Town, to provide an indication of the proportion of children identified who might have been exposed. In addition it compares the identification and referral in Khayelitsha (a decentralised model of MDR-TB care) with the other seven sub-districts (a centralised, hospital-based model) to determine if more children are identified and if there are any implications for delay to be seen.

Methods

Identification and treatment of child contacts

According to provincial and national guidelines, following the diagnosis of DR-TB in an adult, a home visit should be conducted to educate the patient and their family, give advice about infection control and identify symptomatic contacts. A professional nurse oversees this process within each of the eight sub-districts. Children less than five years and HIV-infected children are referred to their local clinic for assessment prior to referral to TCH which serves as the main provincial paediatric DR-TB referral centre. In the Khayelitsha sub-district these children are referred to the specialist outreach clinic, conducted monthly in Khayelitsha. Children from outside the City of Cape Town health district are also sometimes referred to the DR-TB clinic at TCH.

Case identification, data collection and eligibility: source cases

Adult "source cases" (>18 years) treated for MDR-TB in the City of Cape Town Health District from 1 April 2010 to 31 March 2011 were identified from routine TB register data. Source cases were included if they had been started on MDR-TB treatment during the stated time period for sputum smear and/or culture positive pulmonary TB, and had been registered at a TB clinic in one of the eight sub-districts. Adults were excluded if they did not have TB resistant to both isoniazid and rifampicin, or were registered in hospital or prison (i.e. unclear sub-district of origin).

Case identification, data collection and eligibility: child contacts

From 1 May 2010 to 30 June 2011 all children evaluated at the TCH, or at the outreach DR-TB clinic, were prospectively recorded. Children were included in the study if they were either HIV-infected or were less than five years old and had significant contact with a source case with sputum smear and/or culture positive pulmonary MDR-TB. Significant contact was defined as living with or having regular daily interaction with the source case over the preceding six months.

Data analysis

After removing duplicates from the MDR-TB register, probabilistic linking was done using software Registry Plus™ Link Plus (Centers for Disease Control and Prevention, Atlanta, GA, USA) to match adult cases from the register to the names of source cases provided by the parents/caregivers of children attending DR-TB clinics.[450-451] An inclusive algorithm was used allowing the software to use four demographic variables: name, surname, sex and age. Names and surnames were converted using the New York State Identification and Intelligence System, a phonetic coding system that allows for inconsistencies and variations in spelling. The total number of source cases, the number of children assessed and the number of linked source cases were determined. Time to assessment was defined as the time from sputum production in the source case, for the sample that diagnosed MDR-TB, to the child being evaluated at the DR-TB clinic.

Statistical analysis was performed using STATA version 11. Missing data were excluded from analysis. The association between categorical variables was assessed using the χ^2 test or Fisher's exact test, where appropriate. The Mann Whitney test was used to compare quantitative data which were not normally distributed, and data summarised using the median and IQR. The t-test was used to compare normally distributed quantitative data.

Results

Of the 1265 adult MDR-TB source cases registered during the study period, 1221 were included. The sub-district could not be determined for the remaining 41 cases. Six hundred and seventy (55.0%) were male; the median age at diagnosis was 35 years (IQR: 27-44 years). (Table 21) One hundred and eighty nine (15.5% of total) patients were registered in Khayelitsha. Clinical characteristics of the source cases from Khayelitsha vs. other sub-districts were similar, except for the prevalence of HIV infection, which was higher in the Khayelitsha group (70.5% vs. 49.8%; p<0.001).

Two hundred and sixty-five children were evaluated at TCH, or at the outreach DR-TB clinic, during the assessment period. Eleven were excluded as not meeting the criteria of significant contact. Of the 254 included, 146 (57.5%) were linked to 126 source cases; the median number of contacts per source case was 1 (range 1 to 4). Of the 108 unmatched children, 26 (24.1%) were linked to a source case resident outside the City of Cape Town. Of the linked children, a total of 35 children (linked to 31 source cases) were from Khayelitsha and 111 children (linked to 95 source cases) were from the remaining seven sub-districts. Eighty (54.8%) children were male; median age 32 months (IQR: 13-46 months). (Table 22). As expected, children from Khayelitsha were more likely to be Xhosa than from the other sub-districts (100% vs. 36%; p<0.001). Children from Khayelitsha were better nourished with mean weight-for-age z-score 0.07 compared to -0.63 (p=0.012). Other characteristics were similar between the two groups.

Of source cases in Khayelitsha, 16.4% (31/189) led to the assessment of at least one child contact, compared to 9.2% (95/1032) from source cases diagnosed in the other sub-districts (p=0.003). Children in Khayelitsha were seen at a median of 71 days (IQR: 37-121 days) from the date of source case MDR-TB sputum production compared to 90 days (IQR: 56-132 days) in the other sub-districts (p=0.15).

Discussion

In a previous MDR-TB contact study in Cape Town, a mean of 1.7 child contacts five years or less were identified for each source case with sputum-positive TB.[21] Recent TB household studies from Cape Town also indicate a mean of 1.7 children younger than five years identified per drug-susceptible TB source case. (Personal communication: Anneke Hesseling). Based on our findings, it is therefore likely that only a small proportion of possible MDR-TB child

contacts were identified, referred and evaluated by a specialist as is recommended in national and international guidelines. There appears to be some advantage provided by decentralised care, in terms of number of children identified per source case and time for child to be seen, but the number of children evaluated remains low for both models. Furthermore, despite a trend towards children being seen earlier in Khayelitsha, the time to assessment is sub-optimal in the light of the high risk of disease progression in young children.

The reason so few children are evaluated may be explained by a number of factors. First, the definition of child contact used by healthcare workers may not be sufficiently inclusive. If a definition is used where only children living in the same house as the source case are included, fewer contacts will be revealed than if a definition of any significant contact is used, as in our study.[404] It is therefore possible that children are not identified by local healthcare teams. Furthermore, if children are locally identified, then personal, logistic or financial barriers to accessing clinic appointments may occur. In this operational study, we used the source case as the denominator and children evaluated in the specialist clinic as the numerator; we are therefore unable to determine where the attrition occurred. However, studies examining children exposed to drug-susceptible TB have demonstrated that this 'drop off' occurs at every step in the care pathway.[452-453]

Delay in the assessment of child contacts has a number of components. These include the time to diagnosis in the source case, time to identification of child contacts, time for the child to be seen locally and the time for the child to be seen in the specialist clinic. Since we captured the date of sputum production in the source case and the date the child was seen in the specialist clinic we were unable to determine the respective duration of each of these components. However, the delay associated with starting TB treatment has been well explored and is associated with both patient and health system delays.[454-456] The delay from sputum sampling to the initiation of DR-TB treatment initiation has fallen from 72 days in 2005 to 33 days in 2010, in Khayelitsha.[122] In a sample of ten health facilities in the City of Cape Town excluding Khayelitsha, the mean delay was 83 days in 2005-2008 and 53 days in 2008-2011. (Personal communication: Pren Naidoo). The impact of health system strengthening and availability of more rapid diagnostic tests has improved delay (LPA was introduced at the end of 2008) but there is a suggestion that some of the health system delay may be improved by decentralised care.

A limitation to the study is the number of children seen in clinic for which we could not match to a source case from the DR-TB register. Nearly a quarter were from outside the region but

for the rest the reason is unclear. There may have been a matching problem despite our inclusive matching approach; this would likely apply equally to the two models of care compared in our study. It may have been that children were seen during the inclusion period but that the source case was registered outside the dates searched. It may also have been that some of the source cases were primary defaulters and were diagnosed but never started treatment. Finally, the registration of source cases could have been incomplete. Although this is a limitation, we set out to document the proportion of registered MDR-TB source cases in which child contacts were identified and assessed in clinic as per local guidelines. These limitations do not affect the conclusion that child contacts are seen in only a small proportion.

Table 21 - Characteristics of adult multidrug-resistant tuberculosis source cases identified by health district in the period 1 May 2010 – 30 June 2011 (n=1221)

	Khayelitsha (n=189)	**Other sub-districts (n=1032)**	**Total (n=1221)**
Median age in years; n=1211 (IQR)	34 (27-40)	36 (28-44)	35 (27-44)
Male gender; n=1219 (%)	93 (49.5)	577 (56.0)	670 (55.0)
HIV positive; n=1070 (%)*	117 (70.5)	450 (49.8)	567 (53.0)
Positive sputum smear; n=1011 (%)	73 (42.4)	397 (47.3)	470 (46.5)
XDR (%)	16 (8.5)	86 (8.3)	102 (8.4)

HIV: human immunodeficiency virus; XDR: extensively drug-resistant; IQR: inter-quartile range
*Difference in HIV prevalence between Khayelitsha and the other seven sub-districts: $p<0.001$.

Table 22 - Characteristics of child MDR-TB contacts identified by health district and linked to a source case in the period 1 May 2010 – 30 June 2011 (n=146)

		Khayelitsha (n=35)	**Other sub-districts (n=111)**	**Total (n=146)**
Gender	Male (%)	16 (45.7)	64 (57.7)	80 (54.8)
Age (months)	Median (IQR)	31 (12-44)	32 (13-47)	32 (13-46)
Ethnicity (n=145)*	Xhosa (%)	35 (100)	39 (35.5)	74 (51.0)
	Coloured (%)	0	70 (63.6)	70 (48.3)
	White (%)	0	1 (0.9)	1 (0.7)
HIV status (n=140)	Positive (%)	2/34 (5.9)	5/106 (4.7)	7 (5.0)
Previous TB treatment reported by family	Yes (%)	1 (2.9)	11 (9.9)	12 (8.2)
Weight-for-age z-score (n=142)**	Mean (SD)	0.07 (1.49)	-0.63 (1.36)	-0.46 (1.42)
Relationship of source case to child	Mother (%)	14 (40.0)	36 (32.4)	50 (34.3)
	Father (%)	2 (5.7)	18 (16.2)	20 (13.7)
	Grandparent (%)	5 (14.3)	15 (13.5)	20 (13.7)
	Aunt/uncle (%)	7 (20.0)	30 (27.0)	37 (25.3)
	Other (%)	7 (20.0)	12 (10.8)	19 (13.0)
Was the source case the primary caregiver?	Yes (%)	10 (28.6)	30 (27.0)	40 (27.4)
Most intense exposure between child and source case	Sleeps in different house (%)	2 (5.7)	16 (14.4)	18 (12.3)
	Sleeps in same house (%)	19 (54.2)	43 (38.7)	62 (42.5)
	Sleeps in same room (%)	7 (20.0)	19 (17.1)	26 (17.8)
	Sleeps in same bed (%)	7 (20.0)	33 (29.7)	40 (27.4)

HIV: human immunodeficiency virus; IQR: inter-quartile range; SD standard deviation
*Difference between Khayelitsha and the other seven sub-districts: p<0.001
**Difference between Khayelitsha and the other seven sub-districts: p=0.012

Study 6: non-attendance at clinic appointments in child contacts of multidrug-resistant tuberculosis

The following study has been accepted as an article:

- *Zimri K, Hesseling AC, Godfrey-Faussett, Schaaf HS, Seddon JA. Why do child contacts of multidrug-resistant tuberculosis not come to the assessment clinic? Pub Heath Action 2012; 2: 71-75*

Introduction

Following from the previous section which determined that only a small proportion of the eligible child contacts are identified and referred to specialist assessment, this study continues to explore reasons for this. In the paediatric TB literature few studies have quantified the proportion of eligible child contacts brought for assessment following exposure to a case of infectious drug-susceptible TB.[452-453, 457-459] Few studies have examined reasons for non-attendance. Children may not be identified or they may be identified but then not brought to clinic appointments. In other healthcare contexts, the reasons for failure to attend paediatric clinic appointments are complex but include logistical and financial aspects, parental educational status and the attitudes of the parents towards the child, including perceptions regarding the importance of the disease.[460-461] The attrition for child TB contacts appears to occur at every step in the identification and referral cascade.[453] This study aimed to determine potential reasons for clinic non-attendance amongst child contacts of MDR-TB cases, using qualitative and quantitative methodology.

Methods

Study design

The aim of the study was to determine whether there were differences between the children brought for assessment to DR-TB specialist clinics and those not brought. Whilst it was postulated that factors such as distance and cost may be important, it was felt that an initial focus group discussion would be useful to identify potential key variables which could then be examined in a quantitative case-control study.

Focus group discussion

Parents/caregivers were purposively sampled to create a focus group of ten people,[462] to include a mix of genders, ages, residential locations, ethnicity and whether they had brought their children to appointments. The discussion took place on 5th August 2011 and lasted 90 minutes. The semi-structured session was facilitated by KZ to cover a series of broad topics but with open-ended discussion encouraged between participants. The session was recorded, transcribed and translated where needed. The transcript was analysed by KZ and JAS, using standard ethnographic techniques, to determine themes and concepts that led to the design of questionnaires.[463-464]

Study population and inclusion

From the 1st September 2011 a register was created of all children (<5 years or <13 years if HIV-infected) referred to the DR-TB clinic at TCH or outreach clinic, who had been referred as a well child, in significant contact with an infectious case of pulmonary MDR-TB (sputum smear or culture positive) within the preceding six months. This register was compiled from the telephone referrals. The first 50 children who had been referred and who subsequently attended their clinic appointments were recruited following written informed consent from their parent/caregiver (assent in children over seven years of age). Only the first child referred from a household was eligible for inclusion. The first 50 children who had been referred but who failed to attend their clinic appointment were identified, traced and also recruited following consent/assent. Once recruited, a structured interview was conducted with the parents/caregivers. All interviews were conducted by a study nurse (KZ; English and Afrikaans speaking) and research counsellor (English and Xhosa speaking) who asked questions in a standardised manner following training. If the participant did not understand the question, it was repeated, where necessary with explanation from the interviewer. Questionnaire fields included demographics of the household and source case, the logistical and financial implications of attending clinic appointments, together with perceptions of MDR-TB.

Living standards measure

Parents/caregivers were asked a series of questions to determine their assets and disposable income. A well-established market segmentation tool, the Living Standards Measure, has been used widely in South Africa since 1989, devised and subsequently revised, by the South African Advertising Research Foundation.[465] The results from 27 variables are used to create a 'score' from 1 to 14 which reflects the standard of living in a household.

Data classification and analysis

Data were analysed using STATA version 11; missing data were excluded from analysis. Associations were assessed using the χ^2 (or Fisher's exact) test with the effect estimated (OR) and 95% CI calculated. The Mann Whitney test was used to assess associations between non-parametric data, with median and IQR calculated.

Results

Focus group discussion

From the focus group discussion, a number of themes emerged. Some were associated with the physical challenges of getting a child to an appointment.

"The local clinic is easier to go to but to go to Tygerberg Hospital is sometimes difficult sometimes to get there because of money we don't have."
"The weather plays a role if you must go to the MDR clinic because you must wait at the taxi rank or bus stop and sometimes take two to three rides to get there."

Other themes that emerged included the attitude of clinic staff.

"The sisters at the clinic take sometimes very long to give the letter."
"Just like yesterday we were sitting the whole day for the referral letter."
"I just feel some of the staff at the clinic is inexperienced."
"The sister gave us the wrong letter and when we went back to say it is the wrong letter they were more aggressive than we were supposed to be that had to come back for the right letter."

Other concerns were about the appointment itself.

"I feel uncomfortable because my child is very small and some adults – I could hear how they say that some of them don't take their medication. This one man said today, 'I didn't take my medication for more than two weeks'. It would be much better if they could maybe thinking of putting up a mobile clinic for either the adults or the children to see them separately."
"Your first time, you wait very long because of the file."
"I had sleepless nights when I first heard I must take my child to the clinic, I even thought my child is going to die; I didn't know what the doctor is going to say"

Finally, some parents/caregivers felt that personal elements affected whether children were brought to appointments.

"I feel some parents just don't take their children to the clinic because they just don't care. They don't take their children's health seriously".
"Some parents are just plain lazy! They don't want to get up from bed to go to the clinic".
"The other reason is also that some parents found it very difficult to get off from work."
"I think a lot of business people or employers is not informed about the disease"

Quantitative study
Of the first 56 children referred who attended, 50 were included. Of the six not included, three were too old (over five but HIV-negative), one child presented with TB disease and two families left the clinic before the study team could approach them. Of the first 58 children who were referred but who did not attend, 50 were included. Of the eight not included, five were too old, one had moved to a different province and two could not be traced.

Significant risk factors for non-attendance included ethnicity (Coloured vs. Xhosa; OR: 2.82; 95%CI: 1.21-6.59; p=0.01) the mother being the TB source case (OR: 3.78; 95%CI: 1.29-11.1; p=0.02) and cigarettes smoked in the house (OR: 2.37; 95%CI: 1.01-5.57; p=0.04). (Table 23) There were significant logistical and financial differences, including time taken to get to the DR-TB clinic (45 vs. 60 minutes; p=0.002) and cost of transport (18.5 vs. 40 SA Rand; p=0.03). Of those not attending specialist clinic appointments, more had to use multiple minibuses (OR: 3.08; 95%CI: 1.28-7.41; p=0.008). (Table 24)

Families not bringing their children to appointments were more concerned about infection risk whilst waiting to be seen (OR: 2.45; 95%CI: 1.07-5.60; p=0.03). (Table 25) Families failing to attend DR-TB appointments were more likely to feel that they had to wait a long time to be seen at the local clinic (OR: 2.47; 95%CI: 1.07-5.69; p=0.03).

Discussion

As far as can be determined, this is the first study to examine reasons for non-attendance of child contacts of MDR-TB cases. A focus group discussion was conducted to determine appropriate questions to examine quantitatively in a systematic sample of children. Children not brought to appointments were more frequently of Coloured ethnicity and lived in families containing smokers. If the mother was the person with TB, the child was less likely to be

brought. For those attending DR-TB clinic appointments, travel times were shorter, cheaper and required fewer transport changes. Those attending were less concerned about infection risk while waiting to be seen at the DR-TB clinic and were made to wait less at their local clinic.

The reasons for the association between ethnicity and attendance are complicated and may be a surrogate for other socio-economic and cultural characteristics. While details regarding employment, education and living standard were captured, the complex social and cultural implications of ethnicity and lifestyle were not fully investigated. The reason for children being brought less frequently if the mother was the source case may be more easily explained. Mothers were the main carer for the child in the majority of instances and if the mother was unwell or hospitalised, access to evaluation for the child was impaired. Smoking may also be a surrogate for other socio-economic or cultural factors, or it may be that smokers have less money available for transport or feel stigmatised interacting with healthcare services.

Although it is not surprising that fewer children were brought to clinic appointments if the journey was long, expensive or complicated, it is interesting that the Living Standard Measure or education of the parent did not differ between the two groups. Also of note, attendance appeared to be more influenced by the attitudes of staff at local clinics than staff at the DR-TB clinic. This reinforces the significance of quality local care to inform and explain the importance of attending appointments as well as to educate children and their families about the disease.

It is also important to note parental perceptions of MDR-TB. Concerns that either they or their child may be exposed to MDR-TB whilst waiting to be evaluated at either the local or DR-TB clinic may be appropriate; significant rates of hospital acquired infections have been suggested in previous high-profile outbreaks.[466] Even if not justified, such perceptions are important determinants of non-attendance. Consideration should be given to infection control practices and in having children attend local clinic appointments at a separate time or space from adults. Parents/caregivers should also be screened for symptoms when they bring children to appointments to avoid the risk, or the perception of risk, of exposure. Perceptions regarding the danger of MDR-TB disease and its treatment also need to be explored and addressed, as do attitudes to MDR-TB and discrimination against those with MDR-TB. This would include education for both healthcare workers as well as the community.

This observational study employs a combination of qualitative and quantitative research techniques to examine a complex social issue regarding determinants of human behaviour influencing access to health care. The study examines an important topic affecting a vulnerable

and marginalised group. Limitations to the study include the relatively small sample, which may have obscured true associations. The retrospective nature of the study may have allowed recall bias to influence responses from the non-attendees who may have wanted to justify their decisions not to attend. Also, families were only examined in which the child had been identified and referred to the DR-TB clinic. The previous study in the thesis demonstrated that only a small proportion of child contacts of MDR-TB accessed specialist assessment; reasons for non-identification of child contacts are not explored. Finally, children exposed to MDR-TB have not been compared with children exposed to drug-susceptible TB. Some of the issues identified in this study may be specific to MDR-TB but some may be common to all children exposed to TB.

Table 23 - Characteristics of children, households, main carers and source cases of children referred as contacts of multidrug-resistant tuberculosis

	Not attending	Attending	OR (95%CI)	p-value
Median age of child in months (IQR)	35 (25-51)	36 (23-53)	-	0.35
Male gender of child (%)	26 (52)	22 (44)	1.38 (0.62-3.05)	0.43
Coloured ethnicity (%)	30 (60)	17 (34)	2.82 (1.21-6.59)	0.01
Child HIV-infected (%; n=88)	3/40 (7.5)	1/48 (2.1)	3.81 (0.37-39.4)	0.33
Mother main carer for child (%)	44 (88)	41 (82)	1.61 (0.52-4.97)	0.58
Median number of years of education of main carer (IQR)	10 (8-11)	10 (8-11)	-	0.35
Main carer without any paid work (%)	34 (68)	35 (70)	0.91 (0.39-2.14)	0.83
Main carer looks after other children (%)	20 (40)	29 (58)	0.48 (0.21-1.09)	0.07
Male gender of main carer (%)	2 (4)	6 (12)	0.31 (0.06-1.64)	0.27
Male gender of source case (%)	19 (38)	25 (50)	0.61 (0.27-1.37)	0.23
Mother source case (%)	17 (34)	6 (12)	3.78 (1.29-11.1)	0.02
Median LSM score of household (IQR)	6 (6-8)	7 (6-8)	-	0.29
Cigarettes smoked in house (%)	36 (72)	26 (52)	2.37 (1.01-5.57)	0.04
Alcohol drunk in house (%)	27 (54)	27 (54)	1.00 (0.45-2.20)	0.80
Illegal drug use in house (%)	10 (20)	9 (18)	1.14 (0.42-3.11)	1.00

IQR: Inter-quartile range; LSM: Living standard measure; OR: Odds ratio; CO: Confidence interval

Table 24 - Financial and travel implications of accessing care for child contacts of multidrug-resistant tuberculosis

	Not attending	Attending	OR (95% CI)	p-value
Median distance to DR-TB clinic in km (IQR; n=82)	5 (4-8)	6 (2-14)	-	0.77
Median number of minutes taken to travel to DR-TB clinic (IQR; n=93)	60 (45-90)	45 (25-60)	-	0.002
Median cost of travel to DR-TB clinic in SAR (IQR)	40 (20-60)	18.5 (4-50)	-	0.03
More than one minibus taxi required to get to DR-TB clinic (%)	26 (52)	13 (26)	3.08 (1.28-7.41)	0.008

DR-TB: drug-resistant tuberculosis; IQR: Interquartile range; SAR: South African Rand; OR: Odds ratio; CO: Confidence interval

Table 25 - Perceptions of disease amongst parents/caregivers of children referred as contacts of multidrug-resistant tuberculosis

Positive responses to the following questions:	**Not attending**	**Attending**	**OR (95% CI)**	**p-value**
Do you have confidence in the medical staff at your local clinic?	33 (66)	41 (82)	0.43 (0.16-1.10)	0.07
Do you have confidence in the medical staff at the MDR-TB clinic?	48 (96)	49 (98)	0.49 (0.04-5.67)	1.00
Does the weather affect your decision on whether to attend appointments at the MDR-TB clinic?	16 (32)	13 (26)	1.34 (0.56-3.21)	0.51
Do you consider MDR-TB a disease that can kill you?	43 (86)	38 (76)	1.94 (0.68-5.50)	0.31
Do you consider MDR-TB a disease that can be treated successfully?	46 (92)	50 (100)	-	0.12
Do you think that people in your community with MDR-TB are discriminated against?	24 (48)	25 (50)	0.92 (0.42-2.03)	0.84
Do you feel that employers in your community discriminate against people with MDR-TB?	37 (74)	28 (56)	2.24 (0.94-5.30)	0.06
Are you concerned about the risk of being infected with MDR-TB while waiting at the MDR-TB clinic?	30 (60)	19 (38)	2.45 (1.07-5.60)	0.03
Do you think that your child would take anti-TB medicines every day without a problem?	27 (54)	34 (68)	0.55 (0.24-1.26)	0.15
Are you concerned about the side effects of the anti-TB medicines for the child?	30 (60)	24 (48)	1.63 (0.73-3.62)	0.23
Do you feel that you have to wait a long time to be seen at your local clinic?	28 (56)	17 (34)	2.47 (1.07-5.69)	0.03
Do you feel that you have to wait a long time at the MDR-TB clinic?	11 (22)	10 (20)	1.13 (0.43-2.97)	0.81
Do you think that parents should be responsible for preventing children from getting MDR-TB?	46 (92)	45 (90)	1.28 (0.32-5.11)	1.00
Out of ten, for you how important a priority is having your child assessed in the MDR-TB clinic? (Median [IQR])	10 (10-10)	10 (10-10)	-	0.37

MDR-TB: multidrug-resistant tuberculosis; IQR: Interquartile range; OR: Odds ratio; CO: Confidence interval

Study 7: tolerability and toxicity of preventive therapy for child contacts of multidrug-resistant tuberculosis

The following study has been submitted as an article:

- *Seddon JA, Hesseling AC, Finlayson H, Schaaf HS. Toxicity and tolerability of multidrug-resistant tuberculosis preventive treatment in children. (submitted)*

The next two studies in the thesis assess preventive therapy for child contacts of MDR-TB. As mentioned in the section describing context, the provincial policy in the Western Cape is that children who have had significant contact with an infectious case of MDR-TB (pulmonary sputum- or smear-positive microscopy) are provided with preventive therapy for six months irrespective of TST result. The first study describes the tolerability and toxicity of the drugs and the second describes the outcome of children given this regimen.

Introduction

One of the major concerns regarding the provision of preventive therapy in children using drugs other than isoniazid, is potential toxicity. In the treatment of MDR-TB disease, the risk-benefit ratio of potentially toxic therapy is relatively clear. In contrast, this risk-benefit ratio is altered when using potentially toxic preventive therapy in children who are currently well, but are at risk of developing disease in the future.

Suggested medications for MDR-TB preventive therapy in children include the fluoroquinolones, ethambutol, pyrazinamide and ethionamide/prothionamide.[87] Isoniazid, given at a high dose (15-20mg/kg daily), can also be used as some isolates have low-level isoniazid resistance.[90, 92] There has been concern regarding the use of ethambutol in children due to the difficulties in testing for optic nerve toxicity. When given at the dosage now advised (15-25mg/kg daily) this is rare, occurring in less than 0.1% of cases.[167] Caution has been exercised regarding the use of the fluoroquinolones in children, based on early animal model data showing damaging effects to the cartilage growth of young beagles.[164] With extensive paediatric use of fluoroquinolones, mainly in children with cystic fibrosis, few adverse events have however been reported to date.[334-335] Pyrazinamide has been shown to be associated with significant hepatotoxicity in adults when given as preventive treatment [156, 158] while ethionamide/prothionamide commonly cause nausea and vomiting[467] and are associated with

hypothyroidism in children.[262] There are limited published data regarding the tolerability and toxicity of preventive therapy regimens given to children exposed to MDR-TB.

Methods

Standard of care

Following exclusion of TB disease through history, examination and plain film chest radiology, children who were less than five years of age or HIV-infected, with significant exposure to an infectious case of MDR-TB, were given a regimen of MDR-TB preventive therapy, as advised by provincial guidelines. Significant exposure was defined as living with or having regular daily interaction with the MDR-TB source case. If the source case had MDR-TB with susceptibility to the fluoroquinolones, the child was given ofloxacin (15-20mg/kg daily; 200mg tablets: Tarivid, Sanofi-Aventis, Midrand, South Africa; 400mg tablets: Tafloc, Aspen Pharmacare, Durban, South Africa), ethambutol (20-25mg/kg daily; 400mg tablets; Sandoz Ethambutol HCl 400, Sandoz SA [Pty] Ltd, Kempton Park, South Africa) and isoniazid (15-20mg/kg daily; 100mg tablets; Be-Tabs Isoniazid; Be-Tabs Pharmaceuticals [Pty] Ltd, Roodepoort, South Africa) for 6 months. Ofloxacin was the only fluoroquinolone available in the National TB Programme during the study period. Children exposed to MDR-TB resistant to ofloxacin were given only high-dose isoniazid (15-20mg/kg daily) for 6 months. Children were routinely evaluated at two, four, six and twelve months, at the TCH or the community outreach sites, when clinical evaluation and chest radiography were completed. Drugs were dispensed at local community TB clinics where each week parents were provided with seven days of treatment for the child. The parents were then responsible for the daily delivery of medications as tablets or divisions of tablets.

Study population and eligibility

All children routinely evaluated at the TCH or community outreach clinic, were eligible if they had been in significant contact with an infectious case of pulmonary MDR-TB (sputum smear or culture positive) within the preceding six months, had started preventive therapy during May 2010 through April 2011, and had attended at least one follow up appointment. Children were recruited following written, informed consent from the parent/caregiver.

Data collection

Children were routinely seen at two, four and six months; details of any additional, unscheduled consultations were also recorded. Parents/caregivers were interviewed concerning potential drug-related adverse events using a structured questionnaire. Adverse

events were categorised using the DMID system.[415] (Table 26). As arthralgia is not categorised in this classification we allocated five grades consistent with the classification system. If old enough to co-operate, visual toxicity was evaluated with Ishihara charts. Parental impression of visual function was used for children who could not be evaluated in this way.

Data analysis

The most severe grade for each category of adverse event, cumulatively experienced during the six month preventive therapy regimen, was determined. Missing data were excluded from analysis. For analysis, children were categorised into those that experienced only Grade 0 and Grade 1 adverse events and those that experience any Grade 2 or higher adverse event. Patient and treatment characteristics were assessed to determine potential association with toxicity. Age was categorized into those less than two years and those older, based on the age distribution of the sample, and weight-for-age z-scores were divided into those less than -1 standard deviations below the reference population and those greater than -1. Data were analysed using STATA version 11. Associations were assessed using the χ^2 (or Fisher's exact) test and effect estimates (OR) and 95% CI were calculated.

Results

Two hundred and forty-five children were initially eligible; 193 were included. In the 52 children not included, the child was not brought to clinic by an adult who could legally provide consent (n=12), consent was not given (n=2), the child was not brought back for follow up (n=37) or the source case was subsequently found not to have MDR-TB (n=1). The median age was 31 months (IQR: 13-45). The mean weight-for-age z-score was -0.55 standard deviations (SD) from the reference population mean (SD: 1.44). One hundred and two (53.1%) were boys; 9 (4.6%) were HIV-infected and 83 (43.2%) were of Xhosa ethnicity. One hundred and seventy three children (89.6%) were given three drugs for preventive therapy and 20 (10.4%) received isoniazid alone, based on the susceptibility pattern of the adult source case. (

Table 27)

Adverse events are demonstrated in Table 28, with all Grade 3 adverse events shown in Table 29. Of the seven children (3.6%) who experienced a Grade 3 adverse event, three were associated with inadvertent overdosing of ofloxacin. No adverse events necessitated the discontinuation of preventive therapy and all resolved without intervention. The most common Grade 2 or higher adverse events were loss of appetite and nausea (12 children; 6.2%), itchy skin (9 children; 4.7%), disturbance of sleep or mood (7 children; 3.6%) and skin rash (7 children; 3.6%). Risk factors for the development of Grade 2 or more severe adverse events are shown in Table 30. No clinical or treatment characteristics were associated with cumulative adverse events, including HIV infection and concomitant treatment with antiretroviral therapy.

Discussion

This study demonstrates that a regimen of ofloxacin, ethambutol and high-dose isoniazid, given as preventive therapy for children exposed to MDR-TB, is well tolerated and associated with few clinically significant adverse events. The three cases of Grade 3 toxicity associated with inadvertent overdosing of ofloxacin is concerning. In South Africa, at the time the study was conducted, ofloxacin was available in two formulations: 200mg and 400mg. Medications are frequently dispensed as loose tablets within a re-sealable packet with the number of tablets to be taken written on outside. If the packet is refilled with a different strength of tablet, confusion can occur, as was reported for the children in this study. Attention to correct dispensing is essential, both to achieve optimal efficacy and minimise toxicity. Only one Grade 2 or higher episode of joint, muscle or bone pain was noted. Given the historical concerns regarding fluoroquinolone use in children, these findings are reassuring. No hepatotoxicity or visual problems were observed, which is encouraging.

There is limited published evidence regarding clinically significant toxicity for preventive MDR-TB therapy in adults or children. To our knowledge, there are no published studies reporting the systematic evaluation and grading of toxicity of MDR-TB preventive regimens in children. One study described a cohort in which some of the children were given preventive therapy for MDR-TB exposure (n=41) and some were given MDR-TB treatment (n=25).[21] Ethionamide was associated with gastrointestinal adverse events in 49%, necessitating cessation of the drug in four cases. One child developed arthralgia thought to be likely due to ofloxacin, which led to drug discontinuation. In another study, 8 of 22 (24%) children experienced adverse events

thought to be attributable to MDR-TB preventive therapy.[144] Gastrointestinal toxicity was the most common, with two experiencing behavioural changes and one an itchy rash. Two patients had elevated liver function tests necessitating discontinuation of therapy. A systematic review and meta-analysis of children treated for MDR-TB disease showed that 39% of children experience adverse events,[221] but there were limited data regarding the severity of adverse events. Fluoroquinolones, used for indications other than MDR-TB, appear to be well tolerated and have limited toxicity.[334-335] However, treatment duration is usually much shorter for indications other than for MDR-TB preventive therapy. The toxicity of ethambutol (15-25mg/kg daily) and isoniazid (5-10mg/kg daily) have been well studied at the recommended dosages for the treatment of drug-susceptible TB[210] but no studies have evaluated the toxicity of isoniazid given at a high dose in children on treatment of MDR-TB. In children treated for TB meningitis, isoniazid given at high dose (15-20mg/kg daily) was not found to be associated with hepatotoxicity, even when given with three other potentially hepatotoxic drugs (rifampicin, pyrazinamide and ethionamide).[468]

This study has strengths and limitations. A prospectively recruited cohort of children exposed to MDR-TB have been characterised who have received routine preventive therapy. Adverse events were systematically documented and categorised and potential risk factors for toxicity were assessed. Nearly two hundred children were followed over six months, providing nearly one hundred years of patient follow-up time. Toxicity evaluation was elicited by discussion with parents/caregivers who reported perceived adverse events and by clinical evaluation of the children. As the children were mostly less than five years of age it was not possible to elicit symptoms directly from them. Furthermore, routine biochemical or radiological monitoring for potential toxicity was not completed, leading to a possible reduced detection of abnormal liver function or joint abnormality. As detailed ophthalmological examinations were not performed on all children it is also possible that subtle visual changes were missed. Determining the potential cause of toxicity in children on multiple concomitant medications can be challenging. To be certain that a specific drug is responsible, the child should be only receiving one drug, or, if being given a multidrug regimen, all drugs should be stopped and sequentially restarted. As preventive therapy was usually multidrug and was continued in spite of minor toxicity, determining the cause of specific adverse events was not possible. This is particularly important for children concomitantly treated for HIV with cART where multiple medications are given together. That only one child with HIV (out of nine) developed a Grade Two adverse event is reassuring.

Table 26 - Classification of adverse events used in children receiving multidrug-resistant tuberculosis preventive therapy

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Joint, muscle or bone pain	No adverse event	Pain but no interference with function or movement	Moderate pain, affecting function but able to carry out normal activities	Severe pain limiting activities	Disabling pain – unable to carry out activities
Skin Rashes	No adverse event	Small areas of redness /rash	Dry peeling or widespread rash	Wet peeling, ulcers or urticaria	Severe, widespread rash, necrosis needing hospitalization
Itchy skin	No adverse event	Slight itching in localised areas	Severe itching in localised areas	Widespread itching over entire body	Uncontrollable scratching needing hospitalization
Headache	No adverse event	Mild – does not need treatment	Transient/moderate – needs non-narcotic treatment	Severe –responds to narcotics	Intractable pain
Sleeping/mood	No adverse event	Mild anxiety	Moderate anxiety or problems getting to sleep	Severe anxiety, problems getting to sleep or repeated waking	Psychosis, unable to sleep for more than an hour
Lethargy	No adverse event	Activity Reduced but for <48 hours	Slightly irritable or slightly subdued	Very irritable or lethargic	Inconsolable or obtunded
Visual problems	No adverse event	None	Blurred vision or minor visual disturbance lasting less than 1 hour	Repeated episodes of blurring or visual disturbances which resolve	Permanent decrease in visual acuity or field defect
Vomiting	No adverse event	1 episode in 24 hours	2-3 episodes in 24 hours	4-6 episodes in 24 hours	>6 episodes in 24 hours or needing hospitalization
Diarrhoea	No adverse event	Slight change in consistency or frequency of stool	Liquid stool	Liquid stool >4x normal frequency for child	Liquid stool >8x normal frequency for child
Jaundice	No adverse event	Jaundice detectable clinically – bilirubin 1.1 - 1.5 x ULN	Obvious clinical jaundice – bilirubin 1.6 – 2.5 x ULN	Severe jaundice – bilirubin 2.6 – 5 x ULN	Hospitalization – bilirubin >5x ULN
Loss of appetite/nausea	No adverse event	Mild – still eating/drinking well	Moderate - decreased appetite	Severe – little food taken	No solid or liquid food taken

ULN: Upper limit of normal (as determined by reference range for age of child and assay used)

Table 27 - Characteristics of children given preventive therapy as contacts of multidrug-resistant tuberculosis (n=193)

Characteristic		**Number**
Median age (IQR; n=191)		31 (13-45)
Male gender (%; n=192)		102 (53.1)
HIV-infected (%)		9 (4.6)
Mean weight-for-age z-score (SD; n=186)		-0.55 (1.44)
Regimen (%)	Isoniazid, ethambutol, ofloxacin	173 (89.6)
	Isoniazid	20 (10.4)
Ethnicity (%)	Xhosa	83 (43.2)
	Coloured	108 (56.8)
	Other	2 (1.0)

IQR: Inter-quartile range; SD: Standard deviation

Table 28 - Summary of cumulative most severe adverse event in children receiving six months of multidrug-resistant tuberculosis preventive therapy (n=193)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Total
Joint, muscle or bone pain*	183	5	1	0	0	189
Skin Rashes	144	42	6	1	0	193
Itchy skin	151	33	8	1	0	193
Headache*	155	3	2	0	0	160
Sleeping/mood	177	9	4	3	0	193
Lethargy	190	3	0	0	0	193
Visual problems	193	0	0	0	0	193
Vomiting	161	31	1	0	0	193
Diarrhoea	174	18	1	0	0	193
Jaundice	193	0	0	0	0	193
Loss of appetite/nausea	164	17	10	2	0	193

*Total not 193 as some parents stated that they could not determine if the child had experienced the adverse event

Table 29 - Characteristics of children developing Grade 3 adverse events (n=7) on MDR-TB preventive therapy

Age (months)	Gender	HIV status	Regimen	Adverse event(s)	Details
38	Girl	Negative	HEO	Insomnia	Mother reported severe insomnia at the two month appointment but stated that the problem had been evident prior to starting the preventive therapy Preventive therapy continued and sleeping improved
21	Boy	Negative	H	Skin rash and itch	Child had eczema prior to starting preventive therapy Rash and itching started four days after preventive therapy Preventive therapy continued and rash resolved within two weeks
25	Boy	Negative	HEO	Insomnia and hallucinations	At the two month appointment child reported not sleeping due to hallucinations Child had been prescribed 300mg ofloxacin but inadvertently given 600mg by clinic staff Preventive therapy continued at correct dose and symptoms resolved
45	Boy	Negative	HEO	Insomnia	At the two month appointment the mother reported that the child did not sleep at all Child had been prescribed 300mg ofloxacin but inadvertently given 600mg by clinic staff Symptoms resolved on correct dosage
6	Girl	Negative	HEO	Loss of appetite	Appetite reported as normal at the two month appointment, Grade 2 at the four month appointment and Grade 3 at the six month appointment Mother reported that some of the loss of appetite might have been due to teething
38	Girl	Negative	HEO	Insomnia and hallucinations	At the two month appointment the mother reported the child to be having hallucinations and sleep problems Child had been prescribed 300mg ofloxacin but inadvertently given 600mg by clinic staff Symptoms resolved on correct dosage
14	Boy	Negative	HEO	Loss of appetite	Child reported to have Grade 3 appetite loss at two months, then none at 4 months but Grade 1 at six months Preventive therapy continued throughout

H: isoniazid; E: ethambutol; O: ofloxacin

Table 30 - Risk factors for the development of Grade 2 or greater adverse events in children on MDR-TB preventive therapy (n=193)

	Total	Grade 0 or 1	Grade 2 or 3	OR (95%CI)	P-value
Age < 2 years	73/191	62	11	1.22 (0.52-2.83)	0.65
Male gender	102/192	89	13	0.87 (0.38-1.98)	0.73
HIV-infected	9/193	8	1	0.80 (0.09-6.67)	0.83
Weight-for-age z-score <-1	72/186	62	10	0.99 (0.42-2.32)	0.98
Xhosa ethnicity	83/191	72	11	1.03 (0.44-2.40)	0.95
Three drug regimen	173/193	149	24	1.45 (0.31-6.69)	0.63

OR: Odds ratio; CI: Confidence Interval

Study 8: preventive therapy for child contacts of multidrug-resistant tuberculosis

The following study has been prepared as an article:

- *Seddon JA, Hesseling AC, Fielding K, Cox H, Hughes J, Godfrey-Faussett P, Schaaf HS. Preventive treatment for child contacts of MDR-TB (in preparation)*

Introduction

This study describes a cohort of children who were given a multidrug preventive therapy regimen following exposure to an adult with MDR-TB. The study describes the children as well as exploring risk factors for poor outcome.

Methods

Standard of care

As described earlier, following exclusion of TB disease through history, examination and plain film chest radiology, children who are less than five years of age or HIV-infected, with significant exposure to an infectious case of MDR-TB, are given a regimen of MDR-TB preventive therapy, irrespective of TST result, as advised by provincial guidelines. Significant exposure is defined as living with or having regular daily interaction with the MDR-TB source case. If the source case has MDR-TB with susceptibility to the fluoroquinolones, the child is given ofloxacin (15-20mg/kg daily), ethambutol (20-25mg/kg daily) and isoniazid (15-20mg/kg daily) for 6 months. Children exposed to MDR-TB resistant to ofloxacin are given only high-dose isoniazid (15-20mg/kg daily) for 6 months. Children are routinely evaluated at two, four, six and twelve months, at the TCH or the community outreach sites, where clinical evaluation and chest radiography are performed. Drugs were dispensed at local community TB clinics, on either a daily, weekly or monthly basis, dependent on clinic and patient preference. HIV testing is offered to all TB contacts following informed consent from the parent or legal guardian, with assent from the child where appropriate, using ELISA in children older than 18 months or DNA PCR if younger or breast-fed. cART is routinely initiated in all HIV-positive children following appropriate evaluation. TST was completed by injecting two tuberculin units intradermally (purified protein derivative RT23, Statens Serum Institute) with results read at 48-72 hours.

Study population and eligibility

All children evaluated at the TCH or community outreach clinic were eligible if they had been in significant contact with an infectious case of pulmonary MDR-TB (sputum smear or culture positive) within the preceding six months and had started preventive therapy from May 2010 through April 2011.

Data collection

Children were seen in clinic at two, four, six month and 12 months, as well at any additional, unscheduled visits. In addition, during the first half of 2012, children were traced and either recalled to clinic or visited at their local clinic or home by the study team. Where this was not possible, local clinics and families were contacted to confirm that the child was clinically well and were putting on weight. The date of this final interaction was recorded. Follow-up time for the children, therefore, was a minimum of 12 months but up to 24 months. Adherence was measured in three ways with equal weighting given to each in determining overall adherence. The first was three day recall, the second a 30 day visual analogue score and the third confirmation from the local clinic to confirm medication uptake.[469-472] Adherence was divided into those with good adherence (≥80% of doses given) and those with poor adherence (<80% doses given).[473] Study outcomes were: well at the end of the observation period, death of any cause, incident TB disease and lost to follow up. Standardized research definitions were applied to classify incident TB disease.[405] Children with confirmed and probable disease were included. Where patients were lost to follow up their censure date was recorded as the last interaction with the study team.

Data analysis

Data were dual-entered and checked for entry errors. Data were analyzed using STATA software (version 11; Stata Corp, College Station, TX). Cohort analysis was undertaken to examine the rate of cohort failure for different exposures. Incident TB disease and death were classified as outcome failures. Time into the cohort was the date of recruitment and time out of the cohort was the date of death, diagnosis of incident TB, date last seen when lost to follow up or date last seen when well. Due to the small number of cohort failures, exact Poisson analysis was undertaken for a small number of predetermined characteristics of the child and treatment, with rate ratios (RR), 95% CI and p-values calculated.

Results

Of 245 children initially eligible, 215 were included, contacts of 173 MDR-TB source cases. Of the children not included, 12 were brought by an adult who could not provide legal consent, for two children consent was not given and in the remainder (n=16), although the source case was said to have MDR-TB at the initial assessment and the child given MDR-TB preventive therapy, on contacting the clinic, the adult was confirmed to have resistance to only either isoniazid or rifampicin. For the children included, median age was 31 months (IQR: 13-24), and 116 (54%) were boys (Table 31). Mean weight-for-age z-score was 0.64 standard deviations below the reference population and of 207 children tested for HIV, 10 (4.8%) were positive. Children received either a regimen of isoniazid, ethambutol and ofloxacin (n=192; 89%) or isoniazid alone (n=23; 11%) and adherence was good in 165 (77%) children.

The median age of source cases was 32.5 (IQR: 26-40) and 71 (42%) were male. Of 170 (98%) tested for HIV, 59 (35%) were positive. Of 167 with DST to ofloxacin, 11 (7%) were resistant. Ninety-nine (62%) out of 170 with recorded smear results, had 2+ or 3+ sputum smear microscopy results (Table 32).

One child died (0.5%), seven developed incident TB (3%) and four (2%) were lost to follow-up (Table 33). In cohort analysis 248.6 patient years of observation time were included. The rate for poor outcome was 32.2 outcomes (95%CI 16.1-64.3) per 1000 years of patient follow up. Risk factors for poor patient outcome (Table 34) were: HIV positivity (RR: 9.87; 95%CI: 0.97-55.2; p=0.05) and poor adherence (RR: 9.66; 95%CI: 1.73-97.9; p=0.006). Children older than 12 months were less likely to have poor outcomes (RR: 0.16; 95%CI: 0.002-0.81; p=0.02).

Discussion

This study demonstrates that following exposure to an MDR-TB source case and the provision of MDR-TB preventive therapy, in nearly 250 patient years of follow-up, one child died and seven developed incident TB. It is likely that the child that died did not develop TB but died of some other form of illness. Of the seven who developed incident TB, one was exposed to *M. tuberculosis* resistant to ofloxacin and five did not take the medications. Therefore, only two children who were exposed to ofloxacin-susceptible *M. tuberculosis*, who took a three-drug regimen with good adherence, developed TB. Significant risk factors for poor outcome included young age and HIV infection. These risk factors are well described in the drug-

susceptible pediatric TB literature[61, 63] and should prompt enhanced vigilance in these vulnerable populations.

The study was associated with some limitations. As this is an observational study, it is not possible to conclude with certainty that this regimen is effective. It is possible that only this number of children would have developed TB had they been given isoniazid or even no medications at all. However, the pre-chemotherapy data do not support this. In the absence of preventive therapy, 50% of *M. tuberculosis*-infected children less than twelve months developed TB disease.[61] The figure is 20-30% for children between one and two years and 5% for children between two and five years. Although only 40% of our cohort was TST positive, a far higher numbers of children would be expected to develop TB if the regimen was not effective. In addition, the evidence that poor adherence to preventive therapy was strongly associated with poor outcome adds support to the argument that this regimen is effective in reducing the risk of progression from infection to disease. This leads onto the next limitation, in that children were included irrespective of TST status, in line with national and provincial guidelines. The rationale for this is that TST is not a highly sensitive test for *M. tuberculosis* infection and by only including TST positive children a number of infected children are excluded. This is especially true of young and HIV positive children. Also, if a TST is negative at the time of initial assessment, there is a chance that the child may have been infected but is yet to mount an immune response. Rather than use a two stage protocol with all children started on preventive therapy which is then stopped if a second TST at two months is negative, the local policy is for all exposed children to be treated. These entry criteria were employed in this study. It could, therefore, be argued that some of the children in the study did not need to be treated. A final limitation of the study is the limited duration of follow up for the children. All children were followed up for a minimum of twelve months with some followed up for 24 months. Although the vast majority of children who are going to develop disease do so with this time period,[21, 60, 83] it is accepted that some children might progress to disease after the period of observation.

Table 31 - Characteristic of children exposed to a multidrug-resistant tuberculosis source case and placed on preventive therapy (n=215)

Characteristic		
Median age in months (IQR)		31 (13-45)
Gender (%)	Male	116 (54.0)
	Female	99 (46.1)
Ethnicity (%)	Coloured	120 (55.8)
	Xhosa	93 (43.3)
	White	1 (0.5)
	Other	1 (0.5)
Weight in cm (SD)		12.4 (4.3)
Height (SD; n=174)		90.6 (71.0)
Weight-for-age z-score (SD; n=211)		-0.64 (1.50)
Height-for-age z-score (SD; n=162)		-0.98 (1.38)
Weight-for-height z-score (SD; n=117)		0.15 (1.40)
TST (%; n=212)	Positive	85 (40.1)
	Negative	127 (59.9)
TST size in mm for those positive (IQR; n=72)		15.5 (13.5-20)
Evidence of BCG scar (%; n=210)	Yes	170 (81.0)
	No	40 (19.1)
Previous TB disease treatment (%)	Yes	17 (7.9)
	No	146 (92.1)
Previous preventive therapy (%; n=213)	Yes	67 (31.5)
	No	146 (68.5)
HIV (%; n=207)	Positive	10 (4.8)
	Negative	197 (95.2)
On ART at start of preventive therapy (%; n=10)	Yes	8 (80.0)
	No	2 (20.0)
Regimen given (%)	HEO	192 (89.3)
	H	23 (10.7)
Type of medication delivery (%)	Daily	28 (13.0)
	Weekly	157 (73.0)
	Monthly	21 (9.8)
	Other	9 (4.2)
Adherence (%)	Good	165 (76.7)
	Poor	50 (23.3)
Outcome (%)	Died	1 (0.5)
	Incident TB	7 (3.3)
	Well	203 (94.4)
	LTFU	4 (1.9)

SD: standard deviation; TST: tuberculin skin test; BCG: Bacille Calmette–Guérin; TB: tuberculosis; HIV: human immunodeficiency virus; HEO: isoniazid, ethambutol & ofloxacin, H: isoniazid; LTFU: lost to follow up

Table 32 - Characteristics of source case with multidrug-resistant tuberculosis (n=173)

Characteristic		
Median age source case in years (IQR; n=168)		32.5 (26-40)
Gender (%; n=171)	Male	71 (41.5)
	Female	100 (58.5)
Source case DST	Amikacin resistant (n=168)	15 (8.9)
	Ofloxacin resistant (n=167)	11 (6.6)
Smear result (%; n=170)	Negative	22 (12.9)
	Scanty	15 (8.8)
	1+	23 (13.5)
	2+	76 (44.7)
	3+	29 (17.1)
	Positive without smear recorded	5 (2.9)
HIV (%; n=170)	Negative	111 (65.3)
	Positive	59 (34.7)
Median CD4 count (IQR; n=56)		192 (103-350)
On ART at start of MDR-TB treatment (%; n=59)	No	38 (64.4)
	Yes	21 (35.6)
Current smoker (%; n=171)	No	103 (60.2)
	Yes	68 (39.8)
Regular alcohol use (%; n=171)	No	137 (80.1)
	Yes	34 (19.9)
Previous hospital admission (%; n=161)	No	114 (70.8)
	Yes	47 (29.2)
Previously in prison (%; n=166)	No	138 (83.1)
	Yes	28 (16.9)

IQR: inter-quartile range; DST: drug susceptibility test; HIV: human immunodeficiency virus; MDR-TB: multidrug-resistant tuberculosis

Table 33 - Mortality and incident tuberculosis in children given preventive therapy for multidrug-resistant tuberculosis (n=8)

Age	Gender	HIV status	Regimen	Source case	DST of source case	Time to outcome	Adherence	Details
3 months	Girl	Positive	HEO	Mother	MDR	9 months	Poor	Child defaulted preventive therapy when mother was admitted to hospital
12 days	Boy	Negative	HEO	Mother's cousin	MDR	3 weeks	Poor	Baby died after three weeks in what looked like sudden infant death syndrome. Preventive therapy not given at all
34 months	Girl	Negative	HEO	Mother	MDR	2 months	Poor	No preventive therapy given by parents at all
12 months	Girl	Negative	H	Aunt x2, Mother	XDR	1 month	Poor	No preventive therapy given by parents at all
51 months	Boy	Negative	HEO	Aunt	MDR	10 months	Good	
9 months	Girl	Positive	HEO	Mother	MDR	6 months	Poor	Poor adherence following the death of the mother
2 months	Boy	Negative	HEO	Mother	MDR	10 months	Poor	
10 months	Girl	Negative	HEO	Mother	MDR	2 months	Good	

HIV: human immunodeficiency virus; DST: drug susceptibility test; HEO: isoniazid, ethambutol & ofloxacin; H: isoniazid; MDR: multidrug-resistant; XDR: extensively drug-resistant

Table 34 - Assessment of risk factors for poor outcome (death or incident tuberculosis disease) in children exposed to multidrug-resistant tuberculosis and treated with a preventive therapy regimen (n=215)

		Number of events	Years of observation	Incidence rate with 95% CI (events per 1000 person years)	Rate Ratio (95% CI)	p-value
Age	0-12 months	5	51.6	97.0 (40.4-233.0)	1	-
	>12 months	3	197.0	15.2 (4.91-47.2)	0.16 (0.02-0.81)	0.02
Gender	Female	4	108.1	37.0 (13.9-98.6)	1	-
	Male	4	140.5	28.5 (10.7-75.9)	0.77 (0.14-4.13)	0.98
TST	Negative	4	149.6	26.7 (10.0-71.2)	1	-
	Positive	3	96.9	31.0 (10.0-96.0)	1.16 (0.17-6.84)	1.00
HIV status	Negative	6	229.5	26.1 (11.7-58.2)	1.0	-
	Positive	2	7.8	257.9 (64.5-1031.4)	9.87 (0.97-55.2)	0.05
Regimen	HEO	7	225.4	31.1 (14.8-65.2)	1	-
	H	1	23.2	43.0 (6.1-305.5)	1.39 (0.03-10.8)	1.0
Ofloxacin DST of source case	Susceptible	7	225.5	31.0 (14.8-65.1)	1	-
	Resistant	1	14.2	70.6 (9.9-500.9)	2.27 (0.05-17.7)	0.77
Adherence	Good	2	189.7	10.5 (2.6-42.2)	1	-
	Poor	6	58.9	101.8 (45.8-226.7)	9.66 (1.73-97.9)	0.006

CI: confidence interval; TST: tuberculin skin test; HEO: isoniazid, ethambutol & ofloxacin, H: isonizid; DST: drug susceptibility test

Study 9: culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment, and outcome

The following study has been published as an article:

- *Seddon JA, Hesseling AC, Willemse M, Donald PR, Schaaf HS. Culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment and outcome. Clin Infect Dis 2012; 54: 157-66*

The final five studies of the thesis concern the treatment of children with DR-TB. The first two are cohorts of children treated for MDR-TB, the first a cohort of culture-confirmed MDR-TB and the second including confirmed as well as presumptively treated children. The subsequent two studies involve specific body systems, namely MDR-TB meningitis and MDR-TB of the spine. The final study assesses the hearing loss of children being treated for MDR-TB.

Introduction

Treatment outcomes are generally poor in adults with MDR-TB, with favourable outcomes reported in only 60% of those receiving treatment.[190] Even though childhood TB comprises approximately 15-20% of the global TB burden,[17] MDR-TB is poorly studied in children, the literature including mainly case reports or small case series.[58, 89, 144, 191, 193-196, 204-205, 260, 474-475]

The diagnosis of TB in young children is challenging and often delayed.[476] Symptoms and signs may be non-specific, especially in children younger than three years of age and in children infected with HIV. [477] Due to the paucibacillary nature of childhood TB, a microbiological diagnosis is typically made in only 20-40% of cases with radiological evidence of intrathoracic disease.[99] Since DST assessment is only possible following bacteriological confirmation, confirmed MDR-TB in children is infrequent. In the absence of a known MDR-TB source case, children are often initially treated for drug-susceptible TB and MDR-TB treatment started only once treatment is failing, microbiology and DST results become available, or an MDR-TB source case is identified.

Treating children with MDR-TB is complex. Few of the multiple drugs routinely used to treat MDR-TB have been studied in children and guidance on drug regimens, dosages, appropriate monitoring and duration of therapy is frequently extrapolated from adult data. As young

children metabolize drugs more rapidly than adults and generally have paucibacillary disease,[150] this may not always be appropriate. This study describes the clinical presentation, treatment and outcome of a large cohort of children with confirmed MDR-TB and evaluates factors influencing treatment response.

Methods

Eligibility criteria

Children less than 15 years of age were included if they were diagnosed with confirmed MDR-TB between 1st January 2003 and 31st December 2008. DST on all children with culture-confirmed TB was routine during the study period. Follow up was documented until 31st May 2011.

Mycobacterial culture and drug susceptibility testing

Mycobacterial culture was completed at the accredited National Health Systems Reference Laboratory following a standard protocol to prevent mycobacterial cross-contamination. Primary mycobacterial cultures were established by inoculation of routine clinical samples into Middlebrook 7H9 broth-based Mycobacterial Growth Indicator Tubes (MGIT960; Becton Dickinson, Sparks, MD, USA) following a standard protocol for decontamination, while lymph node aspirates, pleural fluid, cerebrospinal fluid and other bodily fluids were directly inoculated. *M. tuberculosis* complex isolates were confirmed through PCR.[430] Phenotypic DST was performed using two different assays which have been shown to yield highly concordant results as previously described.[478]

Definition of tuberculosis episodes and treatment delay

A previous TB episode was defined as standard TB treatment (isoniazid, rifampin and pyrazinamide with or without ethambutol) for at least one month, followed by a symptom-free period of ≥ 6 months (reported by parent/carer and the absence of presentations to any health care providers) before the start of the current MDR-TB episode; a commonly used programmatic definition of a separate episode.[479] A MDR-TB episode was defined as beginning (if MDR-TB was subsequently confirmed) at the child's initial documented presentation to the health care system, when the specimen was obtained confirming MDR-TB, or when TB treatment was commenced. Treatment delay was defined as time from the start of MDR-TB episode to initiation of MDR-TB treatment. Treatment delay could not be determined in children who died or were lost to follow-up prior to start of MDR-TB treatment, and for those treated inadvertently with first-line drugs only.

Clinical data and standard of care

MDR-TB treatment was based on local standard of care, informed by international recommendations and available literature.[16, 102, 197, 268, 433, 480-482] High-dose (15-20mg/kg) isoniazid was used in the majority of cases, as there is evidence that isolates with an *inhA* promoter region mutation usually have a low MIC.[90] An injectable agent, most frequently amikacin (15-30mg/kg), was typically used for six months; capreomycin (15-30mg/kg) was substituted if resistance to amikacin was detected. Ofloxacin (15-20mg/kg), the most effective fluoroquinolone available in the South African National TB Programme, was used. Further drugs were added to result in at least four effective drugs. These included: ethionamide (15-20mg/kg), PAS (150mg/kg), terizidone (10-20mg/kg), co-amoxiclavulanic acid (10-15mg/kg 8 hourly), clarithromycin (7.5-15mg/kg 12 hourly) and linezolid (10mg/kg twice daily). All antituberculosis drugs were given under DOT for the full treatment duration. Most children remained hospitalized during the intensive phase when an injectable was given. Thereafter, children were treated at their local TB clinic with hospital out-patient follow-up every two months. Children with XDR-TB were treated for longer periods, typically for 24 months. If not yet on cART, cART was started in HIV-infected children after the initiation of MDR-TB treatment, consistent with national guidelines.

Clinical data collection

Data were collected through chart review. HIV testing followed written informed consent from the parent or legal guardian with pre- and post-test counselling using ELISA in children >18 months and DNA PCR test in younger and breast-fed children. Immunological staging in HIV-infected children used the WHO classification.[483] Weight was recorded and plotted on National Centre for Health Statistics weight-for-age percentile chart. Malnutrition was classified as weight <3rd percentile for age. Two tuberculin units were injected, intradermally (purified protein derivative RT23, Statens Serum Institute) for TST assessment. Results were read at 48-72 hours with a transverse diameter of ≥10mm considered positive in HIV-uninfected and ≥5mm in HIV-infected children. TB disease severity was defined using CR features following review by a single expert reader, read systematically with standardized recording.[413] Disease was classified as pulmonary if there were any CR changes attributable to TB or if any thoracic samples were positive for *M. tuberculosis*. Extra-pulmonary disease (EPTB) was classified if any imaging (ultrasound, plain film radiology or computerized tomography) demonstrated extra-thoracic TB or if a microbiological sample confirmed TB from a site other than the lungs. Intra-

thoracic radiological features were classified as non-severe (normal, hilar lymphadenopathy, airway compression, lobar/segmental collapse/opacification or pleural effusions) and severe (cavities, miliary opacification or a widespread bronchopneumonic picture).

Outcome

Respiratory samples in children with pulmonary involvement were obtained monthly to monitor response to therapy. Cure for this study was defined as three consecutive negative respiratory cultures obtained at least one month apart with no positive cultures after the first negative result, in the presence of treatment completion. Treatment outcomes were further classified as favourable (cured and treatment completed) or unfavourable (died, lost to follow-up, treatment failure, transferred out). For MDR-TB episodes with an initial sputum positive culture, culture-conversion was defined as the time from initiation of therapy until the first negative culture, provided there were no subsequent positive cultures and at least two further negatives. Two-month culture-conversion is described as it is a frequently-used surrogate marker for final treatment outcome in adult treatment trials.[484]

Statistical analysis

Data were analyzed using STATA version 11. All identifier details were dissociated from clinical data by unique study numbers. Missing data were excluded from analysis. Continuous variables were used for age and delay. Associations between clinical characteristics at presentation were assessed using the χ^2 test (or Fisher's exact test) when comparing categorical data; effect estimates (OR) and 95% CI were calculated. The Mann -Whitney test was used to assess the effect of age and delay given the non-normal distribution of the data. Risk factors for treatment outcomes (two-month culture-conversion, final treatment outcome and death) were assessed in univariate analysis. Multivariate logistic models were used to analyse the relationship between presenting characteristics and outcomes if either the univariate relationships showed significance ($p<0.05$) or where variables were thought to be clinically or epidemiologically relevant. Variables classified as collinear were not used in combination in the model.

Results

During the study period, 111 children with MDR-TB were identified with a median age of 50 months ([IQR: 19-108); all were included. Forty-two samples underwent DST to second-line drugs which identified three MDR-TB cases resistant to amikacin, four resistant to ofloxacin and five to both ofloxacin and amikacin (XDR-TB). An overview of the cohort with treatment

outcomes is provided in Figure 7. Demographic and clinical characteristics at the start of MDR-TB treatment are described in Table 35. The median time to MDR-TB treatment initiation (n=102) was shorter in the presence of a known adult MDR-TB index case (median 58 days [IQR 25-120] vs. 123 days [IQR 67-231]; p<0.001). Fifty-three of 85 (62%) children with a sputum result were smear positive; a positive sputum smear was more common in older children (median 85 months [IQR 25-132] vs. 24 months [IQR 15-59]; p<0.001) and in children with more severe CR changes (OR 9.95 [CI 2.98-33.3]; p<0.001). Of children HIV-tested (n=100, 90.1%), 43 (43%) were positive; 27 (64%) had severe immune suppression. Nineteen children were on cART prior to initiation of MDR-TB treatment; 21 were started afterwards (median time to initiation: 75 days [IQR 18-123]). Fifty children (n=109; 46%) had severe CR changes at diagnosis; children with severe CR changes were older (median 84 months [IQR 27-121] vs. 28 months [IQR 15-68]; p=0.002) [Table 35]

Of the 111 cases, 91 (82%) had a favourable treatment outcome. Of these, three were treated successfully with only first-line drugs: two had cervical lymph node disease and the other only hilar lymphadenopathy. Four patients were transferred to another hospital and three were lost to follow -up. One adolescent, diagnosed with pulmonary XDR-TB, was a repeat defaulter and her sputum never converted. She was declared a treatment failure after two years of intermittent treatment and eventually transferred into adult care. One patient was still on treatment at the end of the study period, having been initially treated for MDR-TB with additional resistance to amikacin according to his bacteriology, and then as XDR-TB based on his mother's bacteriology. The overall mortality was 12% (13 deaths; Table 36) regardless of treatment initiation. Eleven children died during their MDR-TB episode, one was cured of TB but died in the year following the end of treatment and one died following treatment failure.

Of the 88 cases successfully treated with MDR-TB drugs, 79 (89.8%) were alive and well at twelve months after completion of treatment. Of the remaining nine, three had been transferred to another institution, five had been lost to follow-up and one had died. Those successfully treated were treated for a median of 18 months, including median six months on an injectable, and were treated with a median of seven drugs over the total course of treatment. (Table 37; Figure 8-11)

Univariate analysis of clinical features and their association with outcome are shown in Table 38. Malnutrition and severe CR changes were associated with a failure to culture-convert by month two, unsuccessful treatment outcome and death. HIV infection and EPTB were associated with death. Children with positive smears at diagnosis were less likely to have

culture-converted by month two. Multivariable analysis is shown in Table 39. After adjustment for age and smear status (or CR severity), malnutrition at diagnosis predicted failure to culture-convert by two months (OR: 4.49 [CI: 1.32-15.2]; p=0.02). Malnutrition (OR: 15.0 [CI: 1.17-192.5]; p=0.04), HIV infection (OR: 24.7 [CI: 1.79-341.1]; p=0.02) and EPTB (OR: 37.8 [CI: 2.78-513.4]; p=0.006) all independently predicted mortality in a model adjusting for age.

Discussion

This study describes the clinical presentation, treatment and outcomes of children with culture-confirmed MDR-TB under routine clinical conditions in a high TB-burden setting. The data indicate that children with culture-proven MDR-TB tend to be young, malnourished, are frequently HIV-infected and often present with radiological features of advanced disease. Furthermore, the absence of a known MDR-TB source case led to considerable delay in initiation of appropriate therapy. Treatment regimens were long (median 18 months) of which six months included an injectable.

Of key importance is that, despite advanced disease and the presence of EPTB in more than 30%, the majority were treated successfully, with more than 80% favourable outcomes. Important risk factors for clinically and programmatically relevant treatment outcomes are identified, including mortality. HIV infection, malnutrition and extrapulmonary involvement were independent risk factors for death in adjusted analyses. Five of the 13 deaths occurred before MDR-TB was confirmed and appropriate treatment started indicating the importance of early diagnosis. Although severe disease on CR was associated with all outcome measures in univariate analyses, this association was less pronounced in adjusted analyses. These findings suggest that, once identified and treated appropriately with individualized therapy based on available DST, children with MDR-TB have a good prognosis, even with high prevalence of HIV co-infection.

Other reports of MDR-TB in children include a previous study from Cape Town of 39 children with culture-confirmed disease; similar to the present study, treatment delay was common if an MDR index case was not identified.[58] A study from Peru described 38 children treated for MDR-TB, 28 with confirmed disease, and also found considerable delay in the initiation of appropriate therapy.[204] A study from New York of 20 children treated for MDR-TB (six culture-confirmed), demonstrated good outcomes and minimal toxicity.[144] A recent case series of 13 children with culture-confirmed MDR-TB from Johannesburg (54% HIV-infected) indicated high mortality of 30%.[191] Other case reports and small series are reported from other settings. [89, 193-

[196, 205, 260, 474-475] Despite the delay in diagnosis, severe disease at presentation and the need for second-line medications, these studies all describe good outcomes, in dramatic contrast to adult MDR-TB data.[190] Reasons for this contrast are unclear. Children may have less severe and paucibacillary disease, may tolerate and adhere to medications better, may be less frequently HIV-infected or may have less concomitant pathology.

The absence of an identified adult MDR-TB source case was strongly associated with delay in initiation of appropriate treatment in children. Of note, there were 16 (14.4%) children who had a source case documented to have died, failed treatment or who was a re-treatment case, indicating a high MDR risk exposure. These factors highlight the importance of careful history taking from both the child's caregiver and health services regarding adults with known MDR-TB or with known risk factors for MDR-TB.

Since only children with culture-confirmed disease are described, these data are not representative of all children with MDR-TB, many of whom are treated on the basis of MDR-TB contact history or poor clinical response to therapy. As bacteriologic yield is associated with radiological extent of disease,[99] this study likely reports children with more advanced disease. The study reports on children diagnosed with MDR-TB using combined sources of surveillance (laboratory and TB register sources), rather than only those who started therapy (the traditional TB treatment cohort approach). Given this conservative approach, treatment success is likely underestimated in this cohort. The long duration of treatment (median of six months with a second-line injectable medication and 18 months overall) could possibly be reduced in children with less severe disease in future, if adequate evidence from clinical studies becomes available.

In this study, many children had severe disease at initiation of treatment. There was a high proportion with a positive smear and nearly half had cavities, severe bronchopneumonic changes or a miliary opacification on CR. Young children are traditionally considered to have paucibacillary TB and as they generally have a poor tussive force, are considered to pose low infection risk. However, these data indicate that children with culture-confirmed MDR-TB frequently have severe disease, are somewhat older than those with drug-susceptible TB,[254-255] and coupled with high rates of smear-positivity may prove a greater infection risk than previously thought. Infection control should be an important consideration in the management of children with MDR-TB.

This retrospective study has limitations including reliance on routine data. Adverse effects and the tolerability of multiple medications, frequently unpalatable, was not systematically assessed. Although all samples were confirmed MDR, DST to second-line drugs was not routinely completed during the study period. The second-line DST results that were available were reported, but due to inconsistent testing and significant bias in completion of DST meaningful conclusions are difficult to draw. Finally, although treatment outcomes were good, morbidity as a result of MDR-TB disease and treatment is not explored.

Figure 7 - Overview of treatment outcomes in children with MDR-TB (percentages of the total number of children; n=111)

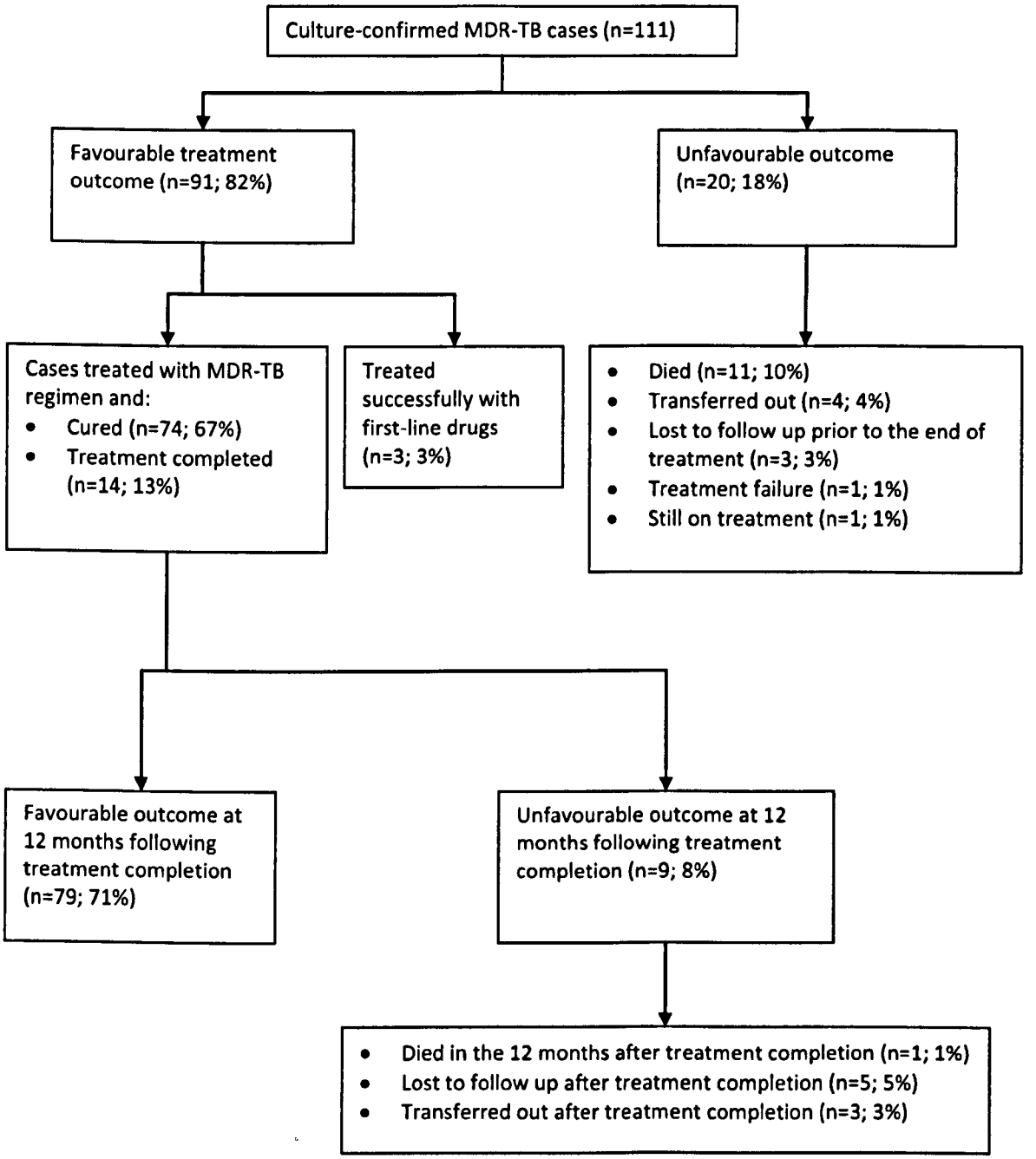


Table 35 - Clinical characteristics at initial presentation in children with bacteriologically confirmed MDR-TB (n=111)

Characteristic	Description	Number (percentage)[1]
Age	Median months (IQR)	50 (19-108)
Male gender		46 (41.4)
Treatment delay (n=105)	Median days (IQR)	91 (51-166)
Index case	None identified	33 (29.7)
	Index case identified with no evidence of MDR-TB	17 (15.3)
	MDR-TB index case indentified	45 (40.5)
	Index case defaulter, treatment failure or died	16 (14.4)
Previous treatment episode		28 (25.2)
Positive Mantoux test (n=89)		63 (70.8)
Weight	<3rd percentile for age	41 (36.9)
	3rd – 10th percentile for age	28 (25.2)
	>10th percentile for age	42 (37.8)
Type of TB	PTB only	73 (65.8)
	EPTB only	12 (10.8)
	Both PTB and EPTB	26 (23.4)
Site of EPTB (n=38) (>1 site possible)	Miliary	7 (18.4)
	Tuberculous meningitis	6 (15.8)
	Pericardial effusion	2 (5.3)
	Pleural effusion	7 (18.4)
	Abdominal	8 (21.1)
	Peripheral lymph node	16 (42.1)
	Bone/joint/spine	9 (23.7)
	Ear	5 (13.2)
Sputum smear positive (n=85)		53 (62.3)
Drug susceptibility test pattern (n=42)	MDR	30 (71.4)
	MDR with resistance to amikacin	3 (7.1)
	MDR with resistance to ofloxacin	4 (9.5)
	XDR	5 (11.9)
CR features (n=109) (> 1 feature present in the majority)	Normal CXR (all had EPTB)	11 (10.1)
	Hilar lymphadenopathy or airways compression	52 (47.7)
	Lobar/segmental collapse/opacification	76 (69.7)
	Large pleural effusion	7 (6.4)
	Cavities	38 (34.9)
	Miliary opacification	7 (6.4)
	Widespread bronchopneumonic changes	21 (19.2)
CR severity (n=109)	Non-severe	59 (54.1)
	Severe	50 (45.9)
Time to sputum conversion (n=74)	Median months (IQR)	2 (1-3)
HIV-infected (tested n=100)		43 (43.0)
HIV immunological stage (n=42)	Not significant[2]	7 (16.7)
	Mild[3]	5 (11.9)
	Moderate[4]	3 (7.1)
	Severe[5]	27 (64.3)
When started on cART (n=43)	Never (all died)	3 (7.0)
	Already on cART at start of MDR TB episode	9 (20.9)
	Started on cART between start of episode and start of MDR-TB treatment	10 (23.3)
	After start of MDR-TB treatment	21 (48.8)
Time from start of MDR-TB treatment to cART initiation (n=21)	Median days (IQR)	75 (18-123)

[1] unless otherwise stated

[2] CD4 value: <11 months: >35%; 12-35 months: >30%; 36-59 months: >25% & >5 years: >500 cells/mm^3

[3] CD4 value: <11 months: 30-35%; 12-35 months: 25-30%; 36-59 months: 20-25% & >5 years: 350-499 cells/mm^3

[4] CD4 value: <11 months: 25-29%; 12-35 months: 20-24%; 36-59 months: 15-19% & >5 years: 200-349 cells/mm^3

[5] CD4 value: <11 months: <25%; 12-35 months: <20%; 36-59 months: <15% & >5 years: <200 cells/mm^3 *or* <15%

Table 36 - Description of deaths (N=13) in children with culture-confirmed MDR-TB (n=111)

Age at death	Gender	HIV status	CD4 count (%)	Site of disease	History	Attributed cause of death
11 years	Male	Unknown		Spinal	Presented with severe disease, underwent surgery and biopsy sample taken, started on first-line treatment but died one month later prior to diagnosis being made	MDR-TB of the spine
6 months	Male	Positive	1652 36%	Pulmonary TB	Died after one month on first-line therapy, MDR-TB diagnosed posthumously only	Hepatotoxicity, HIV, MDR-TB, Down's syndrome, severe cardiac defect, marasmic, bacterial pneumonia
10 years	Female	Positive	37 8%	Disseminated (miliary)	Died after 12 months of MDR-TB treatment	Developed disseminated TB including TB meningitis whilst on full MDR-TB treatment in hospital. Possible XDR-TB
6 years	Male	Positive	33 2%	Pulmonary and abdominal TB	Treated for 10 months with first-line drugs before sample sent for culture. MDR-TB diagnosed posthumously only	Disseminated MDR-TB
9 months	Male	Positive	825 19%	Pulmonary, miliary and lymph node TB	Died after three months of MDR-TB treatment	Multi-system failure, HIV, extensive disseminated TB disease at presentation, sepsis, heart failure, thrombocytopenia
4 years	Female	Positive	Not tested	TB meningitis	Died after three months of MDR-TB treatment	Severe TB meningitis (stage 3)
12 months	Female	Positive	117 6%	Pulmonary TB	Died after five months of MDR-TB treatment	Severe HIV, systemic candida infection, osteomyelitis, respiratory failure, pneumonia, S. aureus sepsis
2 years	Female	Negative		TB meningitis, pulmonary, abdominal and lymph node TB	Died after one month first-line treatment, MDR-TB diagnosed after death	Disseminated MDR-TB meningitis
15 years	Female	Unknown		Pulmonary TB	Died after one month MDR-TB treatment, pre-XDR result returned after death	Congenital myopathy, aplastic anaemia, requiring multiple transfusions, extensive pre-XDR-TB
2 years	Male	Positive	1065 26%	TB meningitis	Given 24 days first-line treatment for stage three, TB meningitis prior to MDR-TB diagnosis. Died after one month MDR-TB treatment	MDR-TB meningitis, HIV
8 years	Female	Unknown		Pulmonary TB	Spastic quadriplegia from previous illness, died 6 days after starting first-line treatment, MDR-TB diagnosed after death	Severe MDR-TB, pre-existing neurological condition making diagnosis challenging
12 years	Female	Negative		Pulmonary TB	Adolescent repeated defaulter with pre-XDR TB. Never sputum culture-converted and declared a treatment failure after 12 months of MDR-TB treatment. Transferred to adult care	Died of pre-XDR TB under the care of adult physician
8 years	Female	Negative		Pulmonary TB	Registered as cured of MDR-TB with 20 months of therapy. Significant lung damage as a result of TB	Developed bronchiectasis and chronic lung abscess. Died within a year of finishing MDR-TB treatment with a bacterial infection.

Table 37 - Treatment characteristics in children successfully treated with an MDR-TB regimen (n=88*)

Median number of drugs used at any point during treatment (range)		7 (4 – 13)
Median duration of intensive phase therapy (range)		6 (0 – 18)**
Median total duration of therapy (range)		18 (8 – 26)
Number of patients using anti-TB drugs (percentage)	Isoniazid (high-dose)	83 (94.3)
	Rifampicin	14 (15.9)
	Pyrazinamide	81 (92.0)
	Ethambutol	82 (93.2)
	Streptomycin	1 (1.1)
	Amikacin	80 (90.9)
	Capreomycin***	6 (6.8)
	Ofloxacin	86 (97.7)
	Ethionamide	86 (97.7)
	Terizidone or cycloserine	57 (64.8)
	PAS***	7 (8.0)
	Clarithromycin	7 (8.0)
	Augmentin	6 (6.8)
	Linezolid***	2 (2.3)

*With percentage unless stated otherwise
**No injectable used in six cases
***Available only from 2007

Figure 8 - Number of drugs used in the treatment of children with culture-confirmed multidrug-resistant tuberculosis

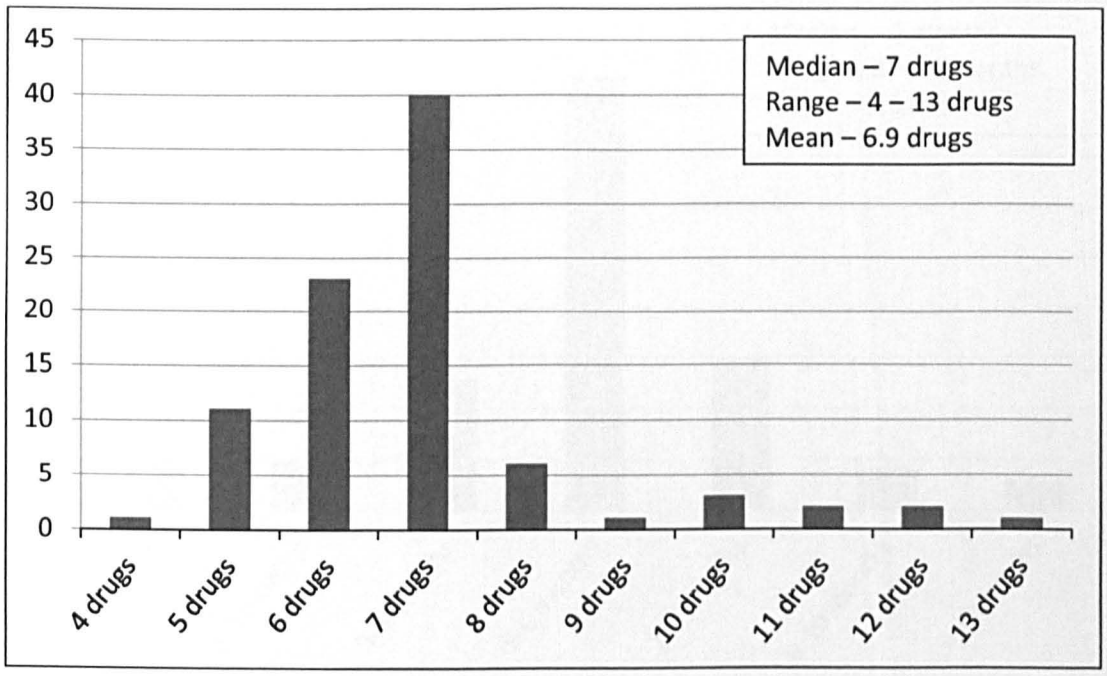


Figure 9 - Drugs used in the treatment of children with multidrug-resistant tuberculosis

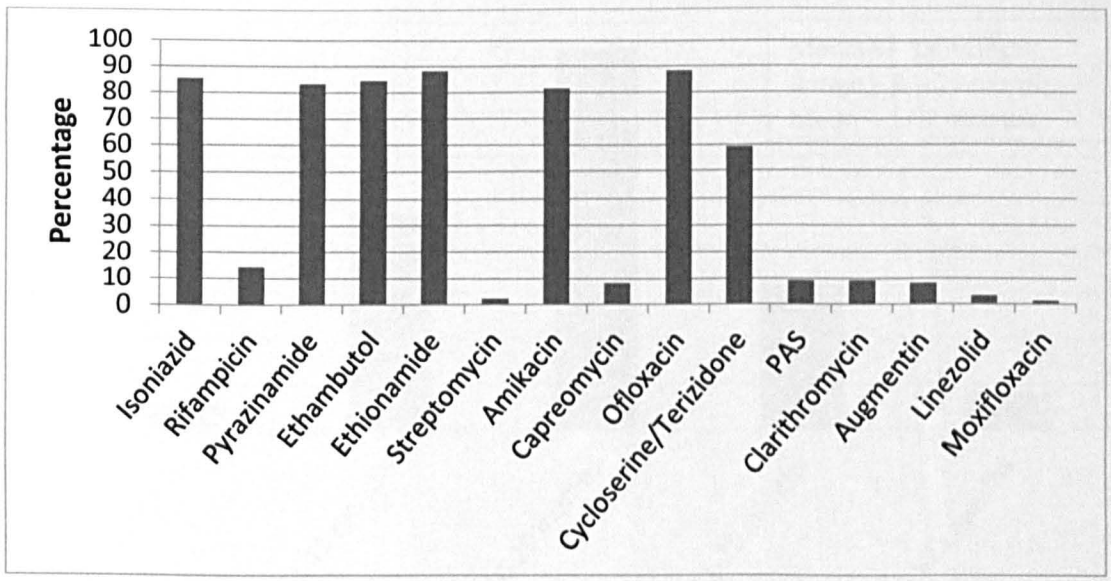


Figure 10 - Length of intensive phase in children treated for multidrug-resistant tuberculosis

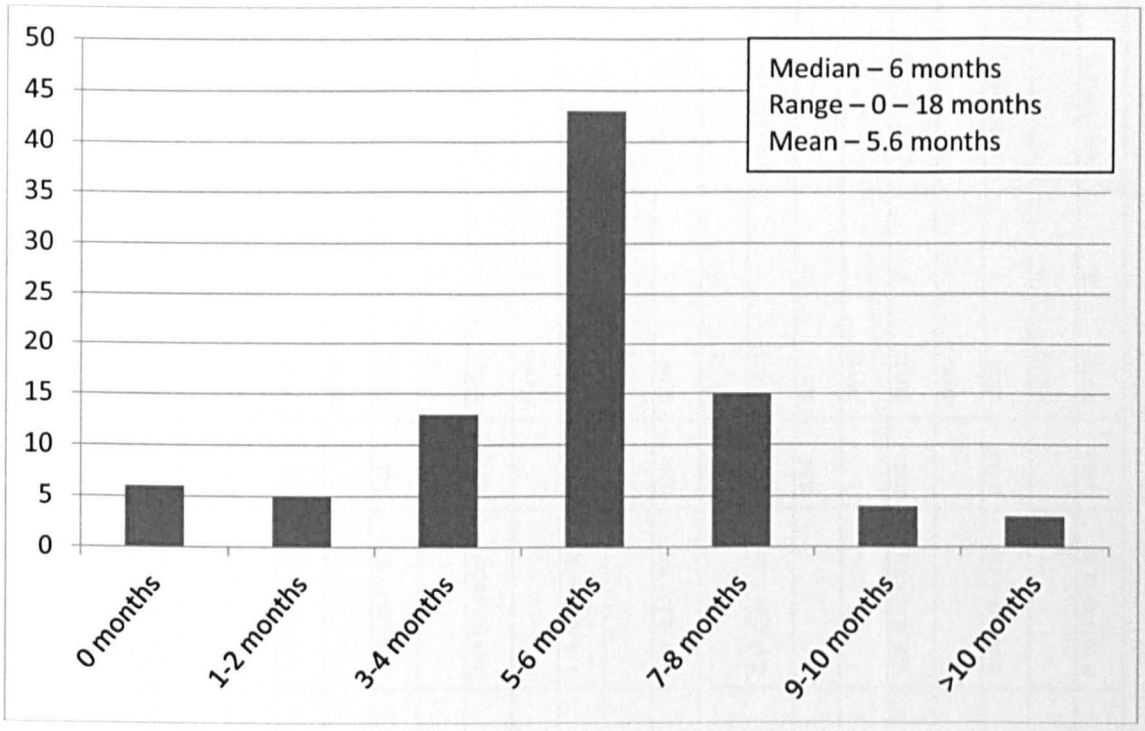


Figure 11 - Total length of treatment in children treated for multidrug-resistant tuberculosis

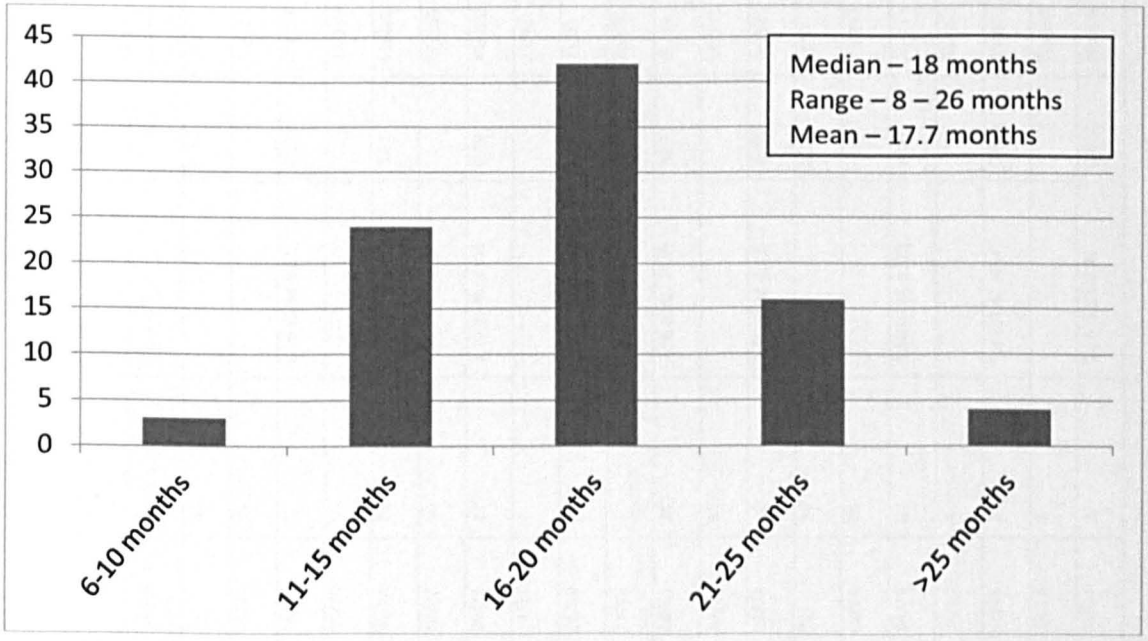


Table 38 - Univariate analysis of clinical features, two month culture-conversion, treatment outcome and death in children with MDR-TB

Characteristic		Failure to culture-convert by month two				Unfavourable treatment outcome				Deaths			
		Number analyzed	Number failing to convert	Odds ratio (95% CI)	P-value	Number analyzed	Number unfavourable outcome	Odds ratio (95% CI)	P-value	Number analyzed	Number dying	Odds ratio (95% CI)	P-value
Age		74	26		0.06	111	20		0.80	111	11		0.97
Gender	Female	46/74	13			65/111	10			65/111	6		
	Male	28/74	13	2.20 (0.80-6.01)	0.11	46/111	10	1.53 (0.57-4.07)	0.39	46/111	5	1.20 (0.34-4.22)	0.78
Nutrition	≥3rd percentile	53/74	14			70/111	8			70/111	2		
	<3rd percentile	21/74	12	3.71 (1.22-11.3)	0.01	41/111	12	3.21(1.15-8.96)	0.02	41/111	9	9.56 (1.79-51.2)	0.001
HIV	Negative	42/71	14			57/100	7			57/100	1		
	Positive	29/71	11	1.22 (0.45-3.31)	0.69	43/100	10	2.16 (0.74-6.36)	0.15	43/100	7	10.9 (1.17-101.0)	0.008
Timing of cART initiation	Before MDR-TB treatment	14/29	4			22/43	5			22/43	4		
	After MDR-TB treatment	15/29	7	2.19 (0.44-10.8)	0.32	21/43	5	1.06 (0.25-4.45)	0.93	21/43	3	0.75 (0.14-3.92)	0.73
Mantoux skin test	Negative	16/62	2			26/89	7			26/89	3		
	Positive	46/62	28	4.50 (0.85-23.7)	0.05	63/89	8	0.39 (0.12-1.27)	0.10	63/89	5	0.66 (0.14-3.03)	0.59
MDR-TB contact	No contact	35/74	13			66/111	15			66/111	8		
	Contact	39/74	13	0.85 (0.32-2.21)	0.73	45/111	5	0.43 (0.14-1.29)	0.12	45/111	3	0.52 (0.13-2.09)	0.35
Treatment delay		74	26		0.25	103	15		0.36	103	8		0.18
Site of TB	No EPTB	60/74	20			73/111	10			73/111	3		
	EPTB	14/74	6	1.50 (0.45-4.97)	0.50	38/111	10	2.25 (0.83-6.11)	0.10	38/111	8	6.22 (1.45-26.6)	0.005
Smear status	Negative	27/68	4			32/85	4			32/85	2		
	Positive	41/68	20	5.48 (1.47-20.4)	0.004	53/85	10	1.63 (0.46-5.77)	0.443	53/85	5	1.56 (0.28-8.68)	0.61
CR severity	Non-Severe	39/74	9			59/109	5			59/109	2		
	Severe	35/74	17	3.15 (1.11-8.92	0.022	50/109	13	3.79 (1.20-12.0)	0.014	50/109	8	5.43 (1.05-28.2)	0.02

Table 39 - Multivariate analysis of clinical and bacteriological features at diagnosis, two month culture-conversion, treatment outcome and death in children with MDR-TB

Characteristic	Number in analysis	OR (95% CI)	P value
Failure to culture-convert by two months*			
Smear positivity	68	3.24 (0.82 – 12.8)	0.10
Malnutrition	68	4.49 (1.32 – 15.2)	0.02
Age	68		0.15
Poor treatment outcome**			
Severe CR changes	99	2.50 (0.68 – 9.19)	0.17
Malnutrition	99	1.87 (0.58 – 6.07)	0.30
HIV positivity	99	1.46 (0.46 – 4.63)	0.52
Age	99		0.51
Death			
EPTB	99	37.8 (2.78 – 513.4)	0.006
Malnutrition	99	15.0 (1.17 – 192.5)	0.04
HIV positivity	99	24.7 (1.79 – 341.1)	0.02
Age	99		0.18

* In an alternative model, holding all variables constant but replacing smear positivity with CR severity, findings were similar (n=74; severe CR changes: OR: 1.88 [CI: 0.61-5.78]; p=0.270, malnutrition: OR: 3.51 [CI: 1.12-11.0]; p=0.031, age: p=0.148)

** In an alternative model, holding all variables constant but replacing severe CR changes with EPTB, findings were similar (n=100; EPTB: OR: 2.59 [CI: 0.86-7.75]; p=0.90, malnutrition: OR: 2.43 [CI: 0.80-7.40]; p=0.115, HIV positivity: OR: 2.43 [CI: 0.65-5.98]; p=0.232, age: p=0.679)

Study 10: the spectrum of presentation, treatment and outcome in children with multidrug-resistant tuberculosis

The following study has been prepared as an article:

- *Seddon JA, Hesseling AC, Godfrey-Faussett, Schaaf HS. The spectrum of presentation, treatment and outcome in children with MDR-TB (in preparation)*

Introduction

This study describes all children treated for MDR-TB over a two year period. It includes children with a confirmed diagnosis as well as children treated presumptively for MDR-TB. The study describes the presentation of the children as well as their treatment, adverse events and the outcome. The study also compares children with severe disease and those with limited disease.

Methods

Eligibility criteria

A register of all children (defined as <15 years in the setting), routinely treated for MDR-TB, was reviewed for children starting treatment from 1 January 2009 until 31 December 2010. As children with RMR-TB (resistant to rifampicin, but susceptible to isoniazid, with or without resistance to other drugs) are treated as MDR-TB due to concerns regarding 'missed' isoniazid resistance on molecular diagnostic tests,[226] children diagnosed with RMR-TB were included. Children with both confirmed MDR-TB as well as presumed MDR-TB were included. A presumed diagnosis was typically made by the attending clinical team if the child was failing a first-line TB regimen with documented good adherence, or if the child had clinical symptoms, signs and radiology of TB with documented close MDR-TB exposure. Children initially started on MDR-TB treatment due to MDR-TB exposure who were subsequently confirmed to have drug-susceptible TB were excluded.

Mycobacterial culture and drug susceptibility testing

Mycobacterial culture (paediatric and adult samples), was completed at the accredited regional National Health Systems Reference Laboratory. Samples were first decontaminated

and then cultured using the Mycobacterial Growth Indicator Tube (MGIT) 960 system (Becton Dickinson, Sparks, MD, USA). The presence of *M. tuberculosis* was confirmed by PCR amplification.[430] Genotypic DST to isoniazid and rifampicin was carried out using LPA (GenoType® MTBDR*plus*; Hain Lifescience, Nehren, Germany), according to the manufacturer's instructions.[432] DST to ethambutol was carried out using the Bactec 460TB system (Becton Dickinson, Sparks, MD, USA), according to international criteria[431] using a concentration of 7.5μg/ml. DST to second-line agents was performed by the indirect proportional method on Middlebrook 7H10 agar using critical concentrations of amikacin 40μg/ml, ofloxacin 2μg/ml and ethionamide 10μg/ml.

Standard of care

MDR-TB treatment was based on local standard of care, based on international guidelines.[16, 197] High-dose isoniazid (15-20mg/kg) was used in almost all children, due to the demonstrated activity against low-level isoniazid resistance.[90-91] An injectable agent, usually amikacin, was added in cases with more severe disease (cavitating lesions, disseminated disease or widespread pulmonary changes on CR); capreomycin was substituted if resistance to amikacin was present. Ofloxacin was used unless resistance had been demonstrated; further drugs were added, to result in the use of at least four effective drugs. These included: ethionamide, PAS, terizidone (equivalent to cycloserine, which was not locally available) and, if necessary, co-amoxiclavulanic acid, clarithromycin and linezolid. All TB drugs were routinely available, free of charge to the patient, through the local TB control programme. cART was started in HIV-infected children, if not already on cART, as soon as possible after the start of MDR-TB treatment.

Data collection

From 1 January 2010, data were collected from patients and their families, following written informed consent, at each outpatient clinic appointment. Data were, therefore, collected both retrospectively and prospectively, as some children had already begun treatment at the start of the data collection period. To document clinical data during 2009, folder reviews were completed at both TCH and BCH. Follow-up continued until 30 June 2012 and included telephone calls and home visits to determine clinical progress and outcome.

Study measures

HIV testing was completed by routine health services following informed consent from the parent or legal guardian, with pre- and post-test counselling using ELISA in children >18 months and DNA PCR test in younger or breast-fed children. The WHO classification was used

for immunological staging in HIV-infected children.[483] Weight and height were recorded at baseline, with weight-for-age, height-for-age and weight-for-height z-scores calculated. Where possible, weight was determined at three, six and twelve months from the start of treatment. As children were not necessarily seen at those time-points, weights taken within window periods around those time points were accepted: one month for the three month weight, six weeks for the six month weight and two months of the twelve month weight. TST (Mantoux, 2 tuberculin units injected intradermally; purified protein derivative RT23, Statens Serum Institute) was used. Results were read at 48-72 hours with a transverse diameter of ≥10mm considered positive in HIV-uninfected and ≥5mm in HIV-infected children.

Case definitions and treatment outcomes

For this study the consensus definitions previously described in the thesis were used regarding exposure to source cases, episode initiation and delay, certainty of diagnosis, site and severity of disease, adverse events and disease outcome. Specifically, an MDR-TB episode was defined as beginning (if MDR-TB was subsequently confirmed or treatment subsequently started) at the child's first presentation to a health care provider, when a specimen was obtained that eventually confirmed MDR-TB or when the child was started on treatment for MDR-TB. Treatment delay was defined as the time from the start of the MDR-TB episode to initiation of MDR-TB treatment. Certainty of MDR-TB diagnosis was divided into confirmed, probable and possible MDR-TB disease using the consensus definitions and based on previous definitions of TB disease certainty.[405] Probable MDR-TB disease was therefore defined as probable TB disease and contact with an MDR-TB source case, while possible MDR-TB was defined as probable TB disease in combination with failure of first-line treatment with confirmed adherence. Severity of disease was classified as severe and non-severe based on criteria by Wiseman et al.[411] Disease was classified as pulmonary if there were any CR changes (including hilar lymphadenopathy attributable to TB) or if any respiratory samples were positive for *M. tuberculosis*. Extrapulmonary TB disease was classified if any imaging (ultrasound, plain film radiology or computerised tomography [CT]) demonstrated evidence of TB outside the thorax or if a microbiological sample confirmed TB from an extrathoracic site. Radiological features of pulmonary TB were classified as non-severe (normal, hilar lymphadenopathy, airway compression, lobar/segmental collapse/opacification or pleural effusions) and severe (cavities, miliary opacification or a widespread bronchopneumonic picture) using radiographic features reviewed by a single expert reader, read in a systematic manner using a standardised reporting and recording form.[413] The family and, where appropriate, the child were asked about adverse events at each clinic appointment; results were recorded using standardised DMID toxicity tables.[415] Hearing was measured at baseline and at monthly intervals using PTA or OAE,

dependent on the age of the child. ASHA guidelines were used to define hearing loss[248] (see later chapter on hearing loss in children treated for MDR-TB for more details of the hearing assessments). The most severe grade of adverse event experienced over the course of treatment, for each category, was determined. MDR-TB treatment outcome was classified as cure, probable cure, treatment completed, failure, death, lost to follow up and transferred out, as defined by the specified definitions.

Statistical analysis

Data were analysed using STATA version 11. Missing data were excluded from analysis. Associations between clinical characteristics at presentation were assessed using the χ^2 test (or Fisher's exact test) when comparing categorical data; effect estimated (OR) and 95% CI were calculated. The Mann Whitney test was used to assess the relationship between categorical data and non-parametric continuous data with median and IQR calculated. The relationship between disease severity and patient and treatment characteristics was determined in univariate analysis.

Results

One hundred and forty nine children were started on treatment for MDR-TB over the two year study period; the median age was 36 months (IQR: 16-66), 69 (46.3%) were male and 32 (21.9%; out of 146 tested) were HIV-infected (Table 40). A culture-confirmed diagnosis was made in 59 children (39.6%); 82 (55.0%) had probable and 8 (5.4%) possible MDR-TB disease. Forty-five (30.2%) children had severe (intra-or extra-thoracic) disease and 23 (50%; of 46 children with sputum culture-positive TB) were also smear-positive.

One hundred and three (69.1%) children were admitted to hospital for a median of 5 months (IQR: 3-7). Of 94 (66.2%) children treated initially with injectable drugs, the median treatment duration was 4 months (IQR: 4-6). The total treatment duration was a median of 13 months (IQR: 11-18). Thirty-six children (24.2%) were cured, 101 (67.8%) were probably cured, one (0.7%) was transferred out, eight (5.4%) were lost to follow-up and three (2.0%) died (Table 41). Of the children with HIV infection (n=32), 14 (43.8%) were cured, 14 (43.8%) were probably cured, 2 (6.3%) died and 2 (6.3%) were lost to follow up. The TB drugs used are documented in Table 42 and documented adverse events reflected in Table 43. One girl developed DRESS syndrome (drug reaction with eosinophilia and systemic symptoms) after one month on MDR-TB treatment. She experienced multiple grade 4 adverse events. Other

than this teenager, there were two grade three reactions (nausea and joint pain) both of which resolved without cessation of treatment.

Children with severe disease were older (54 months [IQR: 18-142] vs. 31.5 months [IQR: 17.5-53.5]; p=0.01) and less frequently had an MDR-TB source case identified (OR: 0.19; 95%CI: 0.08-0.44; p<0.001) compared to children with less severe disease (Table 44). Children with severe disease were more commonly HIV-infected (OR: 6.25; 95%CI: 2.50-15.6; p<0.001), more commonly had extrapulmonary involvement (OR: 5.64; 95%CI: 2.24-14.2; p<0.001) and had poorer nutritional status (mean weight-for-age z-score: -2.11 [SD: 1.61] vs. -0.76 [SD: 1.32]; p<0.001). Children with severe disease were also more likely to have a bacteriologically confirmed TB diagnosis (OR: 8.25; 95%CI: 3.37-20.2; p<0.001), to be admitted to hospital (OR: 9.87; 95%CI: 2.64-36.9; p<0.001), be treated with injectable drugs (OR: 16.3; 95%CI: 3.27-81.3; p<0.001) and to die (p=0.008).

Discussion

In this cohort, with bacteriologically confirmed and clinically diagnosed disease, treatment was overall well tolerated with few significant adverse events. Treatment outcomes were excellent, with over 90% of children cured or probably cured. Many of the children were identified and started on treatment early, following the diagnosis of an MDR-TB source case, illustrating the importance of contact tracing in paediatric case ascertainment. The three children who died either presented late with severe TB disease and concomitant HIV infection or had extensive disease and had defaulted care.

Standardised definitions were used to document the presentation, treatment and outcome and demonstrate that these definitions were found to be robust and easy to use. In comparison to previously described cohorts,[58, 144, 191-192, 204] this study included younger children, included children with a clinical diagnosis in the absence of bacteriological confirmation, described fewer adverse events and documented a higher proportion of children with less severe disease. Treatment outcomes in the present cohort are also better than the outcomes described in a systematic review and meta-analysis of all previous paediatric studies.[221] The prevalence of HIV (21.9%) is lower than that found in the previous study in the thesis (43%) in the same context, which documented only bacteriologically confirmed MDR-TB from 2003-2008. This may be the result of more effective prevention of mother to child transmission programmes or may indicate that HIV disproportionately predisposes to severe (culture-confirmed) TB disease. Systematic reviews of adults with MDR-TB report successful

treatment outcomes in 54-64% of cases, [116, 190] with poor nutrition, alcohol, extensive resistance, standardised (as opposed to individualized) treatment, shorter duration of therapy and male gender found to be associated with poor outcome.

Although it is appreciated that severity of disease is a spectrum, children were categorised as having severe and non-severe disease, based on a classification which considers the anatomical location, extent and local complications of disease.[411] Using this comprehensive research definition of disease severity, successful treatment outcomes are possible in children treated for non-severe disease with a median of 12 months of therapy, with injectable drugs in only 50% of children and many (41%) children treated entirely as outpatients.

For principles of good clinical practice, as well as the need for improved paediatric surveillance data, clinicians should strive to obtain a microbiological diagnosis in children where possible. However, this will, in reality, not always be achieved given the paucibacillary nature of paediatric TB. A subset of children will therefore need to be presumptively treated for MDR-TB disease (as contacts of MDR-TB cases or failing adherent first-line TB treatment) based on symptoms, signs and radiology. In any MDR-TB treatment cohort, as with drug-susceptible TB, there will be a balance between those with confirmed and those with presumed disease. If too many children are treated for presumed disease, it is likely that either not enough commitment is being made to confirming the diagnosis or children are being over-treated for non-TB diseases. If the majority is bacteriologically confirmed, it is likely that clinicians are not treating enough children presumptively. The exact proportion of confirmed and presumed diagnoses will vary dependent on resources, clinical experience, intensity of clinical sampling, the observed spectrum of disease, HIV prevalence, age demographics of society and other factors including patient and health system delays. However, based on this and previous studies, it is likely that the figure confirmed 'should' be between 25-50%.[5, 99, 485-486]

It was challenging to define children who defaulted treatment before the time advised by their attending clinician, but who were found on follow-up, in most over two years later, to be well, free of TB symptoms or signs and growing successfully. They were categorised as "probably cured". These results imply that perhaps not all of the treated children required the advised duration or therapy. Older studies from the drug-susceptible literature suggested that a significant proportion of children with what would be now described as limited disease were cured with either isoniazid given alone or with no drug therapy at all.[61] However, it is not clear which children with non-severe disease would progress to develop more extensive disease with limited or no treatment. Most clinicians today would not feel it was ethical, especially in

the presence of HIV co-infection, young age and poor nutritional status, to withhold treatment from a child with respiratory symptoms and hilar lymphadenopathy on CR, even though it is possible that a proportion could improve without treatment. If health systems carry out more comprehensive and more rapid contact tracing following the diagnosis of MDR-TB source cases, more children will be identified at an earlier stage in the natural history of their disease.

A limitation of this study is that the diagnosis was not confirmed in all children, even though clear research definitions were used. This is, however, the reality of treating children for TB, also for MDR-TB, where a presumptive diagnosis is frequently necessary and appropriate. As this study used data collected as part of routine care, another limitation is missing data. Also, due to the partial retrospective data collection, possible recall bias may have occurred in the description of adverse drug events. In addition, apart from thyroid and renal function, other laboratory investigations were not carried out unless clinically indicated. A further limitation is that comprehensive second-line DST for all children and their source cases were not available. This study described children treated for MDR-TB rather than children with MDR-TB and therefore included children with RMR-TB. Whilst children with RMR-TB may be systematically different from those with MDR-TB, their treatment is not. Finally, the study reports on a relatively short follow-up time. Whilst the first children recruited (at the beginning of 2009) were followed for over three years from the start of treatment, those starting at the end of the study period were followed for eighteen months, some only to the end of treatment. Longer follow-up would be required to assess long-term treatment outcomes including recurrent TB.

Table 40 - Patient demographics in children treated for multidrug-resistant tuberculosis (n=149 unless otherwise stated)

Characteristic		
Median age in months (IQR)		36 (18-66)
Gender	Male	69 (46.3)
	Female	80 (53.7)
Ethnicity	Xhosa	90 (60.4)
	Coloured (mixed ethnicity)	59 (39.6)
Source case	None	20 (13.4)
	MDR-TB	111 (74.5)
	Defaulter	2 (1.3)
	Died	10 (6.7)
	DS[1]	6 (4.0)
Multiple source cases	Yes	36 (24.2)
	No	113 (75.8)
Median delay from start of MDR-TB episode to MDR-TB treatment (IQR)		14 (0-53)
Tuberculin skin test (n=111)	Positive	90 (81.1)
	Negative	21 (18.9)
Previous TB	Yes	13 (8.7)
	No	136 (91.3)
Median weight (IQR; n=142)[2]		12.8 (10.3-19.1)
Median height (IQR; n=124)[2]		87.3 (76.3-107.8)
Mean weight-for-age z-score (SD; n=136)		-0.98 (1.54)
Mean height-for-age z-score (SD; n=118)		-1.05 (1.79)
Mean weight-for-height z-score (SD; n=64)		-0.29 (1.4)
Type of TB	Pulmonary	120 (80.5)
	Extrapulmonary	12 (8.1)
	Both	17 (11.4)
Extrapulmonary involvement (more than one possible; n=39)	Miliary TB	4 (10.3)
	TB meningitis	7 (17.9)
	Abdominal TB	6 (15.4)
	Peripheral lymph node TB	6 (15.4)
	Bone, joint or spinal TB	8 (20.5)
	Other	1 (2.6)
Certainty of diagnosis of DR-TB	Confirmed	59 (39.6)

	Probable	82 (55.0)
	Possible[3]	8 (5.4)
Severity of disease	Non-severe	104 (69.8)
	Severe	45 (30.2)
Sputum smear microscopy result (n=46)	Positive	23 (50.0)
	Negative	23 (50.0)
HIV status (n=146)	Positive	32 (21.9)
	Negative	114 (78.1)
WHO immunological stage (n=32)	Not significant	5 (15.6)
	Mild	3 (9.4)
	Advanced	11 (34.4)
	Severe	13 (40.6)
Timing of ART initiation (n=32)	ART started prior to MDR-TB episode	11 (34.4)
	ART started after start of MDR-TB episode but before MDR-TB treatment	10 (31.3)
	ART started after MDR-TB treatment	11 (34.4)
Median time to start ART after MDR-TB treatment in days (IQR; n=11)		17 (12-35)
Drug resistance of isolate from child or from identified source case[4]	Rifampicin (n=141)	141 (100)
	Isoniazid (n=141)	125 (88.7)
	Ethambutol (n=92)	23 (25.0)
	Ethionamide (n=102)	5 (4.9)
	Amikacin (n=104)[5]	16 (15.4)
	Ofloxacin (n=103)[5]	14 (13.6)
Chest radiograph features at start of MDR-TB treatment (more than one possible; n=148)	Normal	16 (10.8)
	Perihilar infiltrates	32 (21.6)
	Hilar lymphadenopathy or airways compression	81 (54.7)
	Lobar/segmental collapse or opacification	69 (46.6)
	Pleural effusion	10 (6.8)
	Cavities	22 (14.9)
	Miliary picture	4 (2.7)
	Widespread bronchopneumonic changes	15 (10.1)
Chest radiograph severity (n=148)	Non-severe	72 (48.6)
	Severe	76 (51.4)

[1]Source case identified with no risk factors for MDR-TB
[2]At start of MDR-TB treatment
[3]All cases of possible DR-TB diagnosed on basis probable TB and failing first-line therapy
[4]No DST to guide therapy in 8 patients treated for failing first-line regimen with no identified source case
[5]Six children were treated for XDR-TB due to samples from them or their source case demonstrating resistant to both amikacin and ofloxacin

Table 41 - Treatment and outcome in children treated for multidrug-resistant tuberculosis (n=149 unless otherwise stated)

Characteristic		
Admitted to hospital	Yes	103 (69.1)
	No	46 (30.9)
Median duration of admission in months (n=103)		5 (3-7)
Treated with injectable drugs (n=142)[1]	Yes	94 (66.2)
	No	48 (33.8)
Median duration of injectable drug use (n=94;IQR)		4 (4-6)
Median duration of total treatment (n=137 ;IQR)[2]		13 (11-18)
Median weight gain (IQR; kg)	3 months (n=115)	0.6 (0.2-1.5)
	6 months (n=102)	1.4 (0.7-2.2)
	12 months (n=84)	2.9 (1.0-4.0)
Median number of months to culture conversion (n=40)[3]		1 (0.5-2)
Outcome	Cure	36 (24.2)
	Probable cure[4]	101 (67.8)
	Transferred out	1 (0.7)
	Lost to follow up	8 (5.4)
	Died[5]	3 (2.0)

[1]Excludes patients who died or absconded from hospital prior to the end of the prescribed period of injectable use
[2]Excludes patients who died, were transferred out or were lost to follow up
[3]For children with an initial culture-positive sputum sample with at least one follow up culture (excludes culture-positive extrapulmonary cases)
[4]Includes 8 patients who stopped their therapy before indicated but were clinically well at follow up and one patient who all drugs were stopped due to severe DRESS syndrome but found to be well after two years of follow up and discharged
[5]Three children died: 14 year girl, confirmed pre-XDR-PTB and extensive adult-type disease, absconded from hospital after 3 weeks and was lost to follow up, found to have died 12 months later; 6 month boy, presented with abdominal and pulmonary confirmed MDR-TB, measles and HIV with severe immunosuppression, died after three weeks in hospital; 9 year old boy, presented with extensive, confirmed adult-type pulmonary pre-XDR-TB and HIV, CD4 count 7, died after 3 months from sepsis and hypokalaemia

Table 42 - Drug therapy for children treated for multidrug-resistant tuberculosis who completed therapy (n=137)

Drug	Number of patients with drug included in regimen (%)	Median duration of treatment in months (IQR)
Isoniazid	136 (99.3)	13 (11-18)
Rifampicin	16 (11.7)	7.5 (4.5-12)
Pyrazinamide	136 (99.3)	13.5 (11-18)
Ethambutol	121 (88.3)	12 (10-18)
Streptomycin	2 (1.5)	5.5 (4-7)
Amikacin	82 (59.9)	4 (3-6)
Capreomycin	11 (8.0)	4 (4-6)
Ofloxacin	132 (96.4)	13 (10.5-18)
Moxifloxacin	2 (1.5)	18 (17-19)
Ethionamide	135 (98.5)	13 (10-18)
Terizidone	80 (58.4)	17 (12-18.5)
PAS	27 (19.7)	17 (12-18)
Clarithromycin	3 (2.2)	12 (4-18)
Augmentin	3 (2.2)	18 (4-19)
Linezolid	3 (2.2)	16 (4-21)

Table 43 - Adverse events in children treated for multidrug-resistant tuberculosis

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4[1]	Total
Joint, muscle or bone pain	122	11	2	1	1	137
Skin rashes	104	30	2	0	1	137
Itchy skin	110	24	2	0	1	137
Headache	119	16	1	0	0	136[2]
Sleep/mood problem	124	9	3	0	1	137
Lethargy	118	17	1	0	1	137
Visual problem	132	5	0	0	0	137
Vomiting	113	20	3	0	1	137
Diarrhoea	125	10	1	0	1	137
Jaundice	133	1	2	0	1	137
Appetite/nausea	118	14	3	1	1	137
Hearing loss[3]	117	25				142
Thyroxine supplementation provided[4]	110	32				142

[1]One child developed DRESS syndrome after a month on therapy with severe symptoms and signs. All drugs were stopped and it was unclear which drug was responsible. All grade four reactions are from this one child
[2]One mother felt unable to tell if her child had experienced headache
[3]Hearing loss was not graded but categorised as present or not using ASHA criteria
[4]The decision to start thyroxine supplementation was based on elevated thyroid stimulating hormone (TSH) and low free T4 levels

Table 44 - Comparison of characteristics for children with severe and non-severe multidrug-resistant tuberculosis disease (n=149 unless otherwise stated)

	Children with severe disease (n=45)	Children with non-severe disease (n=104)	OR (95% CI)	p-value
Median age (IQR; months)	54 (18-142)	31.5 (17.5-53.5)		0.01
Male gender	22 (48.9)	47 (45.2)	1.16 (0.57-2.34)	0.68
Coloured ethnicity	18 (40.0)	41 (39.4)	1.02 (0.50-2.10)	0.95
Median delay (IQR; days)	39 (9-89)	2 (0-41.5)		<0.001
MDR-TB source case identified	23 (51.1)	88 (84.6)	0.19 (0.08-0.44)	<0.001
Multiple source cases	12 (26.7)	24 (23.1)	1.21 (0.54-2.71)	0.64
Previous TB	6 (13.3)	7 (6.7)	2.13 (0.67-6.82)	0.19
TST positivity (n=111)	16/24 (66.7)	74/87 (71.2)	0.35 (0.12-1.01)	0.04
Mean weight-for-age z-score (SD; n=136)	-2.11 (1.61)	-0.76 (1.32)		<0.001
Extrapulmonary involvement	18 (40.0)	93 (10.6)	5.64 (2.24-14.2)	<0.001
Bacteriologically confirmed TB diagnosis	33 (73.3)	26 (25.0)	8.25 (3.37-20.2)	<0.001
Smear positive (n=46)[1]	19/29 (65.5)	4/17 (23.5)	6.18 (1.38-27.7)	0.007
Severe chest radiographic changes (n=148)	35/45 (77.8)	41/103 (39.8)	5.29 (2.23-12.5)	<0.001
HIV infection (n=146)	20/44 (45.5)	12/102 (11.8)	6.25 (2.50-15.6)	<0.001
Hospital admission	42 (93.3)	61 (58.7)	9.87 (2.64-36.9)	<0.001
Injectable TB drug use (n=142)[2]	39/41 (95.1)	55/101 (54.5)	16.3 (3.27-81.3)	<0.001
Median duration of injectable drug in those treated with injectables (n=94)[2]	6 (4-6)	4 (3-5)		<0.001
Median total duration of therapy (IQR; n=137)[3]	18 (18-20)	12 (10-16)		<0.001
Mortality	3 (6.7)	0 (0)		0.008

[1]Of children who were sputum culture positive
[2]Excludes children who died or absconded from hospital prior to the end of the prescribed period of injectable use
[3]Excludes children who died, were transferred out or were lost to follow up

Study 11: the impact of drug resistance on clinical outcome in children with tuberculous meningitis

The following study has been published as an article:

- *Seddon JA, Visser D, Bartens M, Jordaan A, Victor T, van Furth AM, Schoeman JF, Schaaf HS. Impact of drug resistance on clinical outcome in children with tuberculous meningitis. Pediatr Infect Dis J 2012; 31: 711-6*

Introduction

TBM is a severe form of TB and frequently occurs in early childhood.[487] Haematogenous spread of bacilli from a primary pulmonary focus leads to the development of a Rich focus in the brain. Rupture of this caseous granuloma into the subarachnoid space causes the clinical features of TBM.[488-489] This usually starts insidiously with a prodromal period of non-specific symptoms but as the disease progresses, neck stiffness, loss of consciousness, motor paresis and convulsions invariably follow. The diagnosis is often delayed and only considered once irreversible neurological damage has already occurred.[487, 490] Untreated, the condition is almost universally fatal with a median time to death of 19.5 days.[491] Even for those treated, TBM is associated with high rates of mortality and morbidity; about 80% of children with advanced disease at diagnosis (TBM stage II and TBM stage III) will suffer severe neurological sequelae.[487, 490] TBM is the commonest cause of bacterial meningitis in the Western Cape.[492]

MDR-TBM has very poor outcome[493-496] but there are little data regarding children. The relationship between the *M. tuberculosis* strain and clinical phenotype has been explored in both adults and children with TBM[41, 76, 497] with conflicting results. The relationship between strain type and drug resistance pattern is complex but a strong association exists between drug resistance and the Beijing genotype.[39, 75, 79] The aim of this study is to analyse whether a relationship exists between the drug susceptibility pattern of the infecting *M. tuberculosis* organism and the clinical outcome of TBM in children and to determine if this relationship is influenced by the genotype of the strain.

Methods

Study population and tuberculous meningitis definition

All children admitted to TCH from January 2003 until April 2009, aged 0-13 years, were included if they had either a diagnosis of confirmed TBM (*M. tuberculosis* isolated from the CSF), or of probable TBM with a positive culture of *M. tuberculosis* from a source other than the CSF. Probable TBM was defined as a clinical diagnosis of meningitis, supported by the presence of characteristic CSF findings (pleiocytosis, elevated protein level and reduced glucose level). In addition, two or more of the following criteria were required: recent weight loss, a positive TST, a CR compatible with TB, a cranial CT scan compatible with TBM or finally, household contact with sputum smear-positive pulmonary TB.[490]

Clinical care

In the Western Cape, HIV-uninfected children with TBM are treated with isoniazid (20mg/kg, maximum 400mg daily), rifampicin (20mg/kg, maximum 600mg daily), pyrazinamide (40mg/kg, maximum 2g daily) and ethionamide (20mg/kg, maximum 1g daily) for six months with HIV-infected children treated for nine months. If the child's isolate of *M. tuberculosis,* or that of the source case, is resistant to any of the drugs used in the local TBM treatment regimen, or if the child deteriorates clinically on this regimen, alternative TB treatment is considered. Treatment is tailored to the DST of the child or source case's isolate. If diagnosed in the context of a failing regimen, treatment is directed at the DST of locally prevailing strains. Treatment for HMR-TB involves the addition of a fluoroquinolone and terizidone with treatment for nine months irrespective of HIV status. Treatment of MDR- and RMR-TB includes any first-line drugs to which the organisms are susceptible, a second-line injectable medication, a fluoroquinolone, and further drugs (from WHO classes four and five) to make up at least four effective drugs with good CSF penetration.[102, 197, 433] Treatment for MDR (and RMR)-TB, for both HIV-infected and -uninfected children, typically consists of six months of intensive phase therapy including an injectable medication followed by a further twelve months of oral therapy.

Children are treated as inpatients at TCH or BCH unless social circumstances are assessed and deemed satisfactory for a home-based care programme. MDR-TBM patients are treated in hospital for at least the intensive phase. All children are treated with steroids. An air encephalogram is performed if there is evidence of hydrocephalus on CT scan; if non-communicating, a ventriculoperitoneal shunt is inserted. HIV testing is performed following informed consent from the parent or legal guardian using ELISA if older than 18 months or

DNA PCR if younger or breast-fed. cART is initiated as soon after HIV diagnosis as is possible.TST is performed by injecting two tuberculin units intradermally (purified protein derivative RT23, Statens Serum Institute) with results read at 48-72 hours. A transverse diameter of ≥10mm is considered positive in HIV-uninfected and ≥5mm in HIV-infected children.

Data collection

Every child with culture-confirmed TB at TCH is recorded prospectively in a clinical database with DST to rifampicin and isoniazid routinely performed on a single sample from all children. A list of children with a diagnosis of TBM was extracted from the database. Case notes were retrieved for these children from TCH and BCH to confirm inclusion criteria and extract clinical details. Patients were included if there was complete documentation of presentation, clinical course and outcome. Development Quotient (DQ) was measured at the end of TB treatment using the Bayley test, Griffiths test or Junior South African Individual Scale, dependant on age. Visual testing was performed clinically. In the majority, formal assessments had been performed by a developmental paediatrician but for some children, an outcome was assigned by the study team based on clinical examinations that had been undertaken by paediatric neurologists, general paediatricians, paediatric registrars or medical officers. For those with complete clinical details, isolates underwent spoligotype analysis.

Mycobacterial culture and drug susceptibility testing

Respiratory samples were inoculated into Middlebrook 7H9 broth-based Mycobacterial Growth Indicator Tubes (MGIT; Becton Dickinson, Sparks, MD, USA) following a standard protocol for decontamination, while samples from sterile sites, including CSF, were inoculated directly. *M. tuberculosis* complex isolates were confirmed as *M. tuberculosis* through PCR.[430] From January 2003 until August 2008 conventional, phenotypic DST was by the indirect proportion method.[254] From August 2008 genotypic DST was performed using the GenoType® MTBDR*plus* (Hain Life Science, Nehren, Germany) LPA, carried out according to the manufacturer's instructions.[432]

Spoligotyping

Genotype determination was performed using standardized spoligotyping methodology.[498] Isolates were assigned to specific genotype families according to their spoligotype signature which included the internationally recognized families of Beijing, LAM (Latin American and Mediterranean family), Haarlem, CAS (Central Asian lineage), a group of ill-defined strains of

the T family, LCC (Low Copy Number Clade) and S family.[445, 499] It was not possible to classify some of the remaining strains.

Data classification

The time from first reported symptoms to initiation of TB therapy was recorded. In cases of DR-TBM, the time from the first reported symptoms to appropriate second-line therapy was also determined. TBM stage was classified as TBM stage I (Glasgow Coma Scale [GCS] 15 with no focal neurological signs), TBM stage II (GCS 11-14 or GCS 15 with focal neurology) or TBM stage III (GCS <11).[490] GCS (or modified paediatric GCS) was assessed and recorded at the time of presentation by the attending doctor. HIV immunological staging was based on WHO criteria.[483] Although the identified strains were recorded, strains were classified as simply Beijing or non-Beijing for analysis. DST was recorded as drug-susceptible, HMR, RMR and MDR. Motor function at the end of therapy was classified as normal, hemiparesis or quadriparesis, cognitive function as normal (DQ >80), mild handicap (DQ: 50-80) or severe handicap (DQ<50) and vision as normal, impaired vision or blind. For analysis, we looked at two dichotomous outcome measures: mortality (alive or dead) and clinical outcome (favourable or unfavourable). A child was classed as having an unfavourable outcome if they died or were left with quadriparesis, severe cognitive handicap or blindness.

Statistical analysis

Data were analysed using STATA version 11 with missing data excluded from analysis. Continuous variables were used for age, time to initiation of appropriate therapy and CSF parameters; all other data were categorical. Associations were assessed using the Fisher's exact test when comparing categorical data with the effect estimated (OR) and 95% CI calculated. The Mann Whitney test was used to assess the effect of age, treatment delay and CSF measurements, given the non-normal distribution of the data with median and IQR.

Risk factors for the two outcomes (unfavourable clinical outcome and death) were assessed in univariate analysis. Multivariable models were used to analyse the relationship between risk factors and outcome if either the univariate relationships showed significance ($p<0.05$) or where variables were thought to be clinically relevant. Standard tests for co-linearity were used.

Results

Patient characteristics

One hundred and forty-two children were identified from the database of children with culture-confirmed TB. On review of the clinical records five did not meet the inclusion criteria. Of the remaining 137 cases, comprehensive clinical details could be found on 123 (Figure 12). The baseline clinical characteristics of these patients are demonstrated in Table 45 with the initial investigations, clinical course and outcome in Table 46. For 104 of these patients samples were located and spoligotyping successfully performed. Ninety-eight (79.7%) of the 123 children included in the analysis were tested for HIV, and of these 20 (20.4%) were HIV-infected. Six (30.0%) of the HIV-infected children had severe immunosuppression at the time of TBM diagnosis, and only three (15.0%) were on cART.

Drug resistance, strain type and outcome

Sixteen children (13.0%) had isolates with drug resistance, five MDR (4.1%), ten HMR (8.1%) and one RMR (0.8%). No XDR-TB cases were identified. Univariate analysis showed an association between MDR-TB and both poor clinical outcome (OR 8.97; 95%CI 0.83-4447.5; p=0.04) and death (OR 67.3, 95%CI 5.0-3343; p<0.001) as shown in Table 47. There was no association between Beijing strain and unfavourable outcome (p=0.29) or mortality (p=1.0). In addition, there was no relationship between Beijing strain and any drug resistance (p=0.21) or MDR (p=1.00). A trend towards an association existed between MDR-TB and HIV (OR 6.71, 95%CI 0.69-83.7; p=0.056), but not with TBM stage (p=0.22). Beijing strain was not associated with HIV status (p=0.78) or TBM stage (p=0.14).

Clinical factors and outcome

Children with unfavourable outcome were younger than those with favourable outcome (median age: 21 months [IQR: 7-35] vs. 30 months [IQR: 15-72]; p=0.008). They also had lower CSF lymphocyte counts (median: 35 cells/μl [IQR: 17-61] vs. 75 cells/μl [IQR: 27-159]; p=0.002). CSF lymphocyte counts were not associated with HIV infection (p=0.24) or strain type (p=0.07). TBM stage III (p<0.001), shunt insertion (p=0.002) and intensive care admission (p=0.02) were associated with unfavourable outcome and reflect disease severity (Table 47 & Table 48). HIV infection was associated with death (OR 6.17, 95%CI 1.15-34.1; p=0.02) and for those dying the time from first symptoms to appropriate treatment was longer (median: 22 days [IQR: 6-61] vs. 10 days [IQR: 5-21]; p=0.049). Time from start of symptoms to initiation of appropriate TB treatment was longer for those with any drug-resistance than those with drug-susceptible TBM (median: 31 days [IQR: 13-66] vs. 9 days [IQR: 5-21]; p=0.001). Time to start appropriate

therapy was not influenced by the presence of a known TB source case (p=0.82), the HIV status of the child (p=0.10) or the age of the child (p=0.82).

Multivariable analysis

Following adjustment for HIV status in multivariate analysis (Table 49) the relationship between MDR-TB and death persisted (AOR 63.9, 95%CI 4.84-843.2; p=0.002). Young age (p=0.013) and MDR-TB (AOR 12.4, 95%CI 1.17-132.3; p=0.037) remained independent risk factors for unfavourable outcome. Those with HMR-TB did not have an increased risk of unfavourable outcome after adjustment for age (AOR 0.22, 95%CI 0.03-1.87; p=0.17). The relationship between HIV and death was less significant following adjustment for drug resistance (AOR 6.17, 95%CI 0.92-41.3; p=0.061).

Discussion

Children with TBM in the Western Cape are young, generally present with advanced disease and, if they survive, are usually left with some form of disability. Rates of drug resistance are relatively low but this study has demonstrated that the time from first symptoms of TBM to the child being given appropriate, effective treatment is longer when the child's isolate is resistant to rifampicin and/or isoniazid. Young age is associated with a poor outcome. In this study, Beijing strain was not associated with drug resistance and there was no association between Beijing strain and either poor outcome or death. MDR-TB, however, was strongly associated with both unfavourable outcome and death, even after adjusted analysis.

A study by Thwaites and colleagues demonstrated that adults with TBM were much more likely to die if infected with an organism resistant to both isoniazid and rifampicin but had no increased risk if resistant to isoniazid alone and/or streptomycin.[494] Other work by the same group demonstrated that HIV infection in adults does not change the clinical presentation of TBM but does influence outcome.[500] A case series of adults from KwaZulu-Natal demonstrated that MDR-TBM was often associated with poor outcome[493] and a series from Durban described eight children with MDR-TBM, of whom seven died.[193] Caws and colleagues demonstrated a relationship between Beijing strain and both HIV infection and drug resistance in adults with TBM.[75] However, Maree and colleagues found, as with our study, no association between strain type and either presentation or outcome in an investigation of children with TBM.[76] Other studies have demonstrated a relationship between strain type and disease phenotype in children[77] and in adults[41, 78] and a number of investigations have demonstrated that strain type, and the Beijing strain specifically, is associated with drug resistance.[39, 79-81]

The association between low CSF lymphocyte count and poor outcome in TBM has been demonstrated in other studies.[41, 501] Previous investigations have shown a relationship between different strains and CSF lymphocyte count which we did not demonstrate. The inflammatory response to TBM is the cause of some of the pathology but it is clear from these and previous data that a failure to mount a lymphocyte response is associated with poor outcome.

In the Western Cape, children with TBM are treated with rifampicin, isoniazid, pyrazinamide and ethionamide.[468] This is in contrast to the WHO guidelines which previously recommended rifampicin, isoniazid, pyrazinamide and streptomycin for two months followed by isoniazid and rifampicin for four months[16] but now recommends rifampicin, isoniazid, pyrazinamide and ethambutol for two months followed by rifampicin and isoniazid for ten months.[113] Isoniazid, pyrazinamide and ethionamide penetrate into the CSF well, rifampicin adequately and ethambutol and streptomycin poorly.[502] In addition, a high proportion of MDR-TB cases have evidence of resistance to ethambutol and pyrazinamide,[161] implying that if a strain is MDR, ethambutol and pyrazinamide should not be assumed to be effective. One final factor that needs to be considered is the genotypic basis of drug resistance which is complicated by cross-resistance and co-resistance.[91] Resistance to isoniazid is usually caused by mutations in either the *katG* gene or the *inhA* promoter region. *KatG* mutations are usually associated with total resistance to isoniazid but if the mycobacteria posess an *inhA* promoter region mutation, this usually results in low-level isoniazid resistance which can be overcome by giving isoniazid at a higher dose (15-20mg/kg).[90, 92] *InhA* promoter region mutations, however, usually result in ethionamide resistance. One explanation for the good outcomes in our study for children with HMR-TB might be that until the diagnosis was made and appropriate treatment started, they received a number of effective drugs with good CSF penetration. Using either the old or the new WHO guidelines this would not have been the case.

The majority (63%) of children presenting with TBM had an identified TB source case but few (15%) had been given preventive treatment. In addition to identifying a source case it is vital to determine the DST pattern of that source case to start appropriate preventive treatment or, if disease develops, disease treatment for the child. Although four of the five children with MDR-TB had been given some kind of prior treatment, none had been treated appropriately. As children in contact with MDR-TB have been previously demonstrated to develop TB on isoniazid preventive treatment,[88] the correct preventive treatment for child contacts of MDR-TB remains unclear.[87] Although it is important to strive to obtain a microbiological diagnosis

from the child, in reality only a small proportion of children with TBM have microbiological confirmation with DST. Most children are treated presumptively and unless a source case is identified, *M. tuberculosis* cultured and DST performed, cases of drug-resistant TBM in children will be missed. Where this is HMR-TBM, it is possible that the current local regimen will adequately treat the disease; however in the context of MDR-TBM outcome is poor unless appropriate second-line treatment is initiated rapidly. Of note, although over 85% of children had evidence of BCG vaccination, TBM still occurred. The protective efficacy of BCG remains debated and the need for effective vaccines is a pressing priority. Only 80% of children were tested for HIV, despite prolonged hospitalisation for a condition known to be associated with HIV infection. All children suspected of TBM should be tested for HIV, especially in a region with high HIV prevalence.

This study is retrospective and the data analysed is reliant on collection from routine sources such as case notes and laboratory records. As there were relatively few cases that had drug resistance, statistical analysis may not have revealed associations that may have been evident if a larger proportion of the cases had been DR. The children in this study may not be representative of all children with TBM. First, the study was carried out in a hospital which may have a more severe disease phenotype than those managed in the community. Second, as a positive mycobacterial result was required for inclusion in the study it is possible that the children had more advanced disease than is typical. A further limitation may have been that survival bias occurred with those presenting to TCH having a greater chance of both survival and drug resistance being diagnosed. Finally, the outcome was only recorded at the end of therapy. Longer follow up would have been desirable.

Table 45 - Presenting clinical characteristics (n=123 unless otherwise stated)

		DS (107)	HMR (10)	RMR (1)	MDR (5)	Total (123)
Age (median & IQR in months)		28 (12-56)	25 (21-38)	14	26 (26-50)	27 (13-55)
Male gender (%)		53 (49.5)	5 (50)	0	1 (20)	59 (48.0)
HIV-infected (n=98; %)		16 (19.3)	1 (11.1)	0	3 (60)	20 (20.4)
Evidence of BCG (n=111; %)		83 (85.6)	7 (87.5)	1	4 (80)	95 (85.6)
TB contact history (n=116; %)		61 (60.4)	7 (77.8)	1	4 (80)	73 (62.9)
Preventive/previous treatment (n=122; %)		11 (10.4)	2 (20)	1**	4 (80)*	18 (14.8)
TST positive (n=108; %)		64 (68.8)	7 (77.8)	0	3 (60)	74 (68.5)
Time from start of symptoms to treatment initiation (median & IQR in days)		9 (5-21)	16 (14-30)	3	6 (2-19)	9 (5-21)
Time from start of symptoms to appropriate treatment initiation (median & IQR in days)		9 (5-21)	31 (14-53)	82	19 (6-51)	11 (5-22)
Presenting symptoms (more than one in most cases; %)	Decreased consciousness	57 (53.3)	5 (50)	0	5 (100)	67 (54.5)
	Headache	26 (24.3)	4 (40)	0	0	30 (24.4)
	GI disturbance	16 (15.0)	2 (20)	0	1 (20)	19 (15.5)
	Poor feeding	17 (15.9)	1 (10)	0	0	18 (14.6)
	Seizures	47 (43.9)	5 (50)	0	2 (40)	54 (43.9)
	Vomiting	50 (46.7)	3 (30)	0	2 (40)	55 (44.7)
	Cough	40 (37.7)	4 (40)	1	3 (60)	48 (39.3)
	Weight loss	93 (86.9)	8 (80)	1	5 (100)	107 (87.0)
	Fever	72 (67.3)	8 (80)	1	4 (80)	85 (69.1)
	Irritability	9 (8.4)	0	0	0	9 (7.3)
	Lethargy	30 (28.0)	1 (10)	1	1 (20)	32 (26.0)
	Neck stiffness	23 (21.5)	1 (10)	1	1 (20)	25 (20.3)
TBM stage (%)	I	22 (20.1)	3 (30)	1		26 (21.1)
	II	44 (41.1)	5 (50)	0	1 (20)	50 (40.7)
	III	41 (38.3)	2 (20)	0	4 (80)	47 (38.2)
GCS (median & IQR)		12 (9-15)	14 (11-15)	15	6 (5-11)	12 (9-15)
Cranial nerve abnormalities noted at presentation (%)		52 (48.6)	8 (80)	1	3 (60)	57 (46.3)
Motor abnormalities noted at presentation (%)		72 (67.3)	2 (20)	1	0	85 (69.1)

TB = tuberculosis; TBM = TB meningitis TST = tuberculin skin test; DS = drug-susceptible; HMR = isoniazid monoresistant; RMR = rifampin monoresistant; MDR = multidrug resistant; IQR = inter-quartile range; GCS = Glasgow coma scale

*One child developed TBM whilst on first-line treatment for pulmonary TB. One child was given isoniazid prophylaxis, one child developed TBM whilst on treatment for confirmed MDR-TB (suspicion of XDR-TB). One child had MDR-TB prophylaxis (isoniazid, ethambutol and ofloxacin) from birth but was then re-exposed over a year later and developed MDR-TBM. The final child received no preventive treatment.
**This child was prescribed isoniazid and rifampin prophylaxis at birth, but it was not given. The child presented almost a year later with Stage I TBM. Nearly three months later resistance testing showed RMR, and although clinically well, treatment was changed to MDR-TB treatment. However, she died two months later after sudden deterioration.

Table 46 - Investigations at diagnosis, clinical course and outcome (n=123 unless otherwise stated)

		DS (107)	HMR (10)	RMR (1)	MDR (5)	Total (123)
Diagnosis (%)	Confirmed TBM	23 (21.5)	2 (20)	1	4 (80)	30 (24.4)
	Probable TBM	84 (78.5)	8 (80)	0	1 (20)	93 (75.6)
Strain (n=104; %)	Beijing	30 (33.0)	6 (60)	0	1 (33.3)	37 (35.6)
	LAM	18 (19.8)	2 (20)	0	0	20 (19.2)
	Haarlem	5 (5.5)	1 (1)	1	0	7 (6.7)
	CAS	3 (3.3)	0	0	0	3 (2.9)
	Ill-defined T family	16 (17.6)	0	0	0	16 (15.4)
	LCC	5 (5.5)	0	0	0	5 (4.8)
	S family	7 (7.7)	0	0	2 (66.7)	9 (8.7)
	Undefined	7 (7.7)	0	0	0	7 (6.7)
CSF Lymphocytes (n=116; median & IQR)		57 (24-138)	28 (5-130)	73	75 (35-105)	57 (22-132)
CSF Protein (n=108; median & IQR)		1.3 (0.9-2.1)	1.3 (0.4-2.2)	1.2	8.4 (1.3-15.5)	1.4 (0.9-2.1)
Air encephalogram (n=61; %)	Non-communicating hydrocephalus	19 (34.6)	1 (25)	-	1 (50)	23 (37.7)
	Communicating hydrocephalus	36 (65.5)	3 (75)	-	1 (50)	38 (62.3)
Ventriculoperitoneal shunt inserted (%)		25 (23.4)	2 (20)	0	2 (40)	29 (23.6)
Admitted to intensive care (%)		13 (12.2)	0 (0)	0	4 (80)	17 (13.8)
Duration of admission (median & IQR in days)*		27 (16-38)	32 (18-53)	35	36 (29-40)	28 (17-39)
Motor function amongst survivors at end of therapy (n=112; %)	Normal	54 (53.5)	8 (80)	0	1	63 (56.3)
	Hemiparesis	34 (33.7)	2 (20)	0	0	36 (32.1)
	Quadriparesis	13 (12.9)	0 (0)	0	0	13 (11.6)
Cognitive function amongst survivors at end of therapy (n=112; %)	Normal	37 (36.6)	5 (50)	0	0	42 (37.5)
	Mild handicap	37 (36.6)	4 (40)	0	1	42 (37.5)
	Severe handicap	27 (26.7)	1 (10)	0	0	28 (25.0)
Vision at amongst survivors at end of therapy (n=108; %)	Normal	77 (79.4)	9 (90)	0	1	87 (80.6)
	Impaired vision	15 (15.5)	1 (10)	0	0	16 (14.8)
	Blind	5 (5.2)	0 (0)	0	0	5 (4.6)
Mortality (%)	Survived	101 (94.4)	10 (100)	0	1 (20)	112 (91.1)
	Died	6 (5.6)	0 (0)	1	4 (80)	11 (8.9)
Clinical outcome (%)	Favourable	74 (69.2)	9 (90)	0	1 (20)	84 (68.3)
	Unfavourable	33 (30.8)	1 (10)	1	4 (80)	39 (31.7)

* Duration of admission at Tygerberg Children's Hospital

TB = tuberculosis; TBM = TB meningitis TST = tuberculin skin test; DS = drug-susceptible; HMR = isoniazid monoresistant; RMR = rifampin monoresistant; MDR = multidrug resistant; IQR = inter-quartile range; GCS = Glasgow coma scale; CSF = cerebro-spinal fluid; CAS – Central Asian Strain; LAM – Latin American Mediterranean; LCC – Low-copy-number Clade

Table 47 - Univariate relationship between microbiological factors, clinical characteristics and clinical outcome

		Total number	Favourable Outcome	Unfavourable Outcome	OR (95%CI)	p-value
DST	Drug susceptible	107	74	33	1.00	
	Isoniazid mono-resistant	10	9	1	0.25 (0.01-1.95)	0.28#
	Rifampicin mono-resistant	1	0	1	-	0.32#
	Multidrug resistant	5	1	4	8.97 (0.83-447.5)	0.04#
Strain	Beijing	37	22	15	1.00	
	Non-Beijing	67	47	20	0.62 (0.25-1.58)	0.29#
HIV status of child	Negative	78	54	24	1.00	
	Positive	20	11	9	1.84 (0.59-5.61)	0.29#
Age of child*						0.008##
Gender of child	Female	64	41	23	1.00	
	Male	59	43	16	0.66 (0.28-1.53)	0.34#
BCG status of child	None	16	12	4	1.00	
	Given	95	61	34	1.67 (0.46-7.64)	0.57#
TBM stage of child	I	26	22	4	1.00	
	II	50	47	3	0.35 (0.05-2.30)	0.22#
	III	47	15	32	11.7 (3.10-53.2)	<0.001#
Time to appropriate therapy**						0.98##
CSF Lymphocyte count***						0.002##
CSF Protein****						0.88##
Ventriculoperitoneal shunt inserted	No	94	71	23	1.00	
	Yes	29	13	16	3.80 (1.45-9.94)	0.002#
Admission to intensive care	No	106	77	29	1.00	
	Yes	17	7	10	3.79 (1.16-12.8)	0.02#

OR = odds ratio, CI = confidence interval, TBM = tuberculous meningitis, CSF = cerebrospinal fluid; DST = drug susceptibility test
Unfavourable outcome – death, quadriparesis, severe cognitive handicap or blindness

\# Fisher's Exact test used
\## Mann Whitney test used
* Median age in months: Favourable outcome: 30; Unfavourable outcome: 21
** Median time in days: Favourable outcome: 9; Unfavourable outcome: 14
*** Median count: Favourable outcome: 75; Unfavourable outcome: 35
**** Median value: Favourable outcome: 1.32; Unfavourable outcome: 1.41

Table 48 - Univariate relationship between microbiological factors, clinical characteristics and death

		Total number	Survivors	Deaths	OR (95%CI)	p-value
DST	Drug susceptible	107	101	6	1.00	
	Isoniazid mono-resistant	10	10	0	-	1.0#
	Rifampicin mono-resistant	1	0	1	-	0.06#
	Multidrug resistant	5	1	4	67.3 (5.0-3343)	<0.001#
Strain	Beijing	37	34	3	1.00	
	Non-Beijing	67	61	6	1.11 (0.22-7.32)	1.0#
HIV status of child	Negative	78	74	4	1.00	
	Positive	20	15	5	6.17 (1.15-34.1)	0.02#
Age of child*						0.34##
Gender of child	Female	64	56	8	1.00	
	Male	59	56	3	0.38 (0.061-1.68)	0.21#
BCG status of child	None	16	14	2	1.00	
	Given	95	86	9	0.73 (0.13-7.69)	0.66#
TBM stage of child	I	26	23	3	1.00	
	II	50	49	1	0.15 (0.003-2.12)	0.11#
	III	47	40	7	1.34 (0.27-8.78)	1.0#
Time to appropriate therapy**						0.049##
CSF Lymphocyte count***						0.54##
CSF Protein****						0.16##
Ventriculoperitoneal shunt inserted	No	94	85	9	1.00	
	Yes	29	27	2	0.70 (0.07-3.70)	1.0#
Admission to intensive care	No	106	100	6	1.00	
	Yes	17	12	5	6.94 (1.41-31.6)	0.008#

OR = odds ratio, CI = confidence interval, TBM = tuberculous meningitis, CSF = cerebrospinal fluid; DST = drug susceptibility test

\# Fisher's Exact test used

\## Mann Whitney test used

* Median age in months: Survival: 28; Death: 26

** Median time in days: Survival: 10; Death: 22

*** Median count: Survival: 57; Death: 39

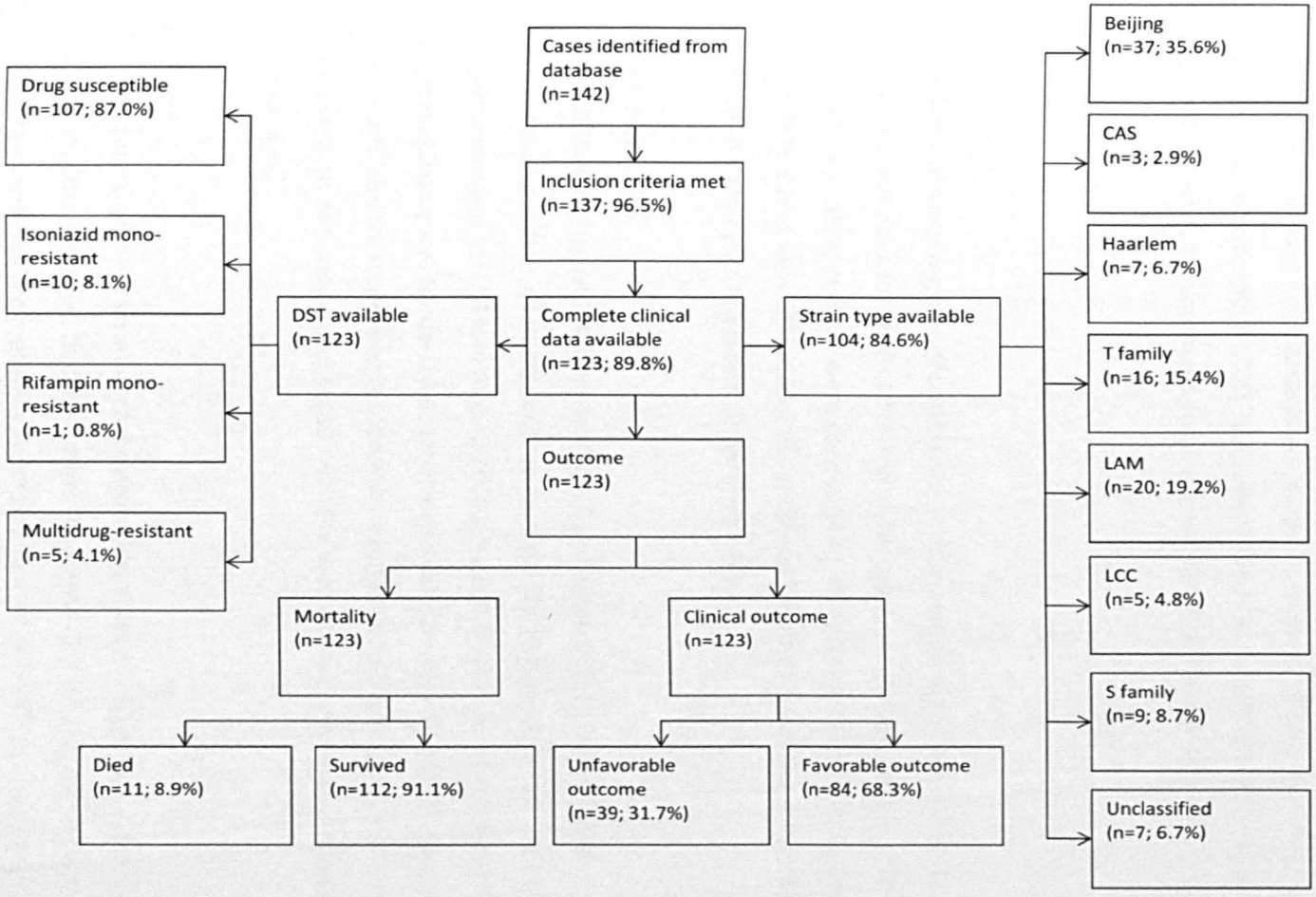
**** Median value: Survival: 1.32; Death: 2.0

Table 49 - Multivariable relationship between drug resistance and outcome

Outcome	Characteristics in model	Variable	Number in analysis	Odds Ratio	95% Confidence Interval	P-value
Unfavourable outcome	Age		122			0.013
	DST	Isoniazid mono-resistant	122	0.22	0.03-1.87	0.17
		Rifampin mono-resistant	122*	-	-	-
		Multidrug-resistant	122	12.4	1.17-132.3	0.037
Mortality	HIV status		88	6.17	0.92-41.3	0.061
	DST	Isoniazid mono-resistant	88**	-	-	-
		Rifampin mono-resistant	88*	-	-	-
		Multidrug-resistant	88	63.9	4.84-843.2	0.002

DST = drug susceptibility test
Unfavourable outcome – death, quadriparesis, severe cognitive handicap or blindness
*Perfectly predicts failure in this model so dropped from analysis
**Perfectly predicts success in this model so dropped from analysis

Figure 12 - Patient identification, inclusion, mycobacterial characteristics and outcome



DST – drug susceptibility test; CAS – Central Asian Strain; LAM – Latin American Mediterranean; LCC – Low-copy-number Clade

Study 12: multidrug-resistant tuberculosis of the spine in children

The following study has been published as an article:

- *Seddon JA, Donald PR, Vlok GJ, Schaaf HS. Multidrug-resistant tuberculsois of the spine in children – characteristics from a high burden setting. J Trop Pediatr 2012; 58: 341-7*

Introduction

Spinal TB in children can be a debilitating disease with potential long-term neurological sequelae. Treatment involves a combination of surgical and medical care with long courses of drug therapy. Few paediatric spinal MDR-TB studies have been published. This study describes the clinical characteristics, management and outcome for children with MDR-TB of the spine.

Methods

Identification of cases

A prospectively maintained register of admissions to BCH was analysed searching for any patient started on treatment for MDR- TB of the spine between January 2004 and December 2010. In addition, laboratory records were consulted to identify any MDR-TB samples from spinal tissue at TCH. Cases were included if the child had a sample taken from the spine that confirmed MDR-TB either phenotypically or genotypically.

Laboratory methods

From January 2004 until August 2008 conventional, phenotypic DST to isoniazid and rifampicin was performed on all paediatric samples culture-positive for *M. tuberculosis*. The indirect proportion technique was used. From August 2008 onwards line probe assay (GenoType® MTBDRplus, Hain Lifescience, Nehren, Germany) was undertaken to test for the presence of *M. tuberculosis* and also for mutations responsible for rifampicin (*rpoB* gene) and isoniazid (*inhA* promoter region and *katG* gene) resistance. The laboratory techniques employed are discussed elsewhere.[432]

Standard of care

Surgery was conducted at the discretion of the attending surgeon. Surgery was indicated to obtain a microbiological diagnosis, to decompress the spinal cord and to correct kyphotic deformity. Children were treated medically according to WHO guidelines, using the DST of the

child to guide treatment when culture-confirmed or that of the source case when diagnosed presumptively. At least four drugs to which the organism was susceptible were used which included pyrazinamide (30-40mg/kg), ethambutol (20-25mg/kg), ofloxacin (15-20mg/kg), amikacin (15-25mg/kg), capreomycin (15-25mg/kg), terizidone (15-20mg/kg), PAS (150-200mg/kg) and isoniazid given at high dose (15-20mg/kg). The injectable drug (amikacin or capreomycin) was typically given for the first six months and the total treatment duration was for a minimum of eighteen months. Regular monitoring of response included radiology with CT or magnetic resonance imaging (MRI), growth and clinical assessment. The monitoring of adverse effects included clinical examination, audiology and blood tests. Follow-up continued to twelve months following the end of therapy.

Results

Eleven children were identified of which four were excluded. Clinical details and presentation are summarised in Table 50. One of the excluded children was diagnosed as having MDR-TB from a gastric aspirate and whilst an inpatient noted to have reduced tone, power and reflexes in lower limbs. An MRI scan of the spine showed changes consistent with TB but as the diagnostic sample was not from the spinal tissue this girl did not meet our inclusion criteria. Two children were excluded as they were only treated presumptively for MDR-TB. In one of these, acid-fast bacilli were seen on a biopsy sample, the child was failing first-line therapy and he had been in contact with an MDR-TB source case. However, the biopsy sample did not grow on culture. The other presumptively-treated child developed spinal TB whilst on first-line therapy. The final child excluded had radiological TB of the spine and was a contact of an MDR-TB source case. He was started on MDR-TB treatment but when the biopsy result from the spine demonstrated drug-susceptible TB he was converted onto first-line therapy.

Of the seven children with culture-confirmed MDR-TB, five were boys and the median age was eight years (range 1.5 to 14). The median delay from start of MDR-TB episode to initiation of appropriate therapy was 36 weeks (range 7 to 76 weeks). One child (child 4) was infected HIV and had a CD4 count of 545 (19%). MDR-TB source cases were not identified for any of the children.

Details of treatment and outcome are shown in Table 51. Injectable treatment was given for a median of 6 months with total treatment duration a median of 18 months. One child died, five completed treatment and one was near the end of therapy at the time of the study. The

medications were well tolerated and although two of the surviving children had marked spinal deformity, none had any significant neurological deficit.

Discussion

All seven cases were associated with significant delay in initiation of appropriate therapy with the diagnosis usually only made after a first-line regimen had failed. Failure to take microbiological samples early on and request appropriate tests was frequently to blame. This is not only important for making the diagnosis of MDR-TB but also for confirming drug-susceptible TB in those suspected of MDR-TB; consequently one child was spared long and unnecessary treatment. All samples taken from children suspected of TB should be sent for culture and DST to at least isoniazid and rifampicin. Although a contact history is important in the diagnosis of TB and particularly MDR-TB, its absence, particularly in older children who may spend more time in the community, does not exclude the diagnosis. A number of the children initially had pulmonary TB which then seems to have spread to the spine in the face of inadequate treatment. All the children, even those with relatively minor involvement, were treated for at least eighteen months and all were operated on, even if only to drain collections of pus or cold abscesses. Where vertebral damage had occurred and deformity was present, posterior fusion was indicated following decompression. The children tolerated both surgical and medical therapy well without the severe adverse effects frequently described in adult.[503] Additionally, the short-term outcome seems to have been reasonable, again contrary to the poor results seen in older patients.[190] The reason for this is not clear but in this series only one child was HIV-infected, a factor commonly associated with poor outcome. Long-term results, however, have not been assessed and for some of the severe cases, morbidity may occur in time.

In the paediatric literature, little has been written concerning MDR-TB and almost nothing with regards to MDR-TB of the spine. Case histories of paediatric MDR-TB of the ankle[504], mastoids[505], femoral head[506] and hip[507] have been described. Case series of sternal TB[508] and non-contiguous spinal TB[509] included some MDR-TB cases and some children. In a further series of 39 paediatric MDR-TB cases, two had osteoarticular TB.[58] Several papers describe case series or surveys of TB in which a proportion are described as paediatric, some spinal and a percentage drug-resistant.[510-511]Lindquist et al. describe a child with MDR-TB of the first cervical vertebra[512] and Hussey et al. describe a case of disseminated MDR-TB with multiple bony foci including the spine.[513] Agashe et al.[514] report 93 osteoarticular TB cases of which five are MDR in children under ten. Pawar et al.[238] describe 25 cases of MDR spinal TB, of which

seven were in children. In all these reports, diagnoses were associated with significant delay. However, most of the studies describe children tolerating therapy well with a good response to medical treatment. Long term follow up was not undertaken.

It is known that following initial infection, seeding to the spine shows preference for the vertebral bodies. There is bony destruction with both caseous and avascular necrosis. The disease, if untreated, is likely to spread directly into adjacent vertebrae, the spinal cord or into the paraspinal muscles causing a cold abscess.[515-516] Mycobacteria can also disseminate into other, more distant, vertebral bodies via the valveless venous plexus system to produce skip or non-contiguous lesions.[509] The neurological consequences of spinal TB are the most concerning and the evolution of a Pott's paraplegia the most severe, occurring in up to 10% of untreated cases.[517-518] Indirect damage can result from either compression on the spinal cord from an abscess or the collapse of a vertebra. Direct damage can result from invasion of the cord by mycobacteria.[519] In addition, even if successfully treated, the patient can suffer major neurological consequences, often many years later, due to spinal cord damage secondary to vertebral collapse, fibrosis or calcification.[516]

Little is known regarding the treatment and outcomes for paediatric spinal MDR-TB. It is, therefore, worth looking at the treatment of drug-susceptible spinal TB in children and drawing comparisons. A series of studies was carried out in the 1960s and 1970s by the Medical Research Council Working Party on Tuberculosis of the Spine.[516] Although medical management alone seems to result in similar outcomes in terms of mycobacterial clearance, there is evidence that benefit is seen with additional surgical drainage, debridement and fixation. Attention has been drawn to the consequences of late onset paraplegia developing in those with extensive bone destruction treated conservatively in childhood.[516]

Controversy exists regarding the length of treatment for drug-susceptible spinal infections. Whilst most evidence suggests that treating for the same duration as pulmonary TB has excellent outcomes in well adhered-to regimens,[520] a couple of studies have shown cases of treatment failure with six month treatment courses.[521] Monitoring therapy with radiology, inflammatory markers (C-reactive protein, erythrocyte sedimentation rate) and immune markers (interferon-γ release assays) are often employed by clinicians in deciding when to stop treatment; only stopping when markers return to normal. It is unclear whether these strategies are beneficial. Both the Joint Tuberculosis Committee of the British Thoracic Society[522] and NICE[137] have stated that a six month regimen is appropriate for drug-susceptible spinal TB. This is similar to the ATS, CDC, IDSA[523] and the AAP.[524] WHO had previously advised

that six months was sufficient,[16] but now recommend twelve months. They concede that this is based on poor quality evidence.[113] It is probably safe to use the same indications for surgery in spinal lesions infected with MDR and drug-susceptible TB and until further evidence emerges, the duration of therapy for MDR spinal disease should be the same as for MDR pulmonary TB.

It appears that both first- and second-line drugs are able to penetrate bone, cartilage, pus, cavity and granuloma, achieving levels well above the MIC for each drug.[108, 525-527] Penetration into cortical and sclerotic bone, although adequate, is much poorer than into other tissues.[528]

Table 50 - Patient characteristics and presentation in children with culture-confirmed multidrug-resistant tuberculosis of the spine

	Gender and age in years at start of MDR TB treatment	Time from start of MDR-TB episode to start of MDR-TB treatment	Known contact	Initial presentation	Rx prior to MDR TB treatment
1	M (8)	Died prior to MDR-TB treatment	None	Progressive paraplegia and marked gibbus formation over preceding two years	Six months of first-line therapy four years earlier and then a further course of first-line therapy from surgery until death
2	M (4)	7 weeks	Father retreatment TB case, no DST undertaken	Three weeks of cough, loss of weight, fever, vomiting and inability to walk	Given first-line therapy from presentation until the results of DST from operative specimen returned
3	F (8)	10 months	None	Pain and deformity in lumbar spine	Six months of first-line therapy then three months no treatment. Recurrence of spinal TB and bilateral psoas abscesses. Drained and MDR-TB grown. 18 months MDR-TB treatment but with uncertain adherence. No treatment for 25 months prior to representation with recurrence of spinal disease. Further nine months of MDR-TB treatment with uncertain adherence prior to development of draining sternal sinus and new spinal lesions. Admitted for full MDR-TB treatment as inpatient
4	M (1.5)	8 months	None	Cough, loss of weight, sweating	Started on first-line therapy but developed worsening respiratory distress 5 months into treatment
5	F (9)	19 months	None	Cough, loss of weight and sweating	Completed six months of first-line therapy. Well for 5 months before developing back pain, night sweats, weight loss and mass in flank. Restarted on first-line therapy but after one month, without improvement, referred for surgical drainage
6	M (14)	5 months	None	Neck pain	Initially given analgesia for sporting injury. The neck pain worsened and he lost weight and developed fever. Three sputum samples were sent for smear (all negative) but no culture. Started presumptively on first-line therapy. Continued deterioration before chest radiograph demonstrated pulmonary TB with likely paravertebral abscess and collapse of T2/T3
7	M (2)	18 months	Mother TB	Spinal deformity	At presentation biopsy sample taken and child started on first-line therapy for spinal TB. Culture result showed MDR-TB but the child could not be traced. Child represented 18 months later with marked deterioration and significant gibbus

(MDR = multidrug-resistant, DST = drug susceptibility testing)

Table 51 - Treatment and outcome in children with culture-confirmed multidrug-resistant tuberculosis of the spine

	Basis of spinal MDR-TB diagnosis	Spinal involvement	Surgery	DST	Length of intensive phase	Total length of treatment	Drugs used	Outcome	Deficit
1	Culture confirmation from spinal tissue sample	T4 to T11 breakdown, large abscess and 90 degree angulation	Posterior decompression and abscess drainage	Resistant to RH	n/a	n/a	n/a	Died	Died
2	Culture confirmation from spinal tissue sample	C5 to T1 with possible myelopathy at C8	Anterior decompression and external fixation with HALO jacket	Resistant to RH	6 months	18 months	H;Z;E;Eto;O;T;A	Treatment completed	Some residual clawing of little and ring fingers of left hand
3	Culture-confirmation on multiple occasions from pus samples	Breakdown at C7/C8 and L5/S1 with repeated collections	Repeated drainage of psoas abscesses but no spinal surgery	Resistant to RH	6 months	18 months	H;Z;E;Eto;O;T;A	Treatment completed	Bilateral high frequency hearing loss. No neurological deficit
4	Culture-confirmation from surgery biopsy specimen	C4/C5 with post and pre-paraspinal abscesses	Drainage of abscesses without spinal surgery	Resistant to RHE	6 months	18 months	H;Z;Eto;O;A	Treatment completed	Free from any neurological symptoms
5	Culture confirmation from pus samples	Lytic lesions T12-L2 bilateral psoas abscesses	Drainage of abscesses	Resistant to RHEA	6 months	18 months	H;Z;Eto;O;T;C	Treatment completed	Slight scoliosis but with full range of movement
6	Culture confirmation from pus sample	Collapse T2/T3 with paraspinal collection T1-T4	Anterior decompression C7-T4 followed two weeks later by posterior fusion	Resistant to RHEO	6 months	Plan for 18 months	H;Z;Eto;A;T;PAS	15 months of treatment completed with a further 3 months planned	Free from any neurological symptoms
7	Culture confirmation from biopsy sample	Collapse of T10/ T11 with 90 degree angulation of spine	Anterior decompression and posterior fusion	Resistant to RH	7 months	24 months	H;Z;E;Eto;O;A;T	Treatment completed	Marked deformity but intact neurology

(R-rifampicin, H-isoniazid, E-ethambutol, Z-pyrazinamide, Eto-ethionamide, O-ofloxacin, A-amikacin, T-terizidone, C-capreomycin, PAS-*para*-aminosalicylic acid)

Study 13: hearing loss in children treated for drug-resistant tuberculosis

The following study has been submitted as an article:

- *Seddon JA, Thee S, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in children treated for mulitdrug-resistant tuberculosis. J Infect (in press)*

Introduction

The aminoglycosides (amikacin and kanamycin), together with capreomycin (a polypeptide), are classified as group two drugs by WHO. These injectable second-line agents are vital for the management of MDR-TB.[197] Although strains resistant to rifampicin but susceptible to isoniazid can be treated with slightly less intense regimens, these RMR cases are usually treated as MDR-TB in most National TB Programmes. This is due to the limitations of modern molecular diagnostic tests which either do not test for isoniazid resistance[529] or miss a significant proportion of cases which have phenotypic resistance.[226] In most circumstances rifampicin resistance is seen as a surrogate for multidrug resistance.

Both the aminoglycosides and polypeptides are known to have adverse effects that include renal and eighth cranial nerve impairment.[214, 241, 530] The effects on the kidneys are thought to be temporary but those on the vestibulo-cochlear system are permanent.[322-323] Hearing loss related to injectable TB drug use usually starts in the high frequencies and if treatment continues, there is progression to the lower frequencies required for communication; however, in some cases severe hearing loss can develop acutely. Hearing is vital not only for effective communication but also for neurological development. Children with hearing deficits have delayed developmental and communication milestones compared to children with normal hearing.[243, 531-532]

Hearing testing for children is performed for two reasons. The first is to identify and quantify hearing loss to enable the provision of support, education, training and hearing aids. The second is to identify hearing loss early, when it is mild and only at high frequencies, so that treatment, where possible, can be changed to prevent further damage. The testing of hearing is challenging in children. PTA is the method of choice for testing adults and allows the testing of different frequencies and amplitudes in both ears independently.[425] PTA is only possible in children on therapy who are able to understand commands and co-operate with testing, which

effectively precludes its use in children younger than five years. As young children are at high risk of developing TB following infection and as young children bear the brunt of the epidemic in many settings,[254] this means that many children are excluded from this form of testing. Auditory brainstem response (ABR) testing is the optimal testing methodology for young children[533] but is only available in South Africa in specialist centres. OAE testing can assess cochlear patency in younger children and is widely available. OAEs are not fully validated for quantifying hearing loss and do not provide as comprehensive an assessment as PTA or ABR.

The frequency and severity of hearing loss is unknown in children treated for MDR-TB with injectable medications. Some data are available for children given these injectable drugs as short antibiotic courses for the treatment of other bacterial infections.[241, 534] Some data regarding ototoxicity are available for adults treated for MDR-TB,[244] but few studies have examined the adverse effects of injectable drugs in children treated for MDR-TB. The aim of this study was to determine the frequency and extent of hearing loss in children treated with an aminoglycoside or polypeptide as part of an MDR-TB regimen.

Methods

Setting

Children with MDR- and RMR-TB present to various regional health centres but once diagnosed and stabilized all children requiring injectable TB medications are transferred to BCH. Routine hearing testing for children treated with injectable TB medications was introduced in 2008. Children are assessed prior to starting injectable drugs and then monthly. If there are challenges to testing or if abnormalities are found, testing is carried out every two weeks. Children are treated for MDR- and RMR-TB with amikacin (20mg/kg once daily IM injection) for between four and six months. Children treated for isolates resistant to amikacin are treated with capreomycin (20mg/kg once daily IM) or streptomycin (20mg/kg once daily IM) dependent on drug susceptibility test results.

Study population

This retrospective study included all children routinely admitted to BCH from January 2009 until December 2010, aged 0-15 years, if they had been a) diagnosed with confirmed MDR- or RMR-TB (*M. tuberculosis* isolated using liquid culture with demonstrated resistance)[535] or presumed MDR- or RMR-TB (a clinical diagnosis of TB in the presence of a drug-resistant source case or the child failing first-line TB therapy), b) were treated with an injectable TB drug for at least a month, and c) had received at least one audiological assessment.

Audiological assessments

Children were assessed using a combination of otoscopy, tympanometry, PTA (including conditioned play audiometry) and/or distortion product otoacoustic emissions (DPOAEs). Otoscopy was used to ensure that there were no anatomical abnormalities and that the external ear canal was clear of occluding wax, foreign bodies or obstruction. A Welch Allyn 262 tympanometer (MFI Medical Equipment Inc. San Diego, USA) was used to assess middle ear function using a 226Hz probe tone. The probe was placed into the child's ear canal ensuring a tight seal with no leakage. Static compliance between 0.2-1.8cm^3, middle ear pressure between +100 and -150 dekapascals, and ear canal volume of 0.2-2.0cm^3 were used. If a type B tympanogram (indicating possible middle ear infection) was noted, the audiologist would notify the attending physician. A five day course of oral antibiotics was usually prescribed before reassessment. If the problem persisted, the child was referred to the ear, nose and throat team.

PTA was performed in a sound-proof booth with calibrated equipment. The AC40 dual channel audiometer (Interacoustics, Assens, Denmark) and the MA51 audiometer (MAICO Diagnostics GmbH, Berlin, Germany) were used. Pure tone air conduction hearing thresholds were obtained for children between six and fifteen years of age, for each ear by testing the octave bands from 250Hz to 8kHz. Audiologists followed the modified Hughson-Westlake procedure[536] (i.e. 10dB down, 5dB up, repeated twice to reliably determine hearing threshold). Stimuli were presented in the following order: 1kHz, 2kHz, 4kHz, 8kHz, repeated at 1kHz, then 500Hz and 250Hz. If there was a difference of 20dB between consecutive frequencies the audiologist would test half octave frequencies, i.e. 750Hz, 1.5kHz, 3kHz and 6kHz. For participants younger than six years, either conditioned play audiometry or DPOAE were performed. For descriptive purposes we considered thresholds of <25dB as normal, 26-40dB as mild, 41-55dB as moderate, 56-70dB as moderately severe, 71-90dB as severe and >90dB as profound hearing impairment.[537-538]

DPOAEs were obtained using an OtoRead^{TM} machine (Interacoustics, Assens, Denmark). A rubber-tipped probe was placed in the external ear canal to create a tight seal. Two simultaneous pure tone signals were then presented to each ear at two different primary frequencies (f1 and f2, where f2 > f1) with f1:f2 ratio of 1.22 and an intensity of 65dB Sound Pressure Level (SPL) and 55dB SPL respectively. Frequencies 2kHz, 4kHz, 6kHz, 8kHz, 10kHz and 12kHz were tested. In order for a child to pass the DPOAE, the emission amplitude needed to

be 6dB or greater above the noise floor. If a child was unable to be tested for any reason, or if the test was abnormal, they were re-tested two weeks later. If the child passed the DPOAE, then they were assessed monthly. DPOAE results were classified as pass, fail or unable to test.

Data collection

BCH admission records were reviewed to identify all patients treated for MDR- and RMR-TB over the study period. Records were compared with data from the audiology department to determine which of the patients had received audiological testing. Clinical records were reviewed to determine the dosage and duration of injectable treatment, demographic and clinical details, as well as audiological and laboratory data.

Data classification and analysis

A distinction was drawn between hearing deficit and hearing loss. Hearing deficit describes the absolute impairment in hearing experienced by a child at treatment completion whereas hearing loss is a measured deterioration in hearing function between two assessments. Children could therefore have hearing deficit at the end of treatment but if previous assessments were not carried out, hearing loss could not be determined. Conversely, it was possible for children to have hearing deficit at the beginning and at the end of treatment, but to experience no hearing loss between assessments.

Hearing deficit assessed by PTA was classified as, at the last hearing assessment, a threshold of greater than or equal to 25dB at any tested frequency, in the presence of normal tympanograms. When testing using OAEs, a classification of hearing deficit was made if the child failed the assessment in the presence of normal tympanograms. When assessed using PTA, hearing loss was classified according to the ASHA guidelines: a) an increase in pure tone thresholds of greater than or equal to 20dB at any one test frequency, b) an increase of greater than or equal to 10dB at any two adjacent test frequencies, or c) a loss of three consecutive frequencies.[248, 425-426] A diagnosis of hearing loss using OAE was made if the child failed the assessment in the presence of normal tympanograms having passed a previous assessment. The classification of hearing loss used is shown in Table 52.

Risk factors for hearing loss were determined by comparing the frequency or mean/median value for children with hearing loss (determined by both PTA and OAE) vs. children without. Chi square (or Fisher's Exact) tests, student t-tests or Mann Whitney tests were used; ORs and 95% CIs calculated.

Results

Patient characteristics

Ninety-four children were included in the study from 113 who were started on injectable treatment for MDR-TB (Figure 13). Median age was 43 months (inter-quartile range [IQR]: 20-110). Forty-five (48%) were boys and 30 (32%) had evidence of extrapulmonary TB. Children were generally malnourished with weight-for-age z-scores a mean of 1.48 standard deviations below the reference mean and median body mass index of 15.5kg/m^2 (IQR: 14.5-17.3). Fifty-two (55%) had a culture-confirmed diagnosis and the majority (62 children; 66%) were treated for MDR-TB. The other children either had disease with more extensive resistance or were started on treatment for MDR-TB but were later confirmed to have less resistant organisms. Twenty-eight children (out of 93 tested; 30%) were HIV-infected of which 20 (71%) were already on cART at the start of TB treatment. Most children (n=82; 87%) were treated with amikacin (Table 53).

Audiological testing

Thirty-six children were assessed using PTA and 58 assessed using OAEs. Hearing deficit is demonstrated in Figure 13, and hearing loss in Figure 14. When combining results of both PTA and OAE testing, 23 (24%) children had hearing loss and 27 (29%) had normal hearing. Forty-four (47%) children could not be classified using this approach. In 7 of the 11 children who had hearing loss determined by PTA (Table 54), hearing loss progressed even after the injectable medication was discontinued.

Assessment of risk factors for hearing loss

A culture-confirmed diagnosis of TB (OR: 4.12; 95%CI: 1.13-15.0; p=0.02) was a significantly associated with hearing loss (Table 55). There was a trend towards the median duration of injectable antibiotic use being longer in children with hearing loss: (164 days; IQR: 119-184 vs. 123; IQR: 70-183; p=0.07).

Discussion

This study demonstrates that both hearing deficit and hearing loss are common in children treated for MDR-TB. The association between hearing loss and culture-confirmed TB disease appears to reflect the extent or severity of disease and might suggest that treating clinicians are more likely to continue injectable drug use in children with extensive pathology. Since the aim was to describe children with definitive hearing loss or normal hearing, a classification

system was developed which precluded the accurate classification of a relatively large number of children. However, despite this conservative estimate, over half of the children had hearing deficit at the end of therapy and a quarter of children experienced hearing loss.

In addition to documenting the risk and degree of hearing loss in children treated for MDR-TB, this study highlights some of the challenges in the assessment of hearing in children, including the classification of hearing deficit and hearing loss. Hearing testing is partially subjective, requires relatively sophisticated equipment, trained staff and co-operative patients. Elements of the frequency (pitch), amplitude (volume), laterality (unilateral or bilateral) and aetiology (sensorineural, conductive or both) need to be considered; all of these factors need to be monitored longitudinally and change classified. The established ASHA criteria were followed to classify whether hearing loss occurred between two PTA assessments. However, a classification system was developed to determine whether children in this study should be classified as having hearing loss or not. This lack of established existing criteria limits meaningful comparisons between different studies.

Several studies have documented the treatment of MDR-TB, mainly in adults; only a handful have systematically assessed hearing loss and analyzed risk factors for ototoxicity. De Jager et al. found no association between clinical or treatment factors and the incidence of hearing loss.[539] Peloquin et al. assessed whether the size and frequency of dosage affected hearing loss and found no association, but demonstrated that older age and cumulative dose were associated with an increased risk.[273] Sturdy et al. found that impaired renal function, older age and the use of amikacin were associated with hearing loss in adults treated for MDR-TB.[540] A number of studies describe cohorts including small numbers of children but few have included those less than ten years of age. The only previous paediatric study examining the adverse effects of children on treatment for MDR-TB describes 38 children treated in Peru; 30 underwent hearing assessments.[204] The testing methodology and classification was not specified; audiology testing was undertaken in children receiving an injectable for more than six months. Two children were found to have mild, high-frequency, conductive hearing loss. Studies of short courses of aminoglycoside use in neonates[319] and children with cystic fibrosis[320] demonstrate limited toxicity but assessment of hearing loss in children receiving longer courses of aminoglycosides following liver transplantation found hearing loss in 15 of 66 children evaluated, using a 35dB loss at one frequency to define hearing loss. [321]

Hearing has particular relevance in children since they need hearing to develop skills and acquire language. The primary means of education is through oral teaching. Hearing loss

during childhood can therefore have profound implications for development.[242-243, 531-532, 541-543] If ototoxicity is identified early, rapid intervention can be implemented.[544-545]

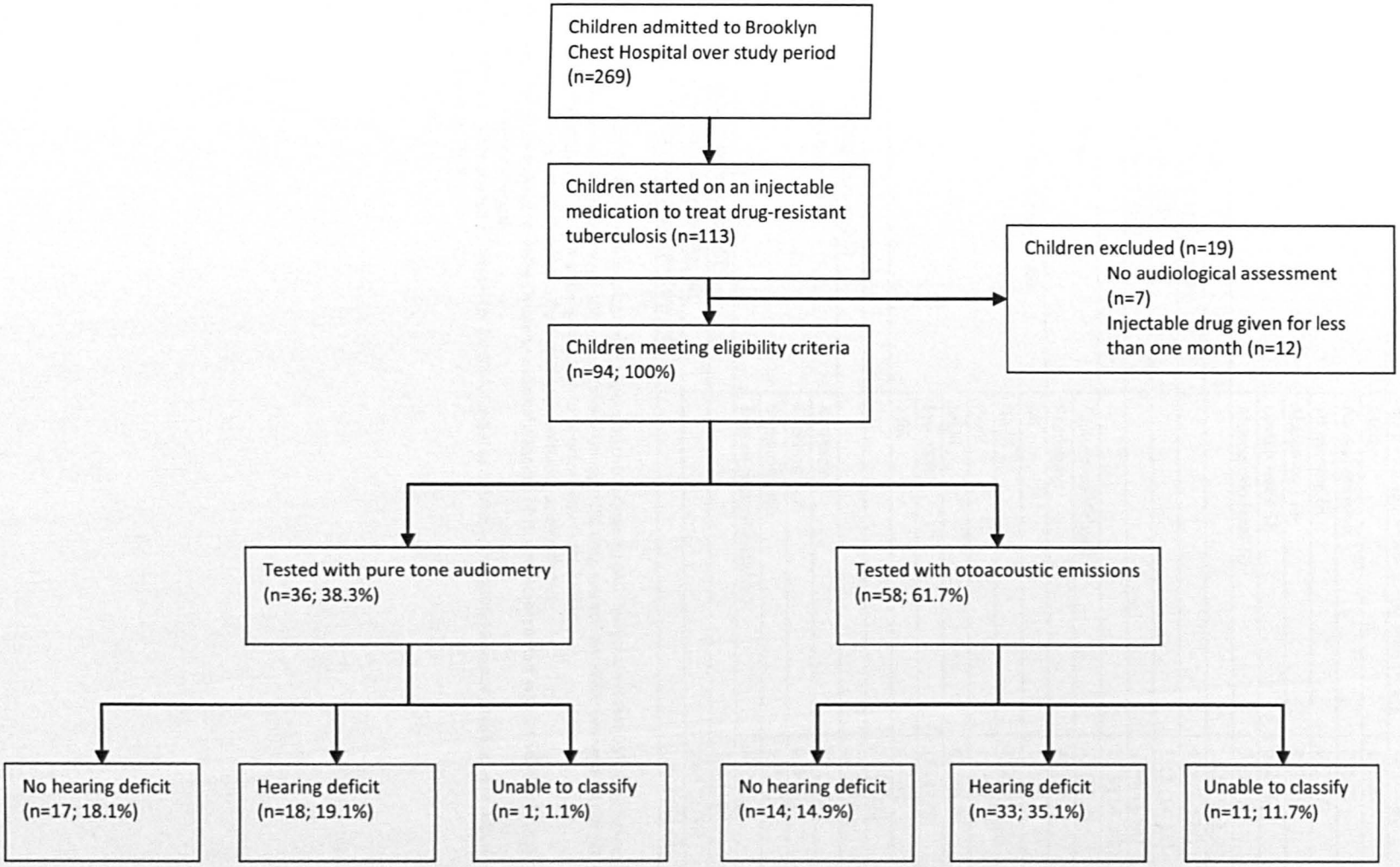
The study has a number of strengths and limitations. It reports the largest study to date documenting hearing loss in children treated for MDR-TB and assess risk factors for hearing loss using a robust classification system. It reports on hearing loss resulting from care provided under routine, programmatic conditions. The retrospective nature of the study limited systematic data collection; therefore some audiological assessments were missing, irregular or incomplete. Clinical parameters were determined from routine data and were incomplete in some instances. The findings may not be representative of all children treated for MDR-TB since only children admitted to hospital are described. Finally, it was not possible to classify and analyse a considerable number of children due to the rigorous classifications used and there was no pharmacokinetic data available for children on the injectable drugs to correlate with toxicity.

Table 52 - Classification of hearing loss using otoacoustic emissions and pure tone audiometry

	Otoacoustic emissions	**Pure tone audiometry**
No hearing loss	• A normal OAE in the last month of therapy or after completing injectable medication	• A normal PTA (all frequencies better than 25dB) in the last month of or after completing injectable medication with no subsequent abnormal tests or • No significant deterioration (as determined by ASHA criteria)[248] between an audiogram performed before or within the first month of therapy and one performed after within the last month of therapy with no subsequent deterioration
Hearing loss	• A normal OAE documented before or during therapy followed by an abnormal OAE in the presence of normal tympanograms	• A significant deterioration (as determined by the ASHA criteria)[248] between an audiogram performed before or during therapy and one performed later during therapy or after completing therapy in the presence of normal tympanograms
Unable to classify	• Normal final OAE but performed before the last month of therapy • Abnormal tympanograms • Abnormal OAE throughout therapy • Unable to test child due to noise or child unable to co-operate	• An abnormal audiogram without an earlier audiogram for comparison • A normal final audiogram (all frequencies better than 25dB) before the last month of therapy • Abnormal tympanograms • Unable to test child due to noise or child unable to co-operate

OAE: otoacoustic emission; PTA: pure tone audiometry; ASHA: American Speech and Hearing Association

Figure 13 - Hearing deficit in children treated for multidrug-resistant tuberculosis with second-line injectable drugs



Percentages calculated from the number of children meeting eligibility criteria (n=94)

Table 53 - Demographic and treatment data in children treated for multidrug-resistant tuberculosis (n=94)

Characteristic		Number (% unless indicated otherwise)
Median age in months (IQR)		43 (20-110)
Male gender		45 (47.9)
Type of TB	Pulmonary	64 (68.1)
	Extrapulmonary	17 (18.1)
	Both extrapulmonary and pulmonary	13 (13.8)
Site of extrapulmonary TB (n=30)	Miliary	1 (3.3)
	Pleural effusion	2 (6.7)
	TB meningitis	8 (26.7)
	Abdominal TB	4 (13.3)
	Lymph node TB	6 (20.0)
	Musculoskeletal TB	9 (30.0)
Median weight in kg (IQR)		13.5 (10.1-21.2)
Median weight/length in cm (IQR) (n=90)		93 (78-121)
Median MUAC in cm (IQR; n=83)		15.3 (14-17)
Mean weight for age z-score (SD)		-1.48 (1.55)
Median BMI (IQR)		15.5 (14.5-17.3)
Certainty of TB diagnosis	Culture-confirmed	52 (55.3)
	Presumed	42 (44.7)
DST of child or source case if diagnosed presumptively	DS*	1 (1.1)
	HMR*	2 (2.1)
	RMR	11 (11.7)
	MDR	62 (66.0)
	Pre-XDR	16 (17.0)
	XDR	2 (2.1)
HIV-infected (n=93)		28 (30.1)
On ART prior to TB diagnosis (n=28)		20 (71.4)
Type of injectable drug given	Amikacin	82 (87.2)
	Capreomycin	9 (9.6)
	Streptomycin	1 (1.1)
	Two or more injectables	2 (2.1)
Mean dose of injectable drug (mg; SD)		320 (189)
Mean dose of injectable drug (mg/kg; SD)		19.4 (2.04)
Mean duration of injectable drug uses (days; SD)		136.2 (51.6)

IQR: inter-quartile range; TB: tuberculosis; MUAC: mid upper arm circumference; BMI: body mass index; DST: drug susceptibility testing; HIV: human immunodeficiency virus; ART: antiretroviral therapy; DS: drug-susceptible; HMR: isoniazid-monoresistant; RMR: rifampicin-monoresistant; MDR: multidrug-resistant; XDR: extensively drug-resistant;
Confirmed diagnosis: *M. tuberculosis* isolated from child with resistance demonstrated
Presumed diagnosis: child treated for MDR-TB due to a clinical diagnosis of TB and either contact with an MDR-TB source case or following failure of first-line therapy
*These three children were started on treatment for MDR-TB due to contact with a MDR-TB source case but were subsequently found to have DS- or HMR-TB

Figure 14 - Hearing loss in children treated for drug-resistant tuberculosis with second-line injectable drugs (n=94)

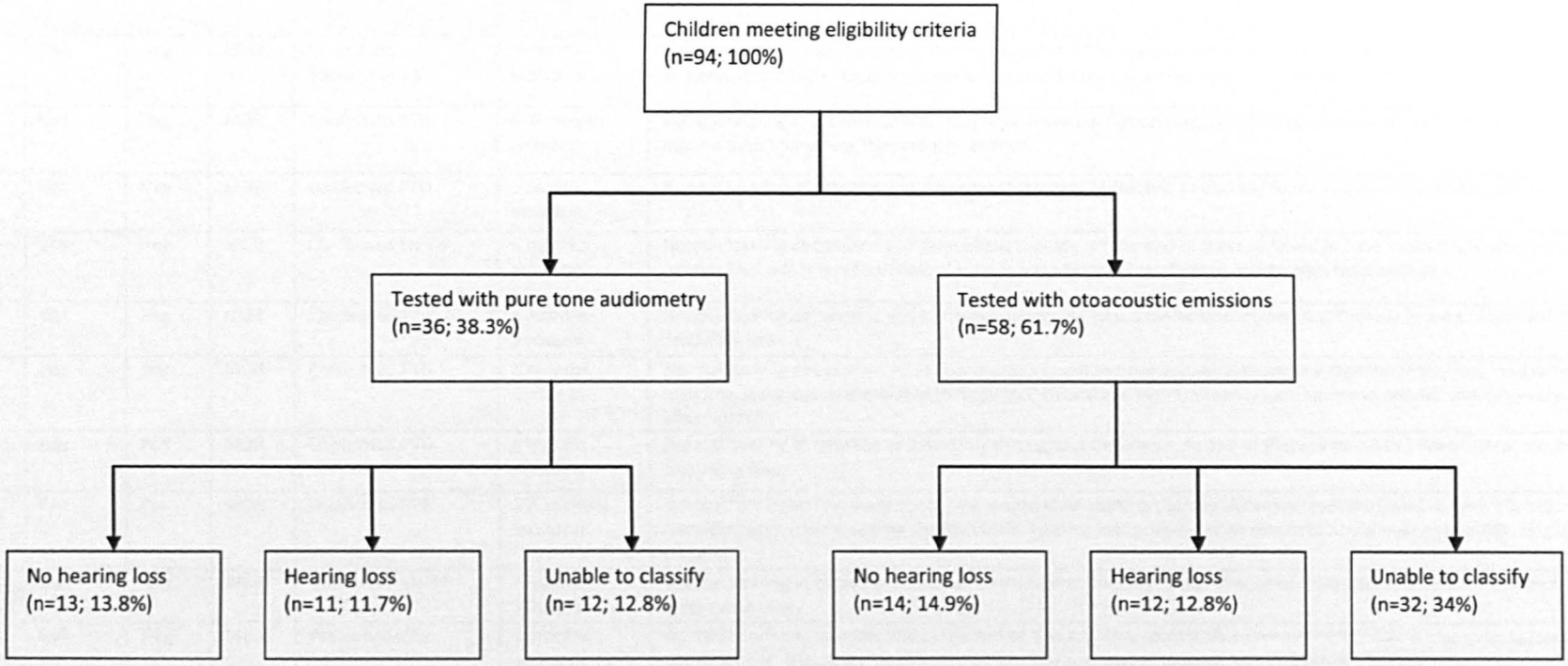


Table 54 - Characteristics of children treated for multidrug-resistant tuberculosis with hearing loss determined using pure tone audiometry (n=11)

Age	Gender	HIV status	DST	Diagnosis	Treatment	Hearing loss
15 yr	Girl	Neg	RMR	Confirmed abdominal TB	2 months amikacin	Unilateral severe high frequency hearing loss at the first assessment carried out one month after the start of treatment. One month later bilateral severe high frequency hearing loss so injectable stopped. No further hearing loss
10 yr	Girl	Pos	MDR	Confirmed PTB	5 ½ months amikacin	Normal hearing at baseline and at monthly intervals whilst on therapy. Moderately severe high frequency hearing loss detected two months after competing injectable treatment
5 yr	Girl	Neg	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and throughout therapy. At the end of therapy found to have unilateral moderate high frequency hearing loss
10 yr	Boy	Neg	MDR	Confirmed LN TB	6 months amikacin	Normal hearing at baseline and throughout therapy. At the end of therapy found to have moderately severe unilateral high frequency hearing loss. A further month later found to have bilateral moderately severe high frequency loss
10 yr	Girl	Neg	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and throughout therapy. Two months after completing therapy found to have unilateral high frequency moderate loss
12 yr	Boy	Pos	MDR	Confirmed PTB	8 months amikacin	Normal hearing at baseline. After four months found to have unilateral moderate high frequency loss, progressing to severe unilateral high frequency loss by the end of therapy and to bilateral high frequency loss, severe in one ear and moderate in the other by 4 months after finishing
13 yr	Boy	Pos	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and monthly throughout treatment. At end of therapy found to have bilateral moderately severe high frequency loss
8 yr	Girl	Pos	MDR	Confirmed PTB	2 ½ months amikacin	Normal hearing at first assessment one month after starting therapy. After two months found to have bilateral moderately severe high frequency loss. After stopping the injectable, hearing loss progressed to severe bilateral hearing loss affecting all frequencies. Hearing aid required
3 yr	Boy	Neg	MDR	Confirmed LN TB	4 months amikacin	Normal hearing at baseline. Found to have moderate unilateral high frequency loss after four months so injectable stopped. No further tests carried out.
12 yr	Girl	Neg	MDR	Presumed PTB	5 months amikacin	Normal hearing at baseline and at the end of therapy. One month after completing injectable medications found to have moderate unilateral high frequency loss, progressing to bilateral high frequency loss (mild in one ear and moderately severe in the other) after a further month
10 yr	Girl	Pos	MDR	Confirmed PTB	4 months amikacin	Found at first assessment (2 months after starting therapy) to have bilateral moderate high frequency loss. By 2 months after completing therapy high frequency loss progressed in one ear to severe

HIV: human immunodeficiency virus; TB: tuberculosis; RMR: rifampicin-monoresistant; MDR: multidrug-resistant; PTB: pulmonary TB; LN: lymph node

Table 55 - Univariate assessment of risk factors of hearing loss in children treated for multidrug-resistant tuberculosis

	Hearing loss (n=23)	**No hearing loss (n=27)**	**OR (95% CI)**	**P-value**
Age in months (IQR)	52 (28-132)	53 (25-120)		0.90
Male gender	9	11	0.94 (0.30-2.95)	0.91
EP involvement	9	6	2.25 (0.63-8.00)	0.20
WFA z-score	-1.07 (-2.29--0.32)	-0.82 (-2.34--0.33)		0.78
BMI in kg/m^2 (IQR)	15.9 (13.9-17.6)	16.1 (14.9-17.3)		0.48
MUAC in cm (IQR)	15.0 (14.0-17.0)	16.4 (14.5-18.1)		0.28
Culture-confirmed diagnosis of TB	17	11	4.12 (1.13-15.0)	0.02
HIV-infected	9	6	2.14 (0.60-7.63)	0.23
Amikacin use	21	24	0.76 (0.11-5.11)	0.78
Mg/kg dose injectable (IQR)	19.6 (18.3-20.4)	19.4 (17.4-20.1)		0.30
Duration of injectable (IQR)	164 (119-184)	123 (70-183)		0.07
Pre-XDR or XDR-TB	4	5	0.93 (0.21-4.01)	0.92

EP: extrapulmonary; WFA: weight-for-age; BMI: body mass index; MUAC: mid upper arm circumference; HIV: human immunodeficiency virus; XDR: extensively drug-resistant; OR: odds ratio; CI: confidence interval

Discussion

The first literature review demonstrated that our understanding of how to manage children exposed to MDR-TB is limited, as few studies have been conducted to guide practice and international policy documents provide little guidance to help the clinician confronted by such a child. The second literature review assessed studies that have described children treated for MDR-TB disease. All are observational and the total numbers are small. High quality, grade one evidence, derived from randomised controlled trials, are lacking and clinicians are forced to draw lessons from these small observational studies, the adult DR-TB literature, the paediatric drug-susceptible literature and combine it with their own clinical experience and judgement. The final literature review discussed the drugs used to treat DR-TB in children. Again, our understanding is incomplete, regarding the properties of the drugs themselves, their toxicity and their interactions with other medications. These reviews led to a series of connected original research studies that explored the cascade from exposure to infection, from infection to disease and from disease to outcome. Below I go through each of these stages and discuss the findings of the research studies in the thesis and how our understanding of DR-TB epidemiology and treatment is affected.

The burden of drug-resistant tuberculosis in children

Study 1 of the thesis documented the burden of drug-resistance amongst children with TB in Cape Town. This study provides two insights. First, a description of the population of children who develop TB and second, a measure of changing trends in paediatric drug-resistance over time. As there are so few studies documenting childhood TB in a systematic manner[546-548] and as recording and reporting can be poor in developing countries,[7] studies such as this, provide an important insight into the epidemic.

This study only described children with culture-confirmed disease and still the median age for this, and previous surveillance periods, was between two and three years. As younger children are less likely to have extensive disease, and thereby less likely to have a confirmed diagnosis, it may be that the age spectrum of all children with TB might be younger. Children were frequently malnourished but it is encouraging that the proportion HIV-infected seems to be

levelling off, possibly as an effective prevention of mother to child transmission programme begins to take effect.

Although the overall proportion of cases that had any drug resistance has remained relatively unchanged over the last few years, rifampicin resistance has increased and with it multidrug resistance. These results are not directly transferable to contexts outside Cape Town but these results provide details not only of the epidemic of drug-resistance in children but also of drug-resistant TB in its entirety due to the sentinel nature of childhood TB.

The final, additional finding of this study that is that although previous studies have raised concerns regarding the use of molecular LPAs for the diagnosis of drug resistance, we found the LPA that is widely used in the Western Cape to be both sensitive and specific when compared to conventional DST.

Risk of infection and disease for child contacts of drug-resistant tuberculosis

Study 2 was a cross-sectional study looking at children presenting to the DR-TB clinic following exposure to an infectious case of MDR-TB. High rates of both infection and disease were seen in these children. A significant risk factor for infection was increasing age, an unsurprising finding which is seen in the drug-susceptible paediatric TB literature and makes biological sense. That Coloured ethnicity was also associated with infection, even in adjusted analysis, is more complex and confirms that TB is, to a great degree, a sociological disease. Alcohol use in the source case was a significant risk for infection in the child but older and HIV-positive source cases seemed to be less infectious. Alcohol abuse in South Africa, and in the Western Cape in particular, is widespread and long-standing. It has complex interactions on both a biomedical and a behavioural level. It is likely that if the DR-TB epidemic is to be brought under control, alcohol use will need to be addressed. High levels of alcohol abuse are also seen in other areas of the world with significant DR-TB control problems, such as Eastern Europe and the former Soviet States; the two problems will need to be managed together. The impact of the age and HIV status of the source case is complex and potentially has implications for infection control and case management. However, due to the complex inter-relationship between multiple exposures, these risk factors need further investigation with larger patient numbers.

Again, consistent with the drug-susceptible literature, younger children and HIV-positive children are at increased risk of developing TB disease, following infection. Alcohol in the household, again seems to influence this progression.

This study highlights the importance of screening children exposed to MDR-TB as a significant proportion will have TB disease that needs to be treated. The study also confirms that young and HIV-infected children are at the highest risk of disease progression following infection and are the most likely to benefit from preventive therapy.

Transmission of drug-resistant *M. tuberculosis*

Studies 3 and 4 describe investigations into a number of families to try to determine the sequence of events that resulted in a number of children developing DR-TB disease. In both studies the children were very likely infected by adults within their families. The transmission dynamics demonstrate the potential for people to transmit strains at different time points in the evolution of molecular resistance. However, when combined with a review of the literature surrounding transmission, these studies, reinforce two lessons. The first is that extensive efforts should be made to isolate the organism in children with clinical/radiological evidence of TB as there is the possibility that the strain is either different or has a different DST to the putative source case. The second, however, is that in spite of this, transmission is usually from the person known to have TB in the family and when confronted with a child with clinical/radiological TB, following sampling, it is appropriate to treat them according to the DST of the strain from that source case.

Models of care for children exposed to drug-resistant tuberculosis

Study 5 assessed what proportion of child contacts of MDR-TB are identified, referred and seen in a specialist clinic as directed by provincial guidelines. This study achieved this by looking at how many source cases led to a child contact being seen rather than what proportion of child contacts are actually seen. However, the magnitude of the discrepancy renders this distinction relatively unimportant and it is fair to draw the conclusion that few of the eligible children were seen. A decentralised model of care demonstrated some advantage over a traditional, hospital-based system but it is clearly not the sole solution.

Study 6 explored reasons why only some of the children identified by health systems access specialist care and found that ethnicity and identity of the source case were important. Unsurprisingly it also discovered that the long, expensive and complex journeys put people off coming to appointments. Families concerned about infection risk were also less likely to attend.

It is clear from these studies that the current provincial guidelines are not being successfully carried out. Some of the reason is likely that resources are limited and the job is large. The identification and referral of well children may not be the greatest priority in a health service that has many other pressing health needs. However, in combination with the results from Study 2 (i.e. the high risk of infection and disease in these contacts) it is concerning. These results suggest that more children should be identified and also that it should be made as easy as possible for children who are identified to access care. Services should be sympathetic, professional and delivered close to the family. Infection control must be addressed and education of health providers and the public at large must improve. Particular assistance should be provided when mothers are diagnosed with MDR-TB as their children are not only very vulnerable to becoming infected and of developing disease but they are also at high risk of not accessing care.

Preventive therapy for child contacts of drug-resistant tuberculosis

Studies 7 and 8 described the provision of a three-drug preventive therapy regimen to child contacts of MDR-TB. Children less than five years or those HIV-infected were given the drugs daily for six months irrespective of TST. This study was not a trial and so efficacy cannot be conclusively determined. The study was observational and carefully documented the standard of care as it was being given in the Western Cape. Study 7 found that adverse events were rare in children given this course of preventive therapy and three out of seven children developing severe adverse events were inadvertently overdosed. The majority of adverse events were skin rashes and itch, sleep/mood disturbances and loss of appetite. All resolved without stopping the medications. Co-administration with cART did not seem to increase the risk of adverse events, even though the number of children with HIV was relatively small.

Study 8 found that most of the children who were prescribed the medications were given them with good adherence. Few of the children provided with preventive therapy developed TB and the only death was in a young child who was thought unlikely to have died of TB. HIV

infection, age less than twelve months and poor adherence to medications were found to be associated with poor outcome.

Taken together these two studies suggest that when confronted with a child who has been exposed to a source case with MDR-TB, a safe option is to give multidrug preventive therapy. Few children given this regimen develop TB. When this is considered alongside Study 2, the provision of preventive therapy to young and HIV-infected children should be strongly considered.

The treatment of drug-resistant tuberculosis

Studies 9 to 12 document children treated for MDR-TB. The first two are large cohorts of children, the first with culture-confirmed MDR-TB and the second with a combination of confirmed and presumed MDR-TB (as well as RMR-TB). The cohort of culture-confirmed MDR-TB frequently had advanced disease with a high proportion smear positive and with severe CR changes. HIV infection was common and in many cases there was a significant delay in the diagnosis and initiation of treatment. In spite of this, successful outcomes were seen in the majority of children. The second cohort, in contrast, included younger children with less severe disease and lower rates of HIV infection. Successful outcomes were even more common than in the previous study and only three children were known to have died – all had severe disease in combination with either HIV infection or treatment default. For children with limited disease, the diagnosis was more often presumed, delay was less (often non-existent), treatment durations were shorter and both hospital admission and injectable drug use was not universal.

These results suggest that even with severe disease, the majority of children can be successfully treated. However, they also advocate for early identification and rapid initiation of treatment in child contacts of MDR-TB who have symptoms, signs and radiology of TB. For this to take place, contact tracing must occur following the identification of adults with MDR-TB. For those children who are identified early and who have non-severe disease, shorter treatments can be given, they can be treated in an ambulatory way and the injectable medication can frequently be withheld.

Study 11 examines children with culture-confirmed TB and a diagnosis of TBM. This study finds that delay was longer in children infected with DR strains and that MDR-TB was associated

with poor outcome and death. Children with HMR-TB had similar outcomes to children with fully susceptible strains. That children with MDR-TBM have poor outcomes is perhaps not surprising as TBM is a severe disease process and if no drugs are provided that have efficacy against the strain, ongoing damage will occur. The results of the HMR-TB are, however, more interesting. It may be that the numbers in the study were not large enough to detect differences and certainly absence of evidence for an effect is not evidence for absence of an effect. However, children in the Western Cape with TBM are treated with four drugs with good CSF penetration for six months and where isoniazid resistance is present, it is likely that the child will still receive at least two effective drugs with good CSF penetration for the whole treatment course. This is unlikely to be the case if the WHO-recommended regimen is given. The absence of an association between strain type and either outcome or drug resistance is also interesting given previously described relationships.

Study 12 describes the presentation, management and outcome for children with culture-confirmed MDR-TB of the spine. The diagnosis was frequently delayed, leading to advanced disease and severe vertebral damage. However, once the diagnosis was made and appropriate treatment instigated, good outcomes were seen. An implication of this study is that when spinal TB does not respond to first-line therapy it is essential that clinical samples are taken and tested for DR-TB.

The adverse effects of drug-resistant treatment

Studies 10 and 13 both assess the adverse effects of second-line TB drugs when given to children with DR-TB disease. Study 10 describes the adverse events experienced in a cohort of treated children. Apart from one girl who developed DRESS syndrome few significant adverse effects were seen. An exception was that an important proportion of children developed hypothyroidism and were given supplementation. Study 13 investigated the effects of the injectable drugs on hearing. It determined that both hearing deficit and hearing loss were common in children treated with injectables. Having culture-confirmed disease was found to be a risk factor for hearing loss. Of note, a number of children developed hearing loss after cessation of their injectable medications.

The implications of these two studies should both reassure and alarm. On the one hand, the majority of the drugs used to treat DR-TB in children are well tolerated and have limited toxicity. This should reassure a clinician when managing such a child. However, the incidence

of hearing loss, a fundamental component of communication, education and the ability to interact with the world, is very high. Regular hearing testing must play a part in the management of any child treated for DR-TB, drugs to mitigate the damaging effects of injectables must be explored and alternative treatments need to be developed.

Conclusions and implications for policy and practice

Many children each year are exposed to DR bacilli and the children most likely to become infected and then develop disease are the most vulnerable. This includes young children, children with HIV infection, children with malnutrition, children from poor backgrounds, and children exposed to alcohol and smoking in their homes. Such children frequently have worse treatment outcomes if they do develop TB disease. Few of these children are identified by routine health services in Cape Town and few started on appropriate preventive therapy. A multidrug preventive therapy regimen is safe and likely effective for well children exposed to MDR-TB. Although confirmation is achieved in fewer than 50% of children with MDR-TB disease, the remaining children can be treated with a presumptive diagnosis. Extensive disease is effectively managed in the same way as adults, but children with limited disease respond well when given fewer drugs and for shorter durations. Although treatments are generally safe and effective particular care should be taken with the injectable medications due to their adverse effects on hearing.

Based on the findings from these studies, from my reading of the literature and from the practical experience that I have accrued over the last three years, I make a number of pragmatic recommendations that could be implemented now into the majority of health programmes in the world.

Recommendation 1

Following the diagnosis of MDR-TB in an adult, a home visit should be undertaken. All children in contact with the source case should be screened with symptom questionnaires. Dedicated staff at a clinic or local healthcare level should provide this service. Particular focus should be placed on child contacts of mothers with MDR-TB as they are at high risk of exposure as well as of failure to access care.

Recommendation 2

Health workers at primary healthcare level should be given gradual and incremental responsibility for the management of MDR-TB exposure and disease in childhood. This will require training courses, followed by outreach services by a specialist, then joint care between

primary care and a specialist and finally care provided by primary care with clear referral and advice pathways to specialists.

Recommendation 3

Children under five years or any child with HIV-infection, following significant contact with an MDR-TB source case, irrespective of TST/IGRA result, should be prescribed a six month course of preventive therapy, to be given daily. The composition of this should be a fluoroquinolone and high-dose isoniazid. Treatment support and supervision should be provided by trained local lay workers.

Recommendation 4

Children with symptoms, signs and/or radiology of TB, with significant exposure to a DR-TB source case, should have extensive microbiological sampling but then should be started on a regimen tailored to the DST of the source case, as a significant proportion of children with DR-TB will never have a confirmed diagnosis.

Recommendation 5

Children with TB of the spine, not responding to first-line treatment should have clinical samples taken from the spine or surrounding tissue to be tested for DR-TB.

Recommendation 6

Children with extensive disease (miliary disease, TBM, disseminated disease, cavitating disease, widespread bronchopneumonic changes on CR) should be managed as adults, irrespective of age. Children with limited disease (hilar or cervical lymphadenopathy) can be managed with fewer drugs, for shorter durations and with either no injectable medication or an injectable given for a shorter period. Ambulatory treatment should be the norm in the majority of cases.

Recommendation 7

All children treated with ethionamide/prothionamide or PAS should have their thyroid function tested regularly whilst on treatment

Recommendation 8

Children treated with an injectable medication should have their hearing tested at baseline using audiological tests (PTA or OAE) with regular testing carried out monthly whilst on treatment. A further test should be carried out six months after completing treatment. Consideration should be given to stopping injectable drugs as soon as is clinically possible.

Recommendation 9

All children treated for TB, with confirmed or presumed resistance to isoniazid, rifampicin or both drugs, should be recorded in a drug-resistant TB register and reported through to a national level.

Recommendation 10

Older children (older than ten years) should be included in all prevalence surveys; currently these focus only in adults. In a sample of sentinel sites, the full age range of children should be included using standardised clinical criteria.

Further research

In the course of this thesis I have reviewed the literature surrounding DR-TB in children and have carried out a number of studies to try to better understand the epidemic. However, there are still many areas that remain unclear. Whilst better vaccines, better diagnostics and an improved knowledge of TB immunology and immunotherapy in children would be beneficial, I have restricted research suggestions to those specific to DR-TB. A number of further areas of research would include the following:

1. An expert group should produce a consensus statement describing a prioritized research agenda
2. Widespread surveillance, using techniques similar to those carried out in Study 1 and with standardised definitions, in a number of different contexts, to better quantify the burden of childhood DR-TB in different setting
3. Operational research to improve the identification and referral of child contacts of DR-TB, by assessing different forms of health system intervention
4. A randomised, controlled trial to compare a preventive therapy regimen, including a fluoroquinolone against isoniazid to prove efficacy
5. An improved understanding of the pharmacokinetics of the second-line TB drugs in children especially when used in combination with cART
6. An improved understanding of the penetration of second-line TB drugs into different body compartments in children, especially CSF, bone, lymph nodes and lung tissue
7. The development of paediatric formulations of novel TB drugs with pharmacokinetic investigations carried out
8. Descriptive cohorts of children treated for MDR-TB in different sites and contexts using standardised definitions for diagnosis, adverse events and outcome
9. Larger, randomised controlled trials of shortened treatment durations for children with limited disease compared to standard of care
10. A randomised controlled trial of WHO recommended therapy vs. Western Cape recommended therapy for the management of children with TBM
11. A trial of aspirin as prevention on aminoglycoside-induced hearing loss in children
12. Assessment of novel and innovative techniques for promoting adherence for children being treated at home for DR-TB

Work dissemination

A list of articles published or in press

- **Seddon JA**, Godfrey-Faussett P, Hesseling, AC, Gie RP, Beyers N, Schaaf HS. Management of children exposed to multidrug-resistant *Mycobacterium tuberculosis*. Lancet Infect Dis 2012; 12: 469-79
- **Seddon JA**, Hesseling AC, Willemse M, Donald PR, Schaaf HS. Culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment and outcome. Clin Infect Dis 2012; 54: 157-66
- **Seddon JA**, Warren R, Enarson DA, Beyers N, Schaaf HS. Drug-Resistant tuberculosis transmission and resistance amplification within families. Emerg Infect Dis. 2012;18:1342-5
- **Seddon JA**, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. Int J Tuberc Lung Dis 2012; 16: 928-33
- **Seddon JA,** Jordaan A, Victor T, Schaaf HS. Discordant drug susceptibility for Mycobacterium tuberculosis within families. Pediatr Infect Dis J 2012; 31: 783-5
- **Seddon JA**, Visser D, Bartens M, Jordaan A, Victor T, van Furth AM, Schoeman JF, Schaaf HS. Impact of drug resistance on clinical outcome in children with tuberculous meningitis. Pediatr Infect Dis J 2012; 31: 711-6
- **Seddon JA**, Hesseling AC, Marais BJ, McIleron H, Donald PR, Schaaf HS. Second-line anti-tuberculosis drugs in children: a review. Tuberculosis 2011; 92: 9-17
- **Seddon JA**, Godfrey-Faussett P, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in patients on treatment for drug-resistant tuberculosis. Eur Respir J 2012; 40: 1277-86
- **Seddon JA**, Donald PR, Vlok GJ, Schaaf HS. Multidrug-resistant tuberculsois of the spine in children – characteristics from a high burden setting. J Trop Pediatr 2012; 58: 341-7
- Ettehad D, Schaaf HS, **Seddon JA**, Cooke G, Ford N. Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infec Dis 2012; 12: 449-56
- Schaaf HS, **Seddon JA**, Caminero JA. Second-line antituberclosis drugs: current knowledge and controversies. Prog Respir Res. 2011; 40: 81-95
- Thee S, **Seddon JA**, Donald PR, Seifart HI, Werely C, Hesseling AC, Rosenkranz B, Magdorf K, Schaaf HS. Pharmacokinetics of isoniazid, rifampicin and pyrazinamide in children younger than two years of age with tuberculosis. Antimicrob Agents Chemother 2011; 55: 5560-7
- **Seddon JA,** Schaaf HS, Hesseling AC. Retooling existing tuberculosis drugs for children. Clin Infect Dis 2012 (in press)
- **Seddon JA,** Hesseling AC, Dunbar R, Cox H, Hughes J, Fielding K, Godfrey-Faussett P Schaaf HS. Decentralised care for child contacts of multidrug-resistant tuberculosis. Pub Health Action 2012; 2: 66-70
- Zimri K, Hesseling AC, Godfrey-Faussett, Schaaf HS, **Seddon JA**. Why do child contacts of multidrug-resistant tuberculosis not come to the assessment clinic? Pub Heath Action 2012; 2: 71-75

- Rose P, Hallbauer U, **Seddon JA**, Hesseling AC, Schaaf HS. Linezolid-containing regimens for the treatment of drug-resistant tuberculosis in South African children. Int J Tuberc Lung Dis 2012; 16: 1588-93
- **Seddon JA,** Thee S, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in children treated for multidrug-resistant tuberculosis. J Infection (in press)
- **Seddon JA,** Furin JJ, Gale M, Del Castillo Barrientos H, Hurtado R, Amanullah F, Ford N, Starke JR, Schaaf HS. Caring for children with drug-resistant tuberculosis: practice-based recommendations. Am J Resp Crit Care Med 2012; 186: 953-964
- Schaaf HS, **Seddon JA**. Epidemiology and management of childhood multidrug-resistant tuberculosis. Clinical Practice (in press)

A list of articles submitted or in preparation

- **Seddon JA,** Perez-Velez CM, Schaaf HS, Furin JJ, Marais BJ, Tebruegge M, Detjen A, Hesseling AC, , Shah S, Adams LV, Starke JR, Swaminathan S, Becerra MC. Consensus statement on research definitions for drug-resistant tuberculosis in children. (submitted)
- **Seddon JA,** Godfrey-Faussett P, Hesseling AC, Schaaf HS, Enarson D. Should preventive treatment be provided to child contact of tuberculosis in high burden settings? (submitted)
- **Seddon JA,** Hesseling AC, Finlayson H, Schaaf HS. Toxicity and tolerability of multidrug-resistant tuberculosis preventive treatment in children. (submitted)
- **Seddon JA**, Godfrey-Faussett P, Hesseling AC, Fielding K, Schaaf HS. Risk factors for infection and disease in child contacts of multidrug-resistant tuberculosis. (submitted)
- **Seddon JA**, Hesseling AC, Fielding K, Cox H, Hughes J, Godfrey-Faussett P, Schaaf HS. Preventive treatment for child contacts of MDR-TB (in preparation)
- **Seddon JA,** Hesseling AC, Godfrey-Faussett, Schaaf HS. The spectrum of presentation, treatment and outcome in children with MDR-TB (in preparation)

Published abstracts, posters and presentations at academic meetings

Published abstracts

- **Seddon JA**, Thee S, Hesseling AC, Schaaf HS. Hearing and renal impairment in children treated for drug-resistant tuberculosis. 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- Zimri K, Hesseling AC, Godfrey-Faussett P, **Seddon JA**. R Reasons for non-attendance for assessment for child contacts of multidrug-resistant tuberculosis. 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- **Seddon JA**, Hesseling AC, Godfrey-Faussett P, Fielding K, Schaaf HS. Risk factors for infection in child contacts of multidrug-resistant tuberculosis. 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- **Seddon JA**, Hesseling AC, Finlayson H, Schaaf HS. Toxicity and tolerability of multidrug-resistant tuberculosis preventive treatment in children. 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- **Seddon JA**, Hesseling AC, Dunbar R, Cox H, Hughes J, Fielding K. Godfrey-Faussett P, Schaaf HS. Decentralised care for child contacts of multidrug-resistant tuberculosis. 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012
- **Seddon JA**, Visser DH, Bartens M, Jordaan AM, Victor TC, van Furth AM, Schoeman JF, Schaaf HS. The impact of drug resistance on clinical outcome in children with tuberculous meningitis. 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012
- **Seddon JA**, Warren RM, Enarson DA, Beyers N, Schaaf HS. Drug-resistant tuberculosis in children is caused by transmission and amplification of resistance within families. 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012
- **Seddon JA**, Hesseling AC Godfrey-Faussett P, Donald PR, Schaaf HS. Review of challenges to treatment: from trials to formulations. 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011.
- **Seddon JA**, Hesseling AC, Dunbar R, Godfrey-Faussett P, Cox H, Hughes J, Schaaf HS. Regional lessons on partnerships for scale-up for child MDR-TB contacts in South Africa. 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011.
- Schaaf HS, **Seddon JA**, Rautenbach C, Hesseling AC. Fourth serial 2-year tuberculosis drug resistance survey in children, Western Cape, South Africa. 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011.
- Thee S, **Seddon JA**, Donald PR, Seifart HI, Hesseling AC, Rosenkranz B, Magdorf K, Schaaf HS. Pharmacokinetics of isoniazid, rinfampicin and pyrazinamide in children younger than two years of age. 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011.
- **Seddon JA**, Donald PR, Schaaf HS. Multidrug-resistant tuberculosis of the spine in children. 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011.
- Schaaf HS, **Seddon JA**, Willemse M, Hesseling AC, Donald PR. Results from the field. MDR-TB in children: clinical features and outcome of culture-confirmed cases. 41st World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Berlin, Germany 11-15 November 2010

Posters at conferences

- *Hearing and renal impairment in children treated for drug-resistant tuberculosis.* 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- *Reasons for non-attendance for assessment for child contacts of multidrug-resistant tuberculosis.* 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- *Risk factors for infection in child contacts of multidrug-resistant tuberculosis.* 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- *Toxicity and tolerability of multidrug-resistant tuberculosis preventive treatment in children.* 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Kuala Lumpur, Malaysia 13-17 November 2012
- *The impact of drug resistance on clinical outcome in children with tuberculous meningitis.* 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012
- *Drug-resistant tuberculosis in children is caused by transmission and amplification of resistance within families.* 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012
- *Multidrug-resistant tuberculosis of the spine in children.* 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011. PC-816-29

Presentations at Conferences

- *Innovative solutions to the challenges of childhood TB.* 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012. Symposium 'Finding solutions to the forgotten epidemic of childhood TB'
- *Decentralised care for child contacts of multidrug-resistant tuberculosis.* 3rd South African TB Conference, Durban, South Africa, 12-15 June 2012. Symposium 'Paediatric TB'
- *Review of challenges to treatment: from trials to formulations.* 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011. Symposium ' Meeting the needs of the most neglected patients: the rising caseload of paediatric drug-resistant tuberculosis'
- *Regional lessons on partnerships for scale-up for child MDR-TB contacts in South Africa.* 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011. Symposium 'Regional lessons on partnerships for scale-up of IPT and contact investigation in children'.
- *Training in reading and classifying CXR findings.* 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union), Lille, France 26-30 October 2011. Workshop 'Child TB training and its role in implementation of child TB management'.

Appendices

Ethical approval and consent forms

All studies in the thesis were approved first by Stellenbosch University Ethics Committees and then by the Ethics Committee of the London School and Hygiene and Tropical Medicine. For studies funding by TREAT TB (through USAID), they were also submitted to, and approved by, the Ethics Committee of the International Union Against Tuberculosis and Lung Disease. Any research carried out on City of Cape Town Health District property was approved by City Health and any research carried out on Western Cape provincial property was approved by the Western Cape Department of Health. A number of the studies requested waiver of consent where it was impossible to trace patients and where data were collected from routine, anonymised patient notes or registers (Study 1, the source cases in Study 5, Study 9, Study 11 and Study 13). These were approved. In all other studies, written informed consent was obtained from the parent/legal guardian with assent from children older than 7 years. Consent forms were generally produced in English, Afrikaans and Xhosa.

In 2003, Professor Schaaf submitted a proposal to the Stellenbosch University Ethical Committee to describe children with tuberculosis at Tygerberg Children's Hospital. This study was approved (2003/005) and renewed each year until I arrived in Cape Town. Following discussion with the Ethics Committee Chair at Stellenbosch University, a number of studies were either conducted under that approval or minor amendments were submitted to cover those studies. These included Studies 1, 4, 9, 10, 12, 13. Existing patient information leaflets and consent forms were used. The original approval from the Ethical Committee of Stellenbosch University is shown on the following pages as well as a form approving ongoing approval for the time when I was carrying out the studies which make up the thesis.

For the studies examining children exposed to DR-TB (Studies 2, 5, 6, 7, 8) a new proposal was submitted to the Ethical Committee. Approval was given (N09/10/280) and is shown in the following pages. An amendment to this study was required as many of the children were brought to clinic appointments by someone who was not the parent or legal guardian. In many families the parent/legal guardian had died and legal transfer of responsibility had not been made. After discussion with the Ethical Committee Chair we submitted an amendment to the effect that we would obtain contact details from the person who had brought the child and then would chase up the parent/legal guardian. If they were not alive, we would obtain consent from the responsible caregiver. Consent forms were produced in English, Afrikaans and Xhosa. The English forms are shown in the following pages.

Study 3 was a sub-study of a larger study (PI: Nulda Beyers) examining the evolution of drug resistance in two communities in Cape Town. Approval is shown in the following pages (N09/05/144). An amendment was submitted to the parent study (which was approved) and consent forms produced in English and Afrikaans (the communities were not Xhosa-speaking). The English version is shown.

Study 11 was a separate study for which a separate proposal was written. Approval is demonstrated below (N10/07/223). A waiver of consent was requested and approved for this retrospective study, examining case notes and previously collected samples.

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

30 Januarie 2003

Dr HS Schaaf
Departement Pediatrie en Kindergesondheid

Geagte dr Schaaf

NAVORSINGSPROJEK: "A PROSPECTIVE EVALUATION OF THE PREVALENCE OF ANTITUBERCULOSIS DRUG RESISTANCE IN CHILDREN IN THE WESTERN CAPE"
PROJEKNOMMER : 2003/005

U aansoek om registrasie en goedkeuring van bogenoemde projek het op 30 September 2002 voor Subkomitee C van die Navorsingskomitee gedien. Die Komitee het in beginsel die projek goedgekeur, maar versoek dat verdere inligting voorsien word.

Hierdie inligting is verskaf en die projek is finaal goedgekeur op 27 Januarie 2003. Die projek is nou geregistreer en u kan voortgaan met die werk. U moet asseblief in verdere korrespondensie na bogenoemde projeknommer verwys.

Ek vestig graag u aandag daarop dat pasiënte wat deelneem aan 'n navorsingsprojek in Tygerberg-hospitaal nie gratis behandeling sal ontvang nie aangesien die PAWK nie navorsing finansieël ondersteun nie.

Die verpleegkorps van die Tygerberg-hospitaal kan ook nie omvattende verpleeghulp met navorsingsprojekte lewer nie weens die swaar werkslading waaronder hulle reeds gebuk gaan. Dit kan dus van 'n navorser verwag word om in sulke gevalle privaat verpleegkundiges te verkry.

Met vriendelike groete

CJ VAN TONDER
NAVORSINGSONTWIKKELING EN –STEUN (TYGERBERG)

CJVT/ev

C:\DOCUMENTS AND SETTINGS\EVISAGIE.000\MY DOCUMENTS\NAVO\PROJECTE\2003\2003-005-001.DOC

Verbind tot Optimale Gesondheid • Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun • Division of Research Development and Support
Posbus/PO Box 19063 • 7505 Tygerberg • Suid-Afrika/South Africa
Tel: +27 21 938 9207 • Faks/Fax: +27 21 933 6330
E-pos/E-mail: cjvt@sun.ac.za

07 July 2010

MAILED

Prof HS Schaaf
Department of Paediatrics and Child Health
Stellenbosch University
P O Box 19063
Tygerberg
7505

Dear Prof Schaaf

"A prospective evaluation of the prevalence of antituberculosis drug resistance in children in the Western Cape."

ETHICS REFERENCE NO: 2003/005

RE : PROGRESS REPORT

At a review panel of the Health Research Ethics Committee that was held on 6 July 2010, the progress report for the abovementioned project has been approved and the study has been granted an extension for a period of one year from this date.

Please remember to submit progress reports in good time for annual renewal in the standard HREC format.

Approval Date: 6 July 2010

Expiry Date: 6 July 2011

Yours faithfully

MRS MERTRUDE DAVIDS
RESEARCH DEVELOPMENT AND SUPPORT
Tel: 021 938 9207 / E-mail: mertrude@sun.ac.za
Fax: 021 931 3352

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot · your knowledge partner

11 February 2010 **MAILED**

Prof HS Schaaf
Department of Paediatrics and Child Health
Stellenbosch University
P O Box 19063
Tygerberg
7505

Dear Prof Schaaf

"Multidrug-resistant TB in children."

ETHICS REFERENCE NO: N09/10/280

RE : APPROVED

At a meeting of the Health Research Ethics Committee that was held on 11 November 2009, the above project was approved on condition that further information is submitted.

This information was supplied and the project was finally approved on 10 February 2010 for a period of one year from this date. This project is therefore now registered and you can proceed with the work.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division: www.sun.ac.za/rds should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.
Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

PARTICIPANT INFORMATION LEAFLET
AND CONSENT FORM FOR USE BY PARENTS/LEGAL GUARDIANS

TITLE OF THE RESEARCH PROJECT: Multidrug-resistant tuberculosis in children

REFERENCE NUMBER: N09/10/280

PRINCIPAL INVESTIGATOR: Professor H Simon Schaaf

ADDRESS: Desmond Tutu TB Centre, Francie Van Zyl Road, Tygerberg 7507

CONTACT NUMBER: 021-9389112/021-9389177

Your child (or foster child if applicable) is being invited to take part in a research project. Please take time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?
This research is studying children who have been exposed to multidrug-resistant tuberculosis.

Tuberculosis (TB) is very common in Cape Town and if a child is unwell with TB (sweating, weight loss, coughing) they need to be treated with four drugs for a number of months. However, it is possible to detect TB infection at an early stage before the child has any problems and feels completely well. To do this it is necessary to find all the children who live in the same house as an adult who is ill with TB. These children are called contacts. Contacts should be examined by a doctor and also have a small injection just under the skin in the arm which is then looked at after two days to see if there is any reaction. If there is any reaction the child may have early TB infection. Children under 5 years of age as well as older children who are HIV-infected are the children at highest risk to develop TB after being in contact with an adult TB case. All child contacts under 5 years of age or HIV-infected contacts of any age should however receive treatment (prophylaxis) if they are otherwise well. The drug that we normally give is called isoniazid.

Because this prevents TB disease from developing it is called preventive treatment. It has been shown that giving this preventive treatment reduces the chance of developing TB disease.

Sometimes people have TB which does not respond to the normal drugs that we give to treat it. When tested in the laboratory the TB bacteria are resistant to these normal drugs. As multidrug-resistant (MDR) TB is resistant to isoniazid it is difficult to know how to treat children who are contacts of an adult with MDR TB. This is why these children (children under 5 years of age and HIV-infected children <14years of age who are in contact with adults who

have MDR TB) are included in this study. In this study we are looking at how to look after these child contacts.

We are going to carry out a study in two parts:

The comparison part. We shall be comparing how effective the hospital system is at finding children needing preventive treatment compared to the community system in Khayelitsha. This will involve the research team coming to your house or meeting you in the local clinic and asking some questions.

The cohort part. This will include all the children who have been in contact with adults with MDR TB. Detailed information will be collected from the carer at the initial clinic visit and the child will be examined. He/she will then be seen every two months for a year. At these reviews the child will be examined, measured and questions will be asked about the child and their health.

As we are going to be carefully recording what is already happening, this study will not involve any tests in addition to those being currently done by the health teams looking after your child (this is the normal treatment of child contacts of MDR TB cases).

What the study will involve is your time, as we shall be asking questions and recording information at each clinic appointment. We also may arrange appointments in addition to those needed if your child were not in the study. These may be at the hospital, local clinic or at your home – whichever is the most convenient and will involve asking questions and examining your child.

Taking part in these studies is entirely optional and if you do not want to take part, your child will be cared for in the usual way. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You can remove your child from the study at any time.

Why has your child been invited to participate?
Your child has been asked to participate as they have been in close contact with an adult who has MDR TB. They may be at risk of developing MDR TB disease themselves and so need to be followed up regularly.

What will your responsibilities be?
If you choose to take part in the study, we would ask that you answer our questions truthfully (all information that you tell us will remain strictly confidential) and bring your child to any arranged appointments to discuss progress and to be examined.

Will your child benefit from taking part in this research?
Taking part in the study will not be of any special benefit to your child. However we hope that the information gathered will help us to plan how best to look after all children exposed to multidrug-resistant TB in the future.

Are there any risks involved in your child taking part in this research?
We shall not be conducting any tests in addition to the ones required by the national programme to look after your child. The decision to prescribe treatment is not part of the study and so any risks associated with these drugs will not be affected by the study. The study team will ask questions at clinic appointments, after clinic appointments or at your home. This may mean that appointments take longer than usual. We do not feel that there will be any risks involved in your child taking part in this research.

If you do not agree to allow your child to take part, what alternatives does your child have?
If you would rather not be in the study, your child will continue to be looked after in the exactly the same way.

Who will have access to your child's medical records?
All the information about your child will be recorded in a way so that they cannot be identified. The information will be kept safe in a locked drawer in a locked office. Only the investigators will see or use the information. If it is used in a publication or thesis the identity of the parents and children will remain anonymous. The study team alone will have access to the information and will keep it in the strictest of confidence.

What will happen in the unlikely event of your child getting injured in any way, as a direct result of taking part in this research study?
As we are observing what is currently happening within the national programme, we do not anticipate that any children will become injured as a result of the study.

Will you or your child be paid to take part in this study and are there any costs involved?
You or your child will not be paid to take part in the study, but your/your child's transport and meal costs will be covered for each study visit. There will be no costs involved for you if your child does not take part.

Is there anything else that you should know or do?
- You can contact Dr Seddon or Prof Schaaf at tel 021-9389177 (Seddon) or 021-9389112 (Schaaf) if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.
- You will receive a copy of this information and consent form for your own records.

Consent to participate in research studies

Declaration by parent/legal guardian

By signing below, I *(name of parent/legal guardian)* ..... agree to allow my child (name of child) ..... who is ..... years old, to take part in a research study entitled ***"Multidrug-resistant tuberculosis in children"***

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older than 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.
- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (*place*) ..... on (*date*) ..... 20.....

Signature of parent/legal guardian **Signature of witness**

Declaration by investigator

I *(name)* ..... declare that:

I explained the information in this document to .....
I encouraged him/her to ask questions and took adequate time to answer them.
I am satisfied that he/she adequately understand all aspects of the research, as discussed above
I did/did not use an interpreter (*if an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (*place*) ..... on (*date*) ..... 20.....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ..... declare that:

I assisted the investigator (*name*) ..... to explain the information in this document to (*name of parent/legal guardian*) ..... using the language medium of Afrikaans/Xhosa.
We encouraged him/her to ask questions and took adequate time to answer them.
I conveyed a factually correct version of what was related to me.
I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) ..... on (*date*) ..... 20....

Signature of interpreter **Signature of witness**

PARTICIPANT INFORMATION LEAFLET
AND ASSENT FORM FOR USE BY CHILDREN

TITLE OF THE RESEARCH PROJECT: Multidrug-resistant tuberculosis in children

REFERENCE NUMBER: N09/10/280

PRINCIPAL INVESTIGATOR: Professor H Simon Schaaf

ADDRESS: Desmond Tutu TB Centre, Francie Van Zyl Road, Tygerberg 7507

CONTACT NUMBER: 021-9389112/021-9389177

You are invited to take part in a study. Please read the information presented here, which will explain what the study is about. Please ask the study staff or doctor any questions about any part of the study that you do not understand. It is important that you understand what this study is about and what it will mean if you take part in the study. Also, your participation is **entirely voluntary** and you are free to say no. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do initially agree to take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be done in the correct ethical way.

What is this research study all about?
This study is going to look at aspects of children who have been exposed to multidrug-resistant tuberculosis (that is TB that does not get better with the normal TB drugs).

Tuberculosis (TB) is very common in Cape Town. Children can live in the same house as adults who have TB – these children are called contacts. Contacts should be examined by a doctor and also have a small injection just under the skin in the arm which is then looked at after two days to see if there is any reaction. If there is any reaction the child may have early TB infection (that means the TB bug is in the body, but you are not yet sick). Children under 5 years of age as well as older children who are HIV-infected are the children at highest risk to develop TB after being in contact with an adult TB case. All child contacts under 5 years of age or HIV-infected contacts of any age should receive treatment (prophylaxis) if they are otherwise well. The drug that we normally use is called isoniazid. This normally protects children from getting sick from TB.

Sometimes people have TB which does not respond to the normal drugs that we give to treat it. This is called multidrug-resistant (MDR) TB. We are not sure how children who have the MDR TB bug in the body, but are not sick, should be treated and this is what this study is about.

We are only going to be looking at what is already happening to children that are in contact with adults with MDR TB. This study will not involve any tests other than those already done by the doctors/nurses looking after children (this is the normal treatment of child contacts of MDR TB cases).

We are only asking for some of your time because we want to ask you and your mother/ caregiver some questions. We will also write all of this down.

Taking part in this study is entirely optional and if you do not want to take part, you will be cared for in the usual way. If you do decide to take part you will be given this information sheet to keep and be asked to sign an assent form. You can ask not to be part of the study at any time.

Why have you been asked to be part of this study?
You have been asked to be part of this study because you have been in close contact with an adult who has MDR TB. You may be at risk of getting MDR TB disease and so you need to be followed up regularly.

What will your responsibilities be?
If you choose to take part in the study, we would ask that you answer our questions truthfully (all information that you tell us will remain strictly confidential).

Will you benefit from taking part in this study?
Taking part in the study will not be of any special benefit to you, but we hope that it will help us to look after all children exposed to multidrug-resistant TB in the future.

Are there any risks involved in taking part in this research?
We are not going to do any tests in addition to the ones that are required by the national programme. The treatment is what you would normally get (no extra drugs). The study team will ask questions at clinic appointments, after clinic appointments or at your home. This may mean that appointments take longer than usual. We do not feel that there will be any risks involved in you taking part in this study.

If you do not agree to take part, what alternatives you have?
If you choose not be in the study, you will be looked after in the exactly the same way.

Who will have access to your medical notes?
The information that we collect about you will be written down in such a way that it cannot be identified as information about you. The information will be kept safe. Only the investigators will see or use the information and will keep it in the strictest of confidence.

Will you be paid to take part in this study?
You will not be paid to take part in the study, but your transport and meal costs will be covered for each study visit.

Is there anything else that you should know?
You can contact Dr Seddon or Prof Schaaf at tel 021-9389177 (Seddon) or 021-9389112 (Schaaf) if you have any further queries or encounter any problems.
You will receive a copy of this information and consent form for your own records.

Assent to participate in research studies

I agree to participate in the study called: "Multidrug-resistant tuberculosis in children" as described in the Information Leaflet

Assent of minor

I (*Name of Child/Minor*)..... have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.
- They have also explained that this study will involve some of the appointments taking longer than if I was not in the study.
- I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

Name of child
(To be written by the child if possible)

Independent witness

06 June 2009

MAILED

Prof N Beyers
Dept of Paediatrics and Child Health
Desmond Tutu TB Centre
Stellenbosch University
Tygerberg
7505

Dear Prof Beyers

"The evolution of drug-resistant tuberculosis in a community."

ETHICS REFERENCE NO: N09/05/144

RE : APPROVAL

It is a pleasure to inform you that the Health Research Ethics Committee has approved the above-mentioned project on 03 June 2009, including the ethical aspects involved, for a period of one year from this date.

This project is therefore now registered and you can proceed with the work. Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division) should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.

Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Committee for Human Research complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

une 2009 15:47 Page 1 c

PARTICIPANT INFORMATION LEAFLET
AND CONSENT FORM FOR USE BY PARENTS/LEGAL GUARDIANS

TITLE OF THE RESEARCH PROJECT: The evolution of drug-resistant tuberculosis in a community

REFERENCE NUMBER: N09/05/144

PRINCIPAL INVESTIGATOR: Professor Nulda Beyers

ADDRESS: Desmond Tutu TB Centre, Francie Van Zyl Road, Tygerberg 7507

CONTACT NUMBER: 021-9389114

Your child (or foster child if applicable) is being invited to take part in a research project. Please take time to read the information presented here, which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?
This research is studying how drug resistance develops in tuberculosis.

Tuberculosis (TB) is very common in Cape Town and is caused by a bug which can be spread from person to person. It can lead to illness such as cough, fever, sweating, weight loss and sometimes death. Normally TB can be treated with four drugs and if these are taken all the time it is usually cured.

Sometimes people have TB which does not respond to the normal drugs that we give to treat it. When tested in the laboratory the TB bacteria are resistant to these normal drugs and is called drug-resistant (DR) TB. At the moment we are not completely sure how DR TB develops.

This research is going to look at how the TB bugs go from being able to be treated by the normal drugs to being resistant. We are planning on looking at how TB spreads in families and then looking at the samples of the bugs in the laboratory to see when the resistance developed. This should help us to tell how resistance occurs.

Why has your child been invited to participate?
Your child has been asked to participate as they have had XDR TB. This form of TB is resistant to lots of the drugs used to treat TB. It is important to discover how they developed XDR TB and who else in the family, household and community had TB beforehand.

What will your responsibilities be?
If you choose to take part in the study, we would ask that you answer our questions truthfully (all information that you tell us will remain strictly confidential). We will ask you and your child

questions about your family, who had TB, what treatment they received and what happened to them. We will also ask some details about their health and about their life such as where they lived and what job they did. If anyone in the household has any symptoms of TB such as sweating, weight loss or cough we will ask them for a sputum sample so that we can test it for TB. We will tell them of the result of these tests and help them get treatment if needed. We also will look in the hospital and clinic records of your child and any household members who have had TB to give us some more information. Finally, we will look at the samples in the laboratory of any household members who have had TB.

Will your child benefit from taking part in this research?
Taking part in the study will not be of any special benefit to you or your child. However we hope that the information gathered will help us to understand how drug resistance develops and might allow us to treat drug-resistant tuberculosis better in the future.

Are there any risks involved in your child taking part in this research?
We shall not be conducting any tests other than asking for sputum samples if people have symptoms. The study team will ask questions which will take up your time and some of the questions may be difficult to answer as they are about family members who may have been unwell or who have even died. We do not feel, however, that there will be any risks involved in you or your child taking part in this research.

If you do not agree to allow your child to take part, what alternatives does your child have?
If you would rather not be in the study, your child will continue to be looked after in the exactly the same way.

Who will have access to your child's medical records?
All the information about your child will be recorded in a way so that they cannot be identified. The information will be kept safe in a locked drawer in a locked office. Only the investigators will see or use the information. If it is used in a publication or thesis the identity of the parents and children will remain anonymous. The study team alone will have access to the information and will keep it in the strictest of confidence.

What will happen in the unlikely event of your child getting injured in any way, as a direct result of taking part in this research study?
As we are asking questions and if necessary asking for sputum samples, we do not anticipate that anyone will become injured as a result of the study. However, the study team have medical training and will help in case of any problem.

Will you or your child be paid to take part in this study and are there any costs involved?
You or your child will not be paid to take part in the study, but your/your child's transport and meal costs will be covered if you need to go anywhere as part of the study. There will be no costs involved for you.

Is there anything else that you should know or do?

- You can contact Dr Seddon at tel 021-9389177 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study team.
- You will receive a copy of this information and consent form for your own records.

Consent to participate in research studies

Declaration by parent/legal guardian

By signing below, I *(name of parent/legal guardian)* ..... agree to allow my child (name of child) ..... who is ..... years old, to take part in a research study entitled **"The evolution of drug-resistant tuberculosis in a community"**

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older than 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.

Signed at (*place*) ..... on (*date*) ..... 20.....

Signature of parent/legal guardian Signature of witness

Declaration by investigator

I *(name)* ..... declare that:

I explained the information in this document to .....
I encouraged him/her to ask questions and took adequate time to answer them.
I am satisfied that he/she adequately understand all aspects of the research, as discussed above
I did/did not use an interpreter (*if an interpreter is used, then the interpreter must sign the declaration below).*

Signed at (*place*) ..... on (*date*) ..... 20.....

Signature of investigator **Signature of witness**

Declaration by interpreter

I *(name)* ..... declare that:

I assisted the investigator (*name*) ..... to explain the information in this document to (*name of parent/legal guardian*) ..... using the language medium of Afrikaans/Xhosa.
We encouraged him/her to ask questions and took adequate time to answer them.
I conveyed a factually correct version of what was related to me.
I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) ..... on (*date*) ..... 20....

Signature of interpreter **Signature of witness**

PARTICIPANT INFORMATION LEAFLET
AND ASSENT FORM FOR USE BY CHILDREN

TITLE OF THE RESEARCH PROJECT: The evolution of drug-resistant tuberculosis in a community

REFERENCE NUMBER: N09/05/144

PRINCIPAL INVESTIGATOR: Professor Nulda Beyers

ADDRESS: Desmond Tutu TB Centre, Francie Van Zyl Road, Tygerberg 7507

CONTACT NUMBER: 021-9389114

You are invited to take part in a study. Please read the information presented here, which will explain what the study is about. Please ask the study staff any questions about any part of the study that you do not understand. It is important that you understand what this study is about and what it will mean if you take part in the study. Also, your participation is **entirely voluntary** and you are free to say no. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do initially agree to take part.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be done in the correct ethical way.

What is this research study all about?
This research is studying how drug resistance develops in tuberculosis.

Tuberculosis (TB) is very common in Cape Town and is caused by a bug which can be spread from person to person. It can lead to illness such as cough, fever, sweating, weight loss and sometimes death. Normally TB can be treated with four drugs and if these are taken all the time it is usually cured.

Sometimes people have TB which does not respond to the normal drugs that we give to treat it. When tested in the laboratory the TB bacteria are resistant to these normal drugs and is called drug-resistant (DR) TB. At the moment we are not completely sure how DR TB develops.

This research is going to look at how the TB bugs go from being able to be treated by the normal drugs to being resistant. We are planning on looking at how TB spreads in families and then looking at the samples of the bugs in the laboratory to see when the resistance developed. This should help us to tell how resistance occurs.

Why have you been asked to be part of this study?
You have been asked to participate as you have had XDR TB. This form of TB is resistant to lots of the drugs used to treat TB. It is important to discover how you developed XDR TB and who else in the family, household and community had TB beforehand.

What will your responsibilities be?
If you choose to take part in the study, we would ask that you answer our questions truthfully (all information that you tell us will remain strictly confidential).

Will you benefit from taking part in this study?
Taking part in the study will not be of any special benefit to you, but we hope that it will help us to understand how drug resistance develops. It may help us to look after children in the future who have drug-resistant TB.

Are there any risks involved in taking part in this research?
We are not going to do any tests and so we do not expect there to be any risks. The study team will ask questions which may be difficult to answer as they are personal and may be about your family. However, we do not feel that there will be any risks involved in you taking part in this study.

If you do not agree to take part, what alternatives you have?
If you choose not be in the study, you will be looked after in the exactly the same way.

Who will have access to your medical notes?
The information that we collect about you will be written down in such a way that it cannot be identified as information about you. The information will be kept safe. Only the investigators will see or use the information and will keep it in the strictest of confidence.

Will you be paid to take part in this study?
You will not be paid to take part in the study, but your transport and meal costs will be covered if you need to go anywhere as part of the study.

Is there anything else that you should know?
- You can contact Dr Seddon at tel 021-9389177 if you have any further queries or encounter any problems.
- You will receive a copy of this information and consent form for your own records.

Assent to participate in research studies

I agree to participate in the study called: **"The evolution of drug-resistant tuberculosis in a community"** as described in the Information Leaflet

Assent of minor

I (*Name of Child/Minor*)..... have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.
- They have also explained that this study will take up some of my time.
- I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

Name of child
(To be written by the child if possible)

Independent witness

30 August 2010 **MAILED**

Prof HS Schaaf
Department of Paediatrics and Child Health
Stellenbosch University
P O Box 19063
Tygerberg
7505

Dear Prof Schaaf

The relationship between clinical outcome and Mycobacterium Tuberculosis drug susceptibility in children with tuberculosis meningitis.

ETHICS REFERENCE NO: N10/07/223

RE : APPROVAL

At a meeting of the Health Research Ethics Committee that was held on 4 August 2010, the above project was approved on condition that further information is submitted.

This information was supplied and the project was finally approved on 24 August 2010 for a period of one year from this date. This project is therefore now registered and you can proceed with the work.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division: www.sun.ac.za/rds should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.
Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

Approval Date: 24 August 2010 Expiry Date: 24 August 2011

Yours faithfully

MR FRANKLIN WEBER
RESEARCH DEVELOPMENT AND SUPPORT
Tel: +27 (0)21 938-9657 / E-mail: fweb@sun.ac.za
Fax: +27 (0)21 931-3352

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax +27 21 931 3352

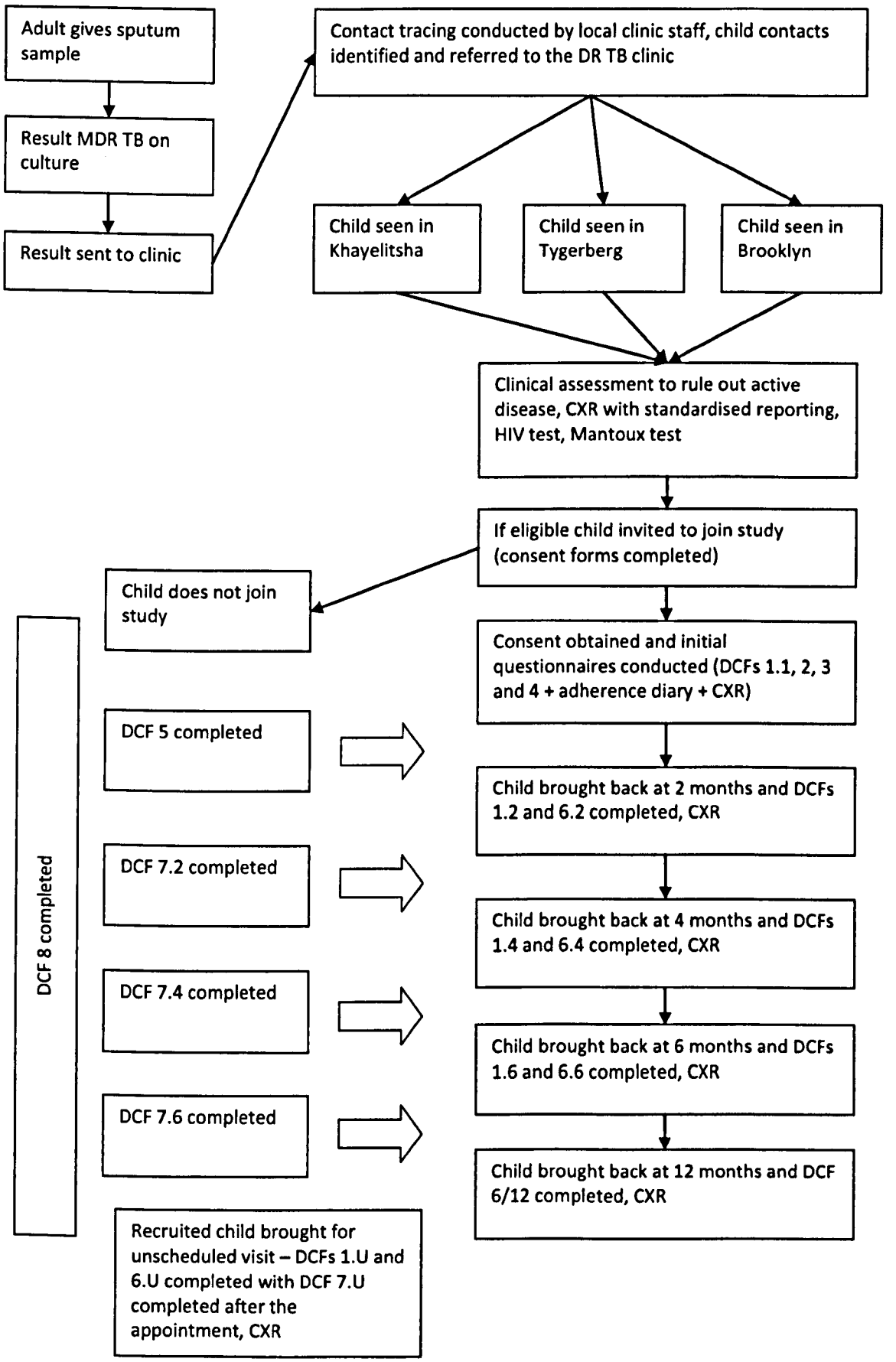
Data capture tools

Children exposed to MDR-TB (Studies 2, 7 and 8)

The studies assessing children exposed to MDR-TB and subsequent preventive therapy (Studies 2, 7 and 8) were captured in a systematic way using data capture forms that I designed. These were based on other forms that are in use in the Desmond Tutu TB Centre for household studies looking at child contacts of drug-susceptible TB. This allows synthesis and comparison between studies. These forms are provided in the following pages and correspond to the diagram on the next page which outlines at which point in the study different forms were completed. The following forms are:

1. DCF1.0 Demographic details at recruitment
2. DCF1.2 Demographic details at 2 months (only one DCF 1 shown)
3. DCF1.4 Demographic details at 4 months
4. DCF1.6 Demographic details at 6 months
5. DCF1.12 Demographic details at 12 months
6. DCF1.U Demographic details at an unscheduled visit
7. DCF2 Child details
8. DCF3 Index case details
9. DCF4 Household details
10. DCF5 Index case details from register
11. DCF6.2 Follow up form for 2 month appointment
12. DCF6.4 Follow up form for 4 month appointment (only one DCF6 shown)
13. DCF6.6 Follow up form for 6 month appointment
14. DCF6.12 Follow up form for 12 month appointment
15. DCF6.U Unscheduled visit form
16. DCF7.2 Adherence form after 2 month appointment (only one DCF7 shown)
17. DCF7.4 Adherence form after 4 month appointment
18. DCF7.6 Adherence form after 6 month appointment
19. DCF7.U Adherence form after unscheduled appointment
20. DCF8 Patient tracking form

Patient Flow for children exposed to multidrug-resistant tuberculosis



Demographic form at recruitment (DCF1.0)

Initial consultation following recruitment

1.0.1. First Name of child	1.0.2. Surname of child
1.0.3. Alternative First name of child	1.0.4. Alternative Surname of child
1.0.5. Date of Birth of child (dd/mm/yyyy)	1.0.6. Hospital number of child
1.0.7. First Name of Main Carer	1.0.8. Surname of Main Carer
1.0.9. Alternative first name of Main Carer	1.0.10. Alternative Surname of Main Carer
1.0.11. Contact telephone number of Main Carer	1.0.12. Alternative telephone number of Main Carer
1.0.13. First Name of Other Carer	1.0.14. Surname of Other Carer
1.0.15. Alternative first name of Other Carer	1.0.16. Alternative Surname of Other Carer
1.0.17. Contact telephone number of Other Carer	1.0.18. Alternative telephone number of Other Carer
1.0.19. Address of child	1.0.20. Alternative address of child
1.0.21. Date Today (dd/mm/yyyy)	1.0.22. Health Clinic
1.0.23. First name of Index Case	1.0.24. Surname of Index Case
1.0.25. Alternative first name of Index case	1.0.26. Alternative Surname of Index case
1.0.27. Registration number on TB register of index case	1.0.28. Contact telephone number of index case

Child Medical Form (DCF2)

Initial consultation following recruitment

2.1. Consent		
2.1.1. Consent for study participation (if no or unknown then do not proceed)	0 No	1 Yes
	-5 Unknown	
2.1.2. Consent to use HIV test result	0 No	1 Yes
	-5 Unknown	
2.1.3. Entry Point	4 Tygerberg	5 Khayelitsha
	6 Brooklyn	-8 Other
2.1.4. Date today (dd/mm/yyyy)		

2.2 Personal Information		
2.2.1. DOB (dd/mm/yyyy)		
2.2.2. Gender	1 Male	2 Female
2.2.3. Ethnicity	4 White	5 Coloured
	8 Xhosa	9 Zulu
	10 Indian	11 Sotho
	-8 Other	
2.2.4. Home Language	1 English	2 Afrikaans
	3 Xhosa	4 Sotho
	5 Zulu	-8 Other

2.3. Medical information – Past tuberculosis history		
2.3.1. Has the child had a TB skin test? (if no or not known go to 2.3.4)	1 Yes	0 No
	-5 Not known	
2.3.2. If a Mantoux test, what was the result? (Positive >/=10 if HIV-uninfected, >/=5 if HIV-infected)	1 Positive	0 Negative
	-5 Unknown	-4 Not applicable
2.3.3. If a Mantoux result was recorded what is the size (mm) (if not recorded go to 2.3.4.)		
2.3.4. Has the child ever been treated for TB disease before? (if no or not known go to 2.3.7)	1 Yes	0 No
	-5 Unknown	
2.3.5. If yes, when was the most recent TB treatment started (dd/mm/yyyy) ?		
2.3.6. Was TB treatment completed?	1 Yes	0 No
	-5 Unknown	
2.3.7. Has the child been given TB preventive treatment before? (if no or unknown go to 2.4.1)	1 Yes	0 No
	2 Currently on treatment	-5 Unknown
2.3.8. If yes, or on treatment, when was the most recent preventive treatment started (dd/mm/yyyy)?		
2.3.9. Was TB preventive treatment completed?	1 Yes	0 No
	2 Currently on treatment	-5 Unknown
2.3.10. Which regimen was prescribed?	1 INH	2 High dose INH
	3 HEO	4 HEthO
	-5 Unknown	

2.4. Medical information – HIV details		
2.4.1. Is the child HIV-infected? (if no or unknown go to 2.5.1)	1 Yes	0 No
	-5 Not known	
2.4.2. If yes, when was the diagnosis made (dd/mm/yyyy)?		
2.4.3. Is the child currently on ART? (if no go to 2.4.7)	1 Yes	0 No
	-5 Unknown	
2.4.4. If yes, which ART? (ring any that apply)	1 AZT	2 3TC
	3 NVP	4 D4T
	5 LPV	6 LPV/Ritonavir
	7 EFV	8 ABC
	9 PI	-8 Other (write in which)
2.4.5. When was ART started (dd/mm/yyyy)?		
2.4.6. What was the CD4 count at ART initiation? (xxxx)		
2.4.7. What was the CD4 percentage at ART initiation? (xx.x%)		
2.4.8. Most recent CD4 count? (xxxx)		
2.4.9. Most recent CD4 percentage? (xx.x%)		
2.4.10. Date of most recent CD4 test (dd/mm/yyyy)?		
2.4.11. Site of HIV care?	1 Government ARV clinic	2 Hospital ARV clinic
	3 Private doctor	-8 Other

2.5. Medical Information – Other Medical issues		
2.5.1 Child Weight (xx.x kg)		
2.5.2. Child Length/Height (xxx cm)		
2.5.3. BCG scar visible?	1 Yes	0 No
	-5 Unknown	
2.5.4. Is the child currently known to be asthmatic?	1 Yes	0 No
	-5 Unknown	
2.5.5. Does the child currently have any chronic bone or joint problems?	1 Yes	0 No
	-5 Unknown	
2.5.6. Does the child currently have a palpable liver?	1 Yes	0 No
	-5 Unknown	
2.5.7. Does the child currently have a palpable spleen?	1 Yes	0 No
	-5 Unknown	
2.5.8. Does the child currently have any clinical signs of chronic lung disease?	1 Yes	0 No
	-5 Unknown	
2.5.9. Is the child's colour vision currently normal?	1 Yes	0 No
	-4 Not possible to test	-5 Unknown

2.6. Details of Exposure		
2.6.1. What is the relationship of the index case to the child? (biological relationships)	1 Mother	2 Father
	3 Grandmother	4 Grandfather
	5 Aunt	6 Uncle
	7 Cousin	8 Sibling
	9 Caregiver other than family	-8 Other
2.6.2. When did exposure to the index case end?	1 More than six months ago	2 More than three months ago but less than six months
	3 Less than three months ago	4 Ongoing
2.6.3. How long was the child exposed to the index case?	1 Less than a week	2 One week to one month
	3 More than one month to three months	4 More than three to six months
	5 More than six months to a year	6 More than a year
2.6.4. Is the index case the child's primary care giver?	1 Yes	0 No
	-5 Unknown	
2.6.5. If not the primary caregiver, is the index case the child's secondary caregiver?	1 Yes	0 No
	-4 Not applicable	-5 Unknown
2.6.6. During the exposure was/is there daily contact between the index case and the child?	1 Yes	0 No
	-5 Unknown	
2.6.7. Does the index case live in the same house as the child?	1 Yes	0 No
	-5 Unknown	
2.6.8. Does the index case sleep in the same room as the child?	1 Yes	0 No
	-5 Unknown	

2.6. Details of Exposure		
2.6.9. Does the index case sleep in the same bed as the child?	1 Yes	0 No
	-5 Unknown	
2.6.10. How many hours on average does the index case spend with the child each day?	1 0-4	2 5-8
	3 9-12	4 >12
2.6.11. Has the child been in contact with more than one index case? (If no, go to 3.1.)	1 Yes	0 No
	-5 Unknown	

2.7. Second index case (if no third index case, go to DCF 3)		
2.7.1. What is the DST pattern of the second index case?	1 Drug susceptible	2 INH/RIF monresistant
	3 MDR	-5 Unknown
2.7.2. What is the relationship of the secondary index case to the child? (biological relationships)	1 Mother	2 Father
	3 Grandmother	4 Grandfather
	5 Aunt	6 Uncle
	7 Cousin	8 Sibling
	9 Caregiver other than family	-8 Other

2.8. Third index case		
2.8.1. What is the DST pattern of the third index case?	1 Drug susceptible	2 INH/RIF monresistant
	3 MDR	-5 Unknown

Index Case Form (DCF3)
Initial consultation following recruitment

3.1. Index Case Information		
3.1.1. DOB (dd/mm/yyyy)		
3.1.2. Gender	1 Male	2 Female
3.1.3. Is the index case HIV-infected?	1 Yes	0 No
	-5 Unknown	
3.1.4. Does the index case smoke currently? (if no go to 3.1.7)	1 Yes	0 No
	-5 Unknown	
3.1.5. How much do they smoke?	1 Does not smoke	2 Less than 5 cigarettes a day
	3 5 – 10 cigarettes a day	4 11 - 20 cigarettes a day
	5 More than 20 cigarettes a day	-5 Unknown
3.1.6. How many years has the index case smoked?	1 Does not smoke	2 Less than six months
	3 Six months to one year	4 More than one year to five years
	5 More than five years	-5 Unknown
3.1.7. Does the index case drink alcohol? (if no go to 3.1.9)	1 Yes	0 No
	-5 Unknown	
3.1.8. How much alcohol does the index case drink	1 Never drinks	2 Drinks less than once a week
	3 Drinks more than once a week	4 Drinks most nights
	5 Drinks every night	-5 Unknown
3.1.9. Has the index case been admitted to a hospital for TB before this episode?	1 Yes	0 No
	-5 Unknown	
3.1.10. Has the index case been in prison	1 Yes	0 No
	-5 Unknown	

Household form (DCF4)

Initial consultation following recruitment

4.1. Household details		
4.1.1. Type of residence	1 Main House	2 Yard House
	3 Flat	-8 Other
4.1.2. Type of housing structure	1 Tin Shack	2 Prefab House
	3 Brick House	4 Container
	5 Wendy House	-8 Other
4.1.3. Number of rooms in house (xx)		
4.1.4. Are there the following assets in the house? (circle any that apply)	1 Electricity	2 A radio
	3 DVD player	4 A television
	5 A refrigerator	6 A bicycle
	7 A motorcycle	8 A car
	9 A cell phone	10 A landline
4.1.5. What is the main source of drinking water?	1 Piped water in the residence	2 Piped water from a public tap
	-8 Other (specify in next box)	
4.1.6. What is the main type of toilet in the household?	1 Flush toilet in the house	2 Shared Flush toilet
	3 Pit latrine	4 VIP latrine
	5 Bush/field toilet	6 Bucket system
	-8 Other (specify in next box)	

4.1. Household details		
4.1.7. Number of persons older than 15 living in the house? (xx)		
4.1.8. Number of persons younger than 15 living in the house? (xx)		
4.1.9. Number of persons younger than 5 living in the house? (xx)		
4.1.10. Number of smokers in the house? (xx)		
4.1.11. Alcohol usage by any adult in the house?	1 No one in the house drinks	2 Adults in the house drink less than once a week
	3 Adults in the house drink more than once a week	4 Adults in the house drink most nights
	5 Adults in the house drink every night	-5 Unknown
4.1.12. Is there a separate room for cooking in the house?	1 Yes	0 No
	-5 Unknown	
4.1.13. What fuel source is mainly used for cooking in the summer?	1 Paraffin stove	2 Wood fire
	3 Coal fire	4 Open flame of other kind
	5 Electric oven	6 Electric heater
	-8 Other (specify in next box)	
4.1.14. What fuel source is mainly used for cooking in the winter?	1 Paraffin stove	2 Wood fire
	3 Coal fire	4 Open flame of other kind
	5 Electric oven	6 Electric heater
	-8 Other (specify in next box)	

Index Case Register Form (DCF5)

To be obtained later from TB register or from

5.1. Register information for the MDR TB episode in the index case		
5.1.1. Register number		
5.1.2. Registration Date (dd/mm/yyyy)		
5.1.3. Gender	1 Male	2 Female
5.1.4. Patient Category	1 N - New patient	2 RC – Relapse (Pulmonary)
	4 RF – Retreatment after Failure (Pulmonary)	6 RD – Retreatment after default (Pulmonary)
	-8 OR – Other previously treated	-5 Unknown
5.1.5. Date of production of sputum sample that diagnosed MDR TB (dd/mm/yyyy)		
5.1.6. Method of diagnosis of MDR TB	1 LPA on sputum sample	2 LPA on culture sample
	3 Conventional DST on culture sample	-5 Unknown
5.1.7. Date of initiation of treatment (dd/mm/yyyy)		
5.1.8. Smear result of sample that diagnosed MDR TB	0 Negative	1 Scanty
	10 1+	20 2+
	30 3+	150 Positive without specifying smear pattern
	-5 Unknown	
5.1.9. Date of most recent sputum sample (dd/mm/yyyy)		
5.1.10. Smear result of most recent sample	0 Negative	1 Scanty
	10 1+	20 2+
	30 3+	150 Positive without specifying smear pattern
	-5 Unknown	

5.2. Resistance pattern of sample at which MDR TB diagnosed (tick one box for each drug)				
	Susceptible	Resistant	Not tested	Unknown
5.2.1 R – rifampicin				
5.2.2 H – isoniazid				
5.2.3 E – ethambutol				
5.2.4 Z – pyrazinamide				
5.2.5 S – streptomycin				
5.2.6 Eth – ethionamide				
5.2.7 A – amikacin				
5.2.8 O – ofloxacin				

5.3. HIV details of index case		
5.3.1. HIV status (if Negative omit next three questions)	1 Positive	0 Negative
	-5 Unknown	
5.3.2. On ART at initiation of MDR TB treatment?	1 Yes	0 No
	-5 Unknown	
5.3.3. Last CD4 count (xxxx)		
5.3.4. Currently on ART?	1 Yes	0 No
	-5 Unknown	

Follow up form (DCF6)

To be completed at the 2 month appointment

6.2.1. Follow up data		
6.2.1.1. Site of consultation	4 Tygerberg	5 Khayelitsha
	6 Brooklyn	-8 Other
6.2.1.2. Date Today (dd/mm/yyyy)		
6.2.1.3. Result of consultation	1 Continue HEO/HEThO/H	2 Complete HEO/HEThO/H
	3 Stop HEO/HEThO/H	4 Diagnosed TB
	-8 Other (specify in next box)	
6.2.1.4. Weight (xx.x kg)		
6.2.1.5. Length/Height (xxx cm)		
6.2.1.6. Mantoux result (Positive >/=10 if HIV-uninfected, >/=5 if HIV-infected)	1 Positive	0 Negative
	2 Not repeated	-5 Unknown
6.2.1.7. If a Mantoux result was recorded what is the size (xx mm) (if not recorded go to 6.2.1.8)		
6.2.1.8. Has the RTH card been brought to the appointment?	1 Yes	0 No
	-5 Unknown	
6.2.1.9. Has the treatment diary been brought to the appointment?	1 Yes	0 No
	3 No treatment diary	-5 Unknown
6.2.1.10. Has the child missed any appointments since last seen? (if no or unknown then go to 6.2.2.)	1 Yes	0 No
	-5 Unknown	
6.2.1.11. If yes, what were the reasons?	1 Forgot	2 Too far
	3 Not enough money for travel	4 Busy
	5 Child ill	6 Carer ill
	-5 Other (specify in next box)	

6.2.2. Medical information – HIV details		
6.2.2.1. Is the child HIV-infected? (if no or unknown go to 6.2.3)	1 Yes	0 No
	-5 Not known	
6.2.2.2. If yes, when was the diagnosis made (dd/mm/yyyy)?		
6.2.2.3. Is the child currently on ART? (if no or unknown go to 6.2.2.6.)	1 Yes	0 No
	-5 Unknown	
6.2.2.4. If yes, which ART? (ring any that apply)	1 AZT	2 3TC
	3 NVP	4 D4T
	5 LPV	6 LPV/Ritonavir
	7 EFV	8 ABC
	9 PI	-8 Other (write in which)
6.2.2.5. When was ART started (dd/mm/yyyy)?		
6.2.2.6. What was the CD4 count at ART initiation? (xxxx)		
6.2.2.7. What was the CD4 percentage at ART initiation? (xx.x%)		
6.2.2.8. Most recent CD4 count? (xxxx)		
6.2.2.9. Most recent CD4 percentage? (xx.x%)		
6.2.2.10. Date of most recent CD4 test (dd/mm/yyyy)?		
6.2.2.11. Site of HIV care?	1 Government ARV clinic	2 Hospital ARV clinic
	3 Private doctor	-8 Other

6.2.3. Adherence		
6.2.3.1. How is the child receiving their medications	1 DOT from clinic	2 DOT from treatment support worker
	3 Parent receives pills weekly from clinic and supervises treatment to child	4 Parent receives pills weekly from treatment support worker and supervises treatment to child
	5 Parent receives pills monthly from clinic and supervises treatment to child	-8 Other
6.2.3.2. Did the child miss or vomit their medications yesterday?	1 Yes	0 No
	-5 Unknown	
6.2.3.3. Did the child miss or vomit their medications the day before yesterday (name day)?	1 Yes	0 No
	-5 Unknown	
6.2.3.4. Did the child miss or vomit their medications the day before that (name day)?	1 Yes	0 No
	-5 Unknown	
6.2.3.5. How many times in the last week have doses been missed?	1 None	2 1 - 2
	3 3 – 4	4 5 – 6
	5 All of them	-5 Unknown
6.2.3.6. When was the last time that medications were missed?	1 Never	2 During the last week
	3 During the last two weeks	4 During the last month
	5 Over a month ago	6 Don't remember
6.2.3.7. In the last 30 days how many doses has the child received? (score from visual scale) (0 – 30) (xx)		
6.2.3.8. From the treatment diary, how many doses have been missed in the last 2 months? (xx)		

6.2.4. Side effects of medications – since the last time the child was seen, have they had any of the following? (Refer to side effect grading sheet)						
	None	Grade 1	Grade 2	Grade 3	Grade 4	Not known
Joint, muscle or bone pain (other than injuries)						
Skin Rashes						
Itchy skin						
Headache						
Sleeping/mood						
Lethargy						
Visual problems						
Vomiting						
Diarrhoea						
Jaundice						
Appetite/nausea						

6.2.5. Clinical examination		
6.2.5.1. Does the child have any bone or joint pain?	1 Yes	0 No
	-5 Unknown	
6.2.5.2. Does the child have a palpable or tender liver?	1 Yes	0 No
	-5 Unknown	
6.2.5.3. Does the child have a skin rash possibly attributable to the medications?	1 Yes	0 No
	-5 Unknown	
6.2.5.4. Is the child's colour vision normal?	1 Yes	0 No
	2 Not possible to test	-5 Unknown

Adherence form (DCF7)

To be completed after 2 month follow-up having telephoned local clinic

7.2.1. Follow up data		
7.2.1.1. How is the child receiving their medications	1 DOT from clinic	2 DOT from treatment support worker
	3 Parent receives pills weekly from clinic and supervises treatment to child	4 Parent receives pills weekly from treatment support worker and supervises treatment to child
	5 Parent receives pills monthly from clinic and supervises treatment to child	-8 Other
7.2.1.2. Over the last month, how many medication pick-ups have been missed?	1 None	2 1 – 2
	3 3 – 4	4 5 - 10
	5 More than 10	-5 Unknown
7.2.1.3. How would the local clinic staff rate the caregiver in respect to giving the medication to the child?	1 Completely reliable	2 Fairly reliable
	3 Fairly unreliable	4 Totally unreliable
	-5 Unknown	

Child Tracking Form (DCF8)

To complete at each consultation

8.1. Recruitment	
8.1.1. Date of recruitment (dd/mm/yyyy)	
8.1.2. Planned date for 2/12 appointment? (dd/mm/yyyy)	
8.1.3. Date TB register consulted to complete DCF 5? (dd/mm/yyyy)	

8.2. Two month visit	
8.2.1. Date of appointment? (dd/mm/yyyy)	
8.2.2. Planned date for 4/12 appointment? (dd/mm/yyyy)	
8.2.4. Date DCF7.2 completed (dd/mm/yyyy)	

8.3. Four month visit	
8.3.1. Date of appointment? (dd/mm/yyyy)	
8.3.2. Planned date for 6/12 appointment? (dd/mm/yyyy)	
8.3.4. Date DCF7.4 completed (dd/mm/yyyy)	

8.4. Six month visit	
8.4.1. Date of appointment? (dd/mm/yyyy)	
8.4.2. Planned date for 12/12 appointment? (dd/mm/yyyy)	
8.4.4. Date DCF7.6 completed (dd/mm/yyyy)	

8.5. Twelve month visit	
8.5.1. Date of appointment (dd/mm/yyyy)	

8.6. Unscheduled visit 1	
8.6.1. Date of appointment (dd/mm/yyyy)	
8.6.2. Planned date for next appointment (dd/mm/yyyy)	
8.6.3. Date DCF7.U completed (dd/mm/yyyy)	

8.7. Unscheduled visit 2	
8.7.1. Date of appointment (dd/mm/yyyy)	
8.7.2. Planned date for next appointment (dd/mm/yyyy)	
8.7.3. Date DCF7.U completed (dd/mm/yyyy)	

8.8. Study outcome	
8.8.1. Date lost to follow up (dd/mm/yyyy)	
8.8.2. Date defaulted (dd/mm/yyyy)	
8.8.3. Date withdrawn from study (dd/mm/yyyy)	
8.8.4. Date completed study (dd/mm/yyyy)	
8.8.5. Date TB diagnosed (dd/mm/yyyy)	
8.8.6. Date of death (dd/mm/yyyy)	

Reasons for non-attendance at clinic (Study 6)

For Study 6 three data capture forms were created following the focus group discussion to explore characteristics of children and their families, the logistical complications of getting to clinic and their perception of DR-TB. These are shown on the following pages

1. DCF1 Demographics
2. DCF2 Travel to clinics
3. DCF3 Perceptions
4. DCF4 Living standards measures

DCF 1 - Demographics		
1.1. Date today (dd/mm/yyyy)		
1.2. Date of birth of child (dd/mm/yyyy)		
1.3. Was the child brought for the appointment?	1. Yes	0. No
1.4. Gender of child	1. Male	2. Female
1.5. Ethnicity	4. White	2. Black
	5. Coloured	-8. Other
1.6. HIV status of child	1. Infected	0. Uninfected
	-5. Unknown	
1.7. Relationship of main carer to child	1. Mother	2. Father
	3. Grandmother	4. Grandfather
	-8. Other	
1.8. Years formal education main carer		
1.9. Paid work of main carer	1. Does not work for pay	2. Occasional work for pay
	3. Regular part-time work for pay	4. Regular full-time work for pay
1.10. Does main carer look after other children?	1. Yes	0. No
1.11. Gender of main carer	1. Male	2. Female
1.12. Gender of index case	1. Male	2. Female
1.13. Relationship of index case to child	1. Mother	2. Father
	3. Grandmother	4. Grandfather
	-8. Other	
1.14 LSM score of household		
1.15. Does anyone in the house smoke	1. Yes	0. No
1.16. Does anyone in the house drink alcohol	1. Yes	0. No
1.17. Does anyone in the house use illegal drugs	1. Yes	0. No

DCF 2 - Travel to clinics		
2.1. Distance to local clinic		
2.2. Time taken to local clinic		
2.3. Cost to local clinic		
2.4. Transport to local clinic	1. Walk	2. Train
	3. Minibus taxi	4. Private Car
	5. More than one ride	-8. Other
2.5. Distance to MDR-TB clinic		
2.6. Time taken to MDR-TB clinic		
2.7. Cost to MDR-TB clinic		
2.8. Transport to MDR-TB clinic	1. Walk	2. Train
	3. Minibus taxi	4. Private Car
	5. More than one ride	-8. Other

DCF 3 - Perceptions		
3.1. Do you have confidence in the medical staff at your local clinic?	1. Yes	0. No
3.2. Do you have confidence in the medical staff at the MDR-TB clinic?	1. Yes	0. No
3.3. Does the weather affect your decision on whether to attend appointments at the MDR-TB clinic?	1. Yes	0. No
3.4. Do you consider MDR-TB a disease that can kill you?	1. Yes	0. No
3.5. Do you consider MDR-TB a disease that can be treated successfully?	1. Yes	0. No
3.6. Do you think that people in your community with MDR-TB are discriminated against?	1. Yes	0. No
3.7. Do you feel that employers in your community discriminate against people with MDR-TB?	1. Yes	0. No
3.8. Are you concerned about the risk of being infected with MDR-TB while waiting at the MDR-TB clinic?	1. Yes	0. No
3.9. Do you think that your child would take anti-TB medicines every day without a problem?	1. Yes	0. No
3.10. Are you concerned about the side effects of the anti-TB medicines for the child?	1. Yes	0. No
3.11. Out of ten, for you how important a priority is having your child assessed in the MDR-TB clinic?		
3.12. Do you feel that you have to wait a long time to be seen at your local clinic?	1. Yes	0. No
3.13. Do you feel that you have to wait a long time at the MDR-TB clinic?	1. Yes	0. No
3.14. Do you think that the parents of children or the health services should be responsible for preventing children from getting MDR-TB?	1. The parents	2. The health services

DCF 4 - Living Standards Measures			
Metropolitan dweller		DVD Player	
Living in a non-urban area		Refrigerator or combined fridge/freezer	
House / Cluster House / Town House		Electric Stove	
Tap water in house / on plot		Microwave oven	
Flush Toilet inside house		Deep Freezer - Free Standing	
Hot running water		Have a washing machine	
Built in Kitchen Sink		Have a tumble dryer	
No Domestic Workers		Dishwashing Machine	
Home security service		M-net / DSTV Subscription	
Cellphones in Household		Home Theatre System	
3 or more Cellphones in Household		Vacuum Cleaner	
Zero or One Radio set in Household		Motor Vehicle in Household	
Hi-Fi / Music centre		Computer - Desktop / Laptop	
Have TV set(s)		Land line (excl. Cellphone)	
VCR			

The spectrum of presentation, treatment and outcome in children with multidrug-resistant tuberculosis (Study 10)

For Study 10 two forms were created. The first was used to capture the presentation and management up until that point in time when the family was consented and interviewed. The second was then completed every time the patient was subsequently seen. This allowed both retrospective and prospective data collection. The first form was CDF 2 and the second DCF 5. For the children on the this treatment study, DCFs 1, 3 and 4 from the studies assessing exposed children were also completed, so that five forms were completed for each child (DCF 5 completed at each follow up appointment)

1. DCF1 Demographic details (completed each time the child was seen)
2. DCF2 Child details at the point when the child was recruited
3. DCF3 Index case details
4. DCF4 Household details
5. DCF5 Follow up forms (completed at each follow up appointment)

2.1. Consent		
2.1.1. Consent for study participation (if no or unknown then do not proceed)	0 No	1 Yes
	-5 Unknown	
2.1.2. Consent to use HIV test result	0 No	1 Yes
	-5 Unknown	
2.1.3. Entry Point	4 Tygerberg	5 Khayelitsha
	6 Brooklyn	-8 Other
2.1.4. Date today (dd/mm/yyyy)		

2.2. Personal Information		
2.2.1. DOB (dd/mm/yyyy)		
2.2.2. Gender	1 Male	2 Female
2.2.3. Ethnicity	4 White	5 Coloured
	8 Xhosa	9 Zulu
	10 Indian	11 Sotho
	-8 Other	
2.2.4. Home Language	1 English	2 Afrikaans
	3 Xhosa	4 Sotho
	5 Zulu	-8 Other

2.3. DR TB Episode		
2.3.1. Date of start of DR TB Episode (see definitions) (dd/mm/yyyy)		
2.3.2. Was the child admitted to hospital? (if no then go to 2.3.6.)	1 Yes	0 No
	-5 Unknown	
2.3.3. Date of admission to hospital (dd/mm/yyyy)		
2.3.4. Date of admission to Brooklyn Hospital (dd/mm/yyyy)		
2.3.5. Date discharged from Brooklyn (dd/mm/yyyy)		
2.3.6. If sent to another hospital, date of discharge from hospital (dd/mm/yyyy)		
2.3.6. Date TB treatment started in DR TB episode (dd/mm/yyyy)		
2.3.7. Date DR TB treatment started (dd/mm/yyyy)		
2.3.8. If finished treatment, date of treatment completion (dd/mm/yyyy)		
2.3.8. If culture-confirmed DR TB, date sample taken that diagnosed DR TB (dd/mm/yyyy)		
2.3.9. What was the child's weight at the beginning of the DR TB episode?	1 <60% expected	2 < 3rd percentile
	3 3rd – 10th percentile	4 > 10th percentile

2.4. Contacts		
2.4.1. Does the child have any TB contacts (if none go to 2.5.)	0 None known	1 One
	2 Two or more	-5 Not known
2.4.2. What is the relationship of the first contact to the child?	1 Mother	2 Father
	3 Sibling	4 Grandparent
	5 Other relative	6 Non-relative
2.4.3. What is the DST of the first contact? (if DST not done/unknown go to 2.4.4. if known go to 2.4.5.)	-5 DST not done/unknown	1 DS TB
	2 RIF mono-resistant TB	3 INH mono-resistant TB
	4 MDR TB	5 MDR + OFL resistant TB
	6 MDR + AMI resistant TB	7 XDR TB
2.4.4. If the first contact has not had a DST or the result is unknown, what has happened to them?	1 Died	2 On first line treatment
	3 Defaulted/ not on treatment	-5 Unknown
2.4.5. What is the relationship of the second contact to the child?	1 Mother	2 Father
	3 Sibling	4 Grandparent
	5 Other relative	6 Non-relative
2.4.6. What is the DST of the second contact? (if DST not done/unknown go to 2.4.7. if known go to 2.5.)	-5 DST not done/unknown	1 DS TB
	2 RIF mono-resistant TB	3 INH mono-resistant TB
	4 MDR TB	5 MDR + OFL resistant TB
	6 MDR + AMI resistant TB	7 XDR TB
2.4.7. If the second contact has not had a DST or the result is unknown, what has happened to them?	1 Died	2 On first line treatment
	3 Defaulted/ not on treatment	-5 Unknown

2.5. Previous Treatment		
2.5.1. Has the child had previous episodes of TB before?	0 Never	1 Once before
	2 Twice or more before	-5 Unknown
2.5.2. For the most recent episode what type of treatment was it?	1 Treatment for DS TB	2 Treatment for DR TB
	3 Prophylaxis for DS TB	4 Prophylaxis for DR TB
2.5.3. For the most recent episode what date did the treatment start? (dd/mm/yyyy)		
2.5.4. For the most recent episode what date did the treatment end? (dd/mm/yyyy)		
2.5.6. For the most recent episode what was the result?	1 Treatment completed	2 Defaulted
	3 Treatment failure	-5 Unknown
2.5.7. For the previous episode what type of treatment was it?	1 Treatment for DS TB	2 Treatment for DR TB
	3 Prophylaxis for DS TB	4 Prophylaxis for DR TB
2.5.8. For the previous episode what date did the treatment start? (dd/mm/yyyy)		
2.5.9. For the previous episode what date did the treatment end? (dd/mm/yyyy)		
2.5.10. For the previous episode what was the result?	1 Treatment completed	2 Defaulted
	3 Treatment failure	-5 Unknown

2.6. Child immunology details		
2.6.1. Largest Mantoux/Tine result during DR TB episode?	1 Positive	2 Negative
	3 Not done/unread	-5 Unknown
2.6.2. If the size of the Mantoux is recorded, what is the size? (xx mm)		
2.6.3. Was a BCG scar noted or recorded as being given in the RTHC?	1 Yes	0 No
	-5 Unknown	

2.7. HIV details		
2.7.1. Is the child HIV-infected? (if no or unknown go to 2.8.)	1 Yes	0 No
	-5 Not known	
2.7.2. If yes, when was the diagnosis made (dd/mm/yyyy)?		
2.7.3. Is the child currently on ART? (if no or unknown go to 2.7.6.)	1 Yes	0 No
	-5 Unknown	
2.7.4. When was ART started (dd/mm/yyyy)?		
2.7.5. What was the CD4 count and percentage at ART initiation? (xxxx, xx.x%)		
2.7.6. What was the CD4 count and percentage at start of DR TB episode? (xxxx, xx.x%)		
2.7.7. Most recent CD4 count and percentage? (xxxx, xx%)		
2.7.8. Date of most recent CD4 test (dd/mm/yyyy)?		

2.8. DR TB		
2.8.1. What type of TB does the child have?	1 PTB	2 EPTB
	3 Both PTB and EPTB	-5 Unknown
2.8.2. If EPTB or both, what type (circle all that apply)	1 Miliary	2 Pleural effusion
	3 Pericardial effusion	4 TBM
	5 Abdominal	6 Lymph node
	7 Bone/joint/spine	8 Disseminated
	9 Other	
2.8.3. Was the child sputum smear-positive at any point in the DR TB episode?	1 Yes	0 No
	-5 Unknown	

2.9. Resistance pattern of sample at which DR TB diagnosed or of the likely index case if diagnosed presumptively (tick one box for each drug)				
	Susceptible	Resistant	Not tested	Unknown
2.9.1 R – rifampicin				
2.9.2 H – isoniazid				
2.9.3 E – ethambutol				
2.9.4 Z – pyrazinamide				
2.9.5 S – streptomycin				
2.9.6 Eth – ethionamide				
2.9.7 A – amikacin				
2.9.8 O – ofloxacin				

2.10. Drugs used				
	Date started	Date stopped	Date restarted	Date stopped
2.10.1 R – rifampicin				
2.10.2 H – isoniazid				
2.10.3 E – ethambutol				
2.10.4 Z – pyrazinamide				
2.10.5 S – streptomycin				
2.10.6 Eto – ethionamide				
2.10.7 Amk – amikacin				
2.10.8 Ofx – ofloxacin				
2.10.9 Cm – capreomycin				
2.10.10 Cs/Trd – cycloserine or terizidone				
2.10.11 Cip – ciprofloxacin				
2.10.12 PAS				
2.10.13 Kl – clarithromycin				
2.10.14 Aug – augmentin				
2.10.15 Lzd – linezolid				
2.10.16 Mfx – moxifloxacin				

2.11. Diagnosis		
2.11.1. On what basis was the DR TB diagnosed?	1 Culture-confirmation	2 TB in contact of DR TB
	3 Failing first line therapy	-5 Unknown

2.12. Side effects of medications – since starting treatment in the DR TB episode has the child had any of the following? (Refer to side effect grading sheet)						
	None	Grade 1	Grade 2	Grade 3	Grade 4	Not known
2.12.1. Joint, muscle or bone pain (other than injuries)						
2.12.2. Skin Rashes						
2.12.3. Itchy skin						
2.12.4. Headache						
2.12.5. Sleeping/mood						
2.12.6. Lethargy						
2.12.7. Visual problems						
2.12.8. Vomiting						
2.12.9. Diarrhoea						
2.12.10. Jaundice						
2.12.11. Appetite/nausea						
2.12.12. Anaemia						
2.12.13. Anaphylaxis						
2.12.14. Hepatotoxicity						

2.13. Hearing and TFTs		
2.13.1. Has there been any hearing impairment (loss of >15db) since starting treatment?	1 None	2 High frequency unilateral loss
	3 High frequency bilateral	4 Mid-range unilateral
	5 Mid-range bilateral	-5 Unknown
2.13.2. Has the injectible had to be stopped early due to hearing loss?	1 Yes	2 No
	3 Unknown	
2.13.3. Has there been any derangement of TFTs?	1 Always normal	2 TSH alone raised once or more
	3 T4 alone low once or more	4 Both TSH raised and T4 low once or more
2.13.4. Has a thyroxine substitute been instituted?	1 Yes	2 No

2.14. Treatment outcome		
2.14.1. What was the treatment outcome?	1 Cured	2 Treatment completed
	3 Treatment failure	4 Defaulted
	5 Died	6 Lost to follow up
	7 Transferred/moved out	8 Still on treatment
	9 Other	
2.14.2. Impairment at the end of treatment?	1 None obvious	2 Chronic lung impairment
	3 Neurological impairment	4 Still on treatment
	5 Died	6 Other

2.15. Culture conversion	
2.15.1. Months to culture conversion if appropriate (time from start of DR TB treatment to date of sampling of first negative culture)	

2.16. Death		
2.16.1. If the patient died, what was the date of death? (dd/mm/yyyy)		
2.16.2. If the patient died, what was the cause of death?	1 TB while on treatment	2 The consequences of TB following treatment
	3 HIV-related illness	4 Other while on treatment
	5 Other following treatment	-5 Unknown

2.17. Radiology (use formal CXR reporting form)		
2.17.1. CXR at diagnosis	1 Normal	2 Hilar nodes/airway compression (mild)
	3 Lobar/segmental collapse/opacification (mod)	4 Pleural effusion – large of loculated (mod)
	5 Small cavities (mod)	6 Brochopneumonic changes (large of severe) (severe)
	7 Miliary (severe)	8 Large cavities (severe)
2.17.2. CXR at end of treatment	1 Normal	2 Improved but not normal
	3 Radiological chronic lung disease	4 Destroyed lobe/lung
	5 Still on treatment	6 Other

DCF 5 (Follow-up form) To be completed at outpatient follow ups or inpatient reviews

5.1. Follow up data		
5.1.1. Site of review	4 Tygerberg	5 Khayelitsha
	6 Brooklyn	-8 Other
5.1.2. Date Today (dd/mm/yyyy)		
5.1.3. If discharged from hospital since last review, date of discharge? (dd/mm/yyyy)		

5.2. Child immunology details		
5.2.1. If a Mantoux/Tine test has been repeated what is the result?	1 Positive	2 Negative
	3 Not done/unread	-5 Unknown
5.2.2. If the size of the Mantoux is recorded, what is the size? (xx mm)		

5.3. Culture conversion	
5.3.1. If culture conversion has occurred since last follow up, months to culture conversion (time from start of DR TB treatment to date of sampling of first negative culture)	

5.4. HIV details		
5.4.1. Is the child HIV-infected? (if no or unknown go to 5.5.)	1 Yes	0 No
	-5 Not known	
5.4.2. If yes, when was the diagnosis made (dd/mm/yyyy)?		
5.4.3. Is the child currently on ART? (if no or unknown go to 2.7.6.)	1 Yes	0 No
	-5 Unknown	
5.4.4. When was ART started (dd/mm/yyyy)?		
5.4.5. What was the CD4 count and percentage at ART initiation? (xxxx, xx.x%)		
5.4.6. What was the CD4 count and percentage at start of DR TB episode? (xxxx, xx.x%)		
5.4.7. Most recent CD4 count and percentage? (xxxx, xx%)		
5.4.8. Date of most recent CD4 test (dd/mm/yyyy)?		

5.5. Drugs used		
	Current drugs (tick)	Date stopped if stopped since last follow up
5.5.1 R – rifampicin		
5.5.2 H – isoniazid		
5.5.3 E – ethambutol		
5.5.4 Z – pyrazinamide		
5.5.5 S – streptomycin		
5.5.6 Eto – ethionamide		
5.5.7 Amk – amikacin		
5.5.8 Ofx – ofloxacin		
5.5.9 Cm – capreomycin		
5.5.10 Cs/Trd – cycloserine or terizidone		
5.5.11 Cip – ciprofloxacin		
5.5.12 PAS		
5.5.13 Kl – clarithromycin		
5.5.14 Aug – augmentin		
5.5.15 Lzd – linezolid		
5.5.16 Mfx – moxifloxacin		

5.6. Side effects of medications – since the last follow up has the child had any of the following (Refer to side effect grading sheet)						
	None	Grade 1	Grade 2	Grade 3	Grade 4	Not known
5.6.1. Joint, muscle or bone pain (other than injuries)						
5.6.2. Skin Rashes						
5.6.3. Itchy skin						
5.6.4. Headache						
5.6.5. Sleeping/mood						
5.6.6. Lethargy						
5.6.7. Visual problems						
5.6.8. Vomiting						
5.6.9. Diarrhoea						
5.6.10. Jaundice						
5.6.11. Appetite/nausea						
5.6.12. Anaemia						
5.6.13. Anaphylaxis						
5.6.14. Hepatotoxicity						

5.7. Hearing and TFTs		
5.7.1. Has there been any hearing impairment (loss of >15db) since the last appointment?	1 None	2 High frequency unilateral loss
	3 High frequency bilateral	4 Mid-range unilateral
	5 Mid-range bilateral	-5 Unknown
5.7.2. Has the injectible had to be stopped early due to hearing loss?	1 Yes	2 No
	3 Unknown	
5.7.3. Has there been any derangement of TFTs since the last appointment?	1 Always normal	2 TSH alone raised once or more
	3 T4 alone low once or more	4 Both TSH raised and T4 low once or more
5.7.4. Has a thyroxine substitute been instituted?	1 Yes	2 No

5.8. Treatment outcome and morbidity		
5.8.1. If the child has finished treatment, what was the outcome?	1 Cured	2 Treatment completed
	3 Treatment failure	4 Defaulted
	5 Died	6 Lost to follow up
	7 Transferred/moved out	8 Other
	9 Still on treatment	
5.8.2. What is the current level of impairment?	1 None obvious	2 Chronic lung impairment
	3 Neurological impairment	4 Other

The impact of drug resistance on clinical outcome in children with tuberculous meningitis (Study 11)

Personal Details			
Hospital number	Study Number		
Date admission to TBH	Date of discharge from TBH		
Date of birth	Gender	M	F

HIV details				
HIV status	Unknown	Positive	Negative	Exposed but negative
CD4 count at diagnosis of TBM (if HIV-infected)		On ART at diagnosis of TBM?	Yes	No

Past medical history			
BCG scar visible/documented on RTHC?	Yes	No	Unknown
Mantoux result	Unknown	Positive	Negative

Time course			
Date symptoms reported to start		Date of admission to hospital	
Date of initiation of treatment		For DR TB, date of initiation of appropriate treatment	
Any known TB contacts?	Yes	No	Unknown
Child given/currently on preventive therapy?	Yes	No	Unknown

Presenting Symptoms (ring those that apply)			
Decreased consciousness	Headache	GI disturbance/diarrhea	Poor feeding
Seizures	Vomiting	Cough	Weight loss
Fever	Change in behavior	Developmental regression	Neck Stiffness

Neurological status at presentation				
TBM stage	Stage one	Stage two	Stage three	Not recorded
GCS (3-15)			Not recorded	
Central nervous system	Normal	Abnormal	Unknown	
Peripheral nervous system	Normal	Abnormal	Unknown	
Hemiplegia	Yes	No	Unknown	
Raised ICP?	Yes	No	Unknown	

Microbiology			
Source of positive result	Gastric washings	Sputum	CSF
	Biopsy	BAL	Other
Resistance pattern	DS	RMP mono-resistant	INH mono-resistant
	MDR	XDR	Unknown
Smear result	Positive	Negative	Unknown
Strain type	Beijing	LAM	Haarlem
	X	Other	Unknown

CSF result									
Macro appearance		Clear		Bloody		Turbid			
RBC (cells/μL)		PMN (cells/μL)		Lymphocytes (cells/μL)		Glucose (mmol/L)		Protein (g/L)	

Other investigations			
CXR	Normal	Signs of TB	Not done
CT brain	Normal	Signs of TBM	Not done
Air Encephalogram	Communicating	Non-communicating	Not done

Inpatient progress		
Neurosurgery	Not done	Shunt
ICU admission	Admitted ICU	Not admitted ICU

Drugs used						
Steroids used	Yes		No		Unknown	
All TB drugs used	INH	PZA	RMP	ETH	EMB	AMI/KAN
	OFL	TER/CYC	PAS	CAP	LNZ	AUG

	Outcome				
Motor function	Normal	Hemiparesis	Quadriparesis	Died	Unknown
Cognitive outcome	Normal	Mild handicap	Moderate/severe handicap	Died	Unknown
Vision	Normal	Impaired vision	Blindness	Died	Unknown

Hearing loss in children treated for drug-resistant tuberculosis (Study 13)

Clinical Details		
1.1.Study Number		
1.2.Hospital Number		
1.4. Date of Birth (dd/mm/yyyy)		
1.5. Date of Admission to BCH (dd/mm/yyyy)		
1.6. Gender	1. Male	2. Female
1.7. Type of TB	1. PTB	2. EPTB
	3. Both PTB and EPTB	
1.8. Ethnicity	4. White	5. Coloured
	8. Xhosa	-8. Other
1.9. Site of EPTB	1. Miliary	2. Pleural Effusion
	3. Pericardial Effusion	4. TBM
	5. Abdominal TB	6. LN TB
	7. Bone/Joint TB	-8. Other
1.10. Weight at admission to BCH (xx.x kg)		
1.11. Length at admission to BCH (xxx cm)		
1.12. MUAC at admission to BCH (xx.x cm)		
1.13. Diagnosis of TB	1. Confirmed	2. Presumed
1.14. DST	1. Not done	2. DS
	3. IMR	4. RMR
	5. MDR	6. Pre-XDR
	7. XDR	-8. Other
1.15. HIV status	1. Positive	2. Negative
	-5. Unknown	
1.16. Date Started HAART (dd/mm/yyyy)		

2. Treatment Details		
2.1. Injectable given	1. Amikacin	2. Capreomycin
	3. Streptomycin	4. Kanamycin
2.2. Dosage of injectable given (xxx mg)		
2.3. Date injectable started (dd/mm/yyyy)		
2.4. Date injectable completed (dd/mm/yyyy)		
2.5. Number of doses given (xx)		
2.6. TB drugs used during injectable phase	1. Isoniazid	2. Rifampicin
	3. Pyrazinamide	4. Ethambutol
	5. Ofloxacin	6. Ethionamide
	7. Terizidone	8. PAS
	9. Clarithromycin	10. Augmentin
	11. Linezolid	-8. Other

3. Renal Function			
Potassium		Creatinine	
Date	Result	Date	Result

Review

Management of children exposed to multidrug-resistant *Mycobacterium tuberculosis*

James A Seddon, Peter Godfrey-Faussett, Anneke C Hesseling, Robert P Gie, Nulda Beyers, H Simon Schaaf

Children exposed to multidrug-resistant (MDR) *Mycobacterium tuberculosis* are at risk of developing MDR tuberculosis. Where treatment is available, it is lengthy, expensive, and associated with poor adherence and notable morbidity and mortality. Preventive treatment effectively lowers the risk of disease progression for contacts of individuals with drug-susceptible tuberculosis, but this strategy is poorly studied for contacts of people with MDR tuberculosis. In this Review we discuss the management of child contacts of source cases with MDR tuberculosis. We pay particular attention to assessment, existing international guidelines, possible preventive treatments, rationales for different management strategies, and the interaction with and implications of HIV infection.

Lancet Infect Dis 2012; 12: 469–79

Published Online February 27, 2012 DOI:10.1016/S1473-3099(11)70366-8

Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK (J A Seddon MRCPCH, Prof P Godfrey-Faussett FRCP); and Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa (J A Seddon, Prof A C Hesseling PhD, Prof R P Gie FCP (Paeds), Prof N Beyers PhD, Prof H S Schaaf MD (Paed))

Correspondence to: Dr James A Seddon, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa jseddon@sun.ac.za

Introduction

Nearly half a million new cases of multidrug-resistant (MDR) tuberculosis are estimated to occur each year,[1] including extensively drug-resistant (XDR) tuberculosis. These forms of the disease are associated with high mortality, particularly in people living with HIV infection.[2] MDR tuberculosis is defined as disease caused by *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid, and XDR as tuberculosis caused by *M tuberculosis* resistant to both these drugs as well as a fluoroquinolone and an injectable second-line antituberculosis medication.[3]

Tuberculosis control programmes have traditionally focused on case-finding and treatment of infectious patients, most of whom are adults. From a public health perspective, this approach must remain the priority because it will lessen disease transmission and, therefore, the number of new infections. To decrease future disease burden and improve clinical care at an individual level, however, these strategies need to be complemented with the identification and treatment of people who are at a high risk of first becoming infected and then of progressing to disease after contact with infectious individuals.[4] Young children and immunosuppressed people are at the highest risk of progressing to disease after infection. Few studies have investigated the management of children exposed to MDR tuberculosis, and there is no consensus about the use of preventive treatment. In this Review we discuss existing international guidelines for the management of child contacts of individuals with MDR tuberculosis, whether preventive treatment could have a role, and what the possible treatments and rationales might be for different management strategies.

Tuberculosis pathophysiology and immunology

After exposure to aerosolised *M tuberculosis*, some children will become infected, after which the adaptive immune system might clear the infection, fail to contain it, or reach an equilibrium in which the immune system is unable to eradicate the infection but prevents progression to disease. The definitions and pathophysiological bases of tuberculosis infection are subjects of much debate. The terms latent tuberculosis infection, latent tuberculosis, and tuberculosis latency are all used. In line with our academic work and paediatric practice, in this Review we use the term tuberculosis infection to cover the spectrum from recent infection with *M tuberculosis*, before an immune response is mounted, to an established state of equilibrium. A proportion of individuals with tuberculosis infection will at some point develop tuberculosis disease.[5] The overall risk of progression is greatest in the first 2 years after infection, and for young children progression occurs within 1 year in 90% of cases.[6,7] Data collected before the era of chemotherapy show that changes seen on chest radiography spontaneously resolve without treatment in a proportion of children with tuberculosis infection. In this Review, however, as in our clinical practice, we use the term tuberculosis disease to refer to symptomatic illness or any radiographic changes on chest radiography that are consistent with tuberculosis.

Traditionally, the only means of detecting tuberculosis infection was if the patient had a history of exposure and a positive tuberculin skin test (TST) result. The crude antigen mixture used in TSTs, however, does not completely differentiate between BCG, *M tuberculosis*, and environmental, non-tuberculous mycobacteria.[8] An immune response might take up to 3 months to develop, and the size of induration can be affected by HIV infection,[9] malnutrition, and other causes of immunosuppression (eg, viral infections, neoplastic disorders, or steroid use).[8] Sensitivity and specificity are difficult to measure in the absence of a gold standard, but when sensitivity is measured against confirmed tuberculosis disease, results are variable. Some tests, such as the interferon-γ-release assays (IGRAs), measure the amount of interferon γ released by T cells or the number of T cells that release interferon γ after stimulation by *M tuberculosis*-specific antigens (eg, ESAT-6, CFP-10, or TB7.7). Large numbers of studies have assessed these in-vitro tests, and in some contexts they seem to show increased sensitivity in confirmed tuberculosis cases or against an exposure gradient.[10] Specificity does not seem to be substantially affected by previous BCG vaccination or exposure to non-tuberculous

Concise Clinical Review

Caring for Children with Drug-Resistant Tuberculosis

Practice-based Recommendations

James A. Seddon[1,2], Jennifer J. Furin[3], Marianne Gale[4], Hernan Del Castillo Barrientos[5,6], Rocío M. Hurtado[7,8,9], Farhana Amanullah[10], Nathan Ford[11], Jeffrey R. Starke[12], and H. Simon Schaaf[1,13]; on behalf of the Sentinel Project on Pediatric Drug-Resistant Tuberculosis

[1]Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa; [2]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Division of Infectious Diseases, TB Research Unit, Case Western Reserve University School of Medicine, Cleveland, Ohio; [4]Médecins Sans Frontières, Sydney, Australia; [5]Servicio de Neumología, Instituto Nacional de Salud del Niño, Lima, Perú; [6]Unión Nacional Contra La Tuberculosis, Lima, Perú; [7]Massachusetts General Hospital, Boston, Massachusetts; [8]Global Health Committee, Boston, Massachusetts; [9]Harvard Medical School, Boston, Massachusetts; [10]The Indus Hospital, Karachi, Pakistan; [11]Médecins Sans Frontières, Geneva, Switzerland; [12]Department of Pediatrics, Baylor College of Medicine, Houston, Texas; and [13]Tygerberg Children's Hospital, Cape Town, South Africa

The management of children with drug-resistant tuberculosis (DR-TB) is challenging, and it is likely that in many places, the roll-out of molecular diagnostic testing will lead to more children being diagnosed. There is a limited evidence base to guide optimal treatment and follow-up in the pediatric population; in existing DR-TB guidelines, the care of children is often relegated to small "special populations" sections. This article seeks to address this gap by providing clinicians with practical advice and guidance. This is achieved through review of the available literature on pediatric DR-TB, including research studies and international guidelines, combined with consensus opinion from a team of experts who have extensive experience in the care of children with DR-TB in a wide variety of contexts and with varying resources. The review covers treatment initiation, regimen design and treatment duration, management of comorbid conditions, treatment monitoring, adverse events, adherence promotion, and infection control, all within a multidisciplinary environment.

Keywords: pediatrics; child; drug resistance

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

With increasingly available rapid diagnostic techniques, more children are likely to be diagnosed with drug-resistant tuberculosis. Guidance is lacking to assist the clinician in caring for children with drug-resistant tuberculosis.

What This Study Adds to the Field

This article draws on the published literature and available guidelines, combining this with the consensus opinion of authors who have extensive experience in the management of children with drug-resistant tuberculosis. It provides guidance on regimen selection, the management of comorbid conditions and adverse events, and how to monitor treatment response. It discusses the promotion of adherence, how to involve other disciplines, and the role of infection control.

(Received in original form June 4, 2012; accepted in final form September 2, 2012)

This work was supported by grant GHN-A-00-08-00004-00 from TREAT TB, USAID (J.A.S. and H.S.S.), the Sir Halley Steward Trust (J.A.S.), the South African Medical Research Council (H.S.S.), and the National Research Foundation of South Africa (H.S.S.).

Author Contributions: J.A.S. created the initial draft of the manuscript, revised the manuscript and approved the final version. J.J.F. revised and edited the manuscript and approved the final version. M.G. revised and edited the manuscript and approved the final version. H.D.C.B. revised and edited the manuscript and approved the final version. R.M.H. revised and edited the manuscript and approved the final version. F.A. revised and edited the manuscript and approved the final version. N.F. performed the systematic review of the literature, revised and edited the manuscript, and approved the final version. J.R.S. revised and edited the manuscript and approved the final version. H.S.S. revised and edited the manuscript and approved the final version.

CME will be available for this article at http://ajrccm.atsjournals.org or at http://cme.atsjournals.org

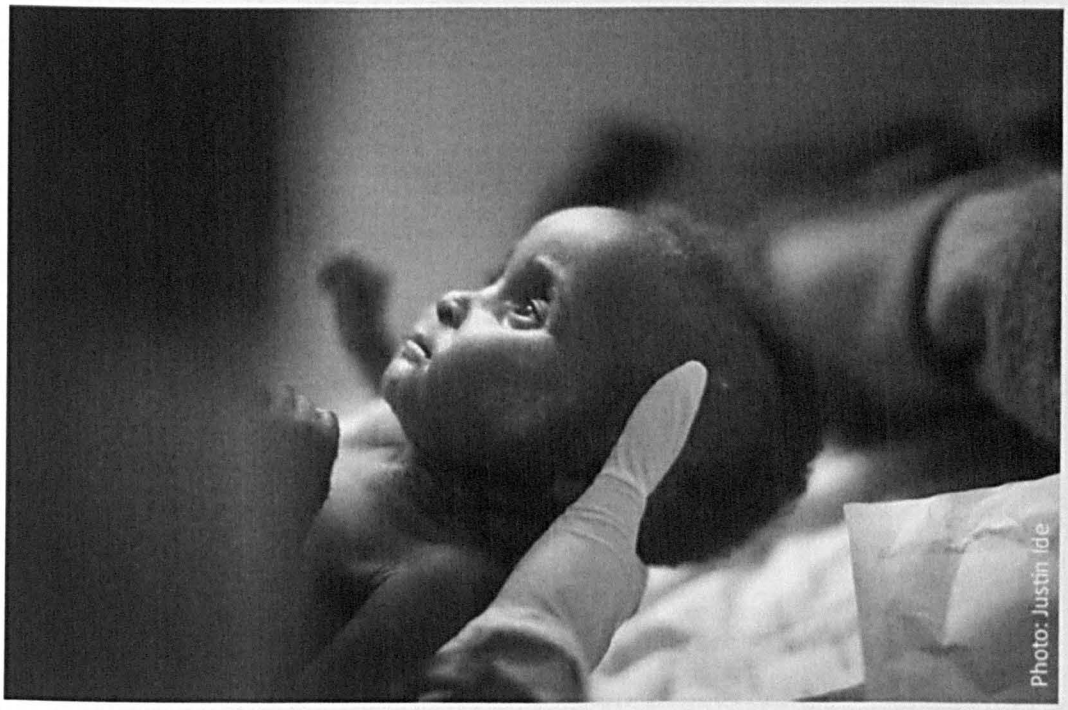
Correspondence and requests for reprints should be addressed to James Seddon, M.B.B.S., M.A., D.T.M.&H., Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, South Africa. E-mail: jseddon@sun.ac.za

Am J Respir Crit Care Med Vol 186, Iss. 10, pp 953–964, Nov 15, 2012

Originally Published in Press as DOI: 10.1164/rccm.201206-1001CI on September 13, 2012
Internet address: www.atsjournals.org

The World Health Organization (WHO) estimates there are 650,000 prevalent cases of multidrug-resistant tuberculosis (MDR-TB) globally (*see* Table 1 for definitions) (1). Because children (< 15 yr of age) comprise up to 20% of the TB caseload in high-burden settings (2–4), the number of children with drug-resistant TB (DR-TB) is undoubtedly high. Data regarding this vulnerable population, however, are lacking: a recent systematic review of children with MDR-TB was only able to include eight studies from five countries (5). Few children with DR-TB are diagnosed, and fewer still are started on appropriate treatment. This failure of appropriate management occurs for several reasons. First, confirmation of the diagnosis for all forms of TB in children is limited by the difficulty in obtaining appropriate diagnostic specimens (6). In many contexts, WHO-endorsed, rapid genotypic tests are being rolled out (7, 8), and, for the majority of regions that did not previously carry out comprehensive culture and drug susceptibility testing (DST), the number of diagnosed cases of pediatric DR-TB will increase. Second, due to misperceptions regarding the toxicity of the second-line TB medications in children, some clinicians are hesitant to use these drugs to treat unconfirmed disease. Finally, there are few practice-based recommendations on the optimal care of children with DR-TB. Existing global guidelines relegate the care of pediatric DR-TB to a one- or two-page

Management of Multidrug-Resistant Tuberculosis in Children: A Field Guide



USAID
FROM THE AMERICAN PEOPLE

TB CARE II

Sentinel Project

First Edition: November, 2012

This handbook is made possible by the support of the American people through the United States Agency for International Development (USAID). The contents of this report are the sole responsibility of TB CARE II and The Sentinel Project on Pediatric Drug-Resistant Tuberculosis and do not necessarily reflect the views of USAID or the United States Government.

Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis

Dena Ettehad, H Simon Schaaf, James A Seddon, Graham S Cooke*, Nathan Ford*

Summary

Background **Paediatric multidrug-resistant (MDR) tuberculosis is a public health challenge of growing concern, accounting for an estimated 15% of all global cases of MDR tuberculosis. Clinical management is especially challenging, and recommendations are based on restricted evidence. We aimed to assess existing evidence for the treatment of MDR tuberculosis in children.**

Methods **We did a systematic review and meta-analysis of published and unpublished studies reporting treatment outcomes for children with MDR tuberculosis. We searched PubMed, Ovid, Embase, Cochrane Library, PsychINFO, and BioMedCentral databases up to Oct 31, 2011. Eligible studies included five or more children (aged ≤16 years) with MDR tuberculosis within a defined treatment cohort. The primary outcome was treatment success, defined as a composite of cure and treatment completion.**

Results **We identified eight studies, which reported treatment outcomes for a total of 315 patients. We recorded much variation in the characteristics of patients and programmes. Time to appropriate treatment varied from 2 days to 46 months. Average duration of treatment ranged from 6 months to 34 months, and duration of follow-up ranged from 12 months to 37 months. The pooled estimate for treatment success was 81·67% (95% CI 72·54–90·80). Across all studies, 5·9% (95% CI 1·3–10·5) died, 6·2% (2·3–10·2) defaulted, and 39·1% (28·7–49·4) had an adverse event. The most common drug-related adverse events were nausea and vomiting. Other serious adverse events were hearing loss, psychiatric effects, and hypothyroidism.**

Interpretation **The treatment of paediatric MDR tuberculosis has been neglected, but when children are treated outcomes can be achieved that are at least as good as those reported for adults. Programmes should be encouraged to report outcomes in children to improve the knowledge base for care, especially as new drugs become available.**

Funding **None.**

Lancet Infect Dis 2012; 12: 449–56

Published Online February 22, 2012 DOI:10.1016/S1473-3099(12)70033-6

See Comment page 425

*These authors share last authorship

Faculty of Medicine, Imperial College London, UK (D Ettehad BSc, G S Cooke DPhil); Desmond Tutu Tuberculosis Centre, Faculty of Health Sciences, Stellenbosch University, South Africa (H S Schaaf MD, J Seddon MBBS); Tygerberg Children's Hospital, South Africa (H Simon Schaaf); Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK (J A Seddon); Africa Centre for Health and Population Studies, University of KwaZulu-Natal, South Africa (G S Cooke); Médecins Sans Frontières, Geneva, Switzerland (N Ford PhD); and Centre for Infectious Disease Epidemiology and Research, University of Cape Town, South Africa (N Ford)

Correspondence to: Dr Nathan Ford, Médecins Sans Frontières, 78 rue de Lausanne, 1211 Geneva, Switzerland nathan.ford@msf.org

Introduction

An estimated 12 million people worldwide have tuberculosis, of whom about 650 000 have multidrug-resistant (MDR) disease.[1] Childhood tuberculosis is estimated to account for 10–15% of the global tuberculosis burden,[2] and probably accounts for a similar proportion when considering only drug-resistant disease. The highest rates of paediatric MDR tuberculosis are reported in low-income countries,[3] and in some regions the incidence of MDR tuberculosis has risen sharply in the past two decades—eg, in the Western Cape, South Africa, the proportion of culture-confirmed cases of tuberculosis with multidrug-resistance has tripled in the past 15 years from 2·3% to 7·3%.[3]

MDR tuberculosis is underdetected in children. Diagnosis of drug resistance needs mycobacterial culture and drug susceptibility testing (DST),[4] but the difficulty in obtaining respiratory secretions, such as sputum or gastric aspirates, or specimens of extrapulmonary tuberculosis from young children,[5] along with the fact that up to half of all children with a clinical diagnosis of tuberculosis disease are smear-negative and culture-negative, makes microbiological confirmation challenging.[6] Strict programmatic requirements for microbiological confirmation of drug resistance combined with insufficient recognition of the importance of taking into account DST patterns from adult source cases can lead to substantial delays in diagnosis and initiation of appropriate treatment.[7] These delays could lead to progression of disease, increased risk of infectiousness of children, greater risk of disease complications such as tuberculous meningitis, and higher rates of morbidity and mortality.[8,9]

Paediatric drug-resistant tuberculosis is a neglected concern, with few children being treated relative to the estimated disease burden.[10] WHO guidelines for the treatment of drug-resistant tuberculosis in adults are based on evidence from meta-analyses of individual patients' data.[11] However, recommendations for children are based on expert opinion, drawing on data from case series and cohort studies,[12,13] often with small sample sizes. Consequently, variation exists in programmatic choices of treatment regimens, with the choice of drugs informed by previous drug exposure and DST results.[14] Because of uncertainties about diagnosis and the best treatment regimens, and concerns about the toxic effects associated with MDR tuberculosis treatment, health-care providers are cautious about treating paediatric MDR tuberculosis.

We did a systematic review and meta-analysis of the available evidence for treatment outcomes in children

Eur Respir J 2012; 40: 1–1
DOI: 10.1183/09031936.00044812

REVIEW

Hearing loss in patients on treatment for drug-resistant tuberculosis

James A. Seddon*,#, Peter Godfrey-Faussett#, Kayleen Jacobs¶, Adam Ebrahim¶, Anneke C. Hesseling* and H. Simon Schaaf*,+

ABSTRACT: **The treatment of drug-resistant (DR)-tuberculosis (TB) necessitates the use of second-line injectable anti-TB drugs which are associated with hearing loss. Hearing loss affects communication and the development of language and social skills in children. This review describes the pathophysiology of hearing loss and the testing methodologies that can be employed. It is the first paper to systematically review the literature regarding hearing loss in those treated for DR-TB. In the studies identified, the methodology used to test for and to classify hearing loss is inconsistent and children and those with HIV are poorly represented. This review describes existing guidelines and suggests management strategies when hearing loss is found. It describes the challenges of testing hearing in the developing world contexts where the majority of patients with DR-TB are treated. Finally it makes the recommendation that a standardised testing methodology and classification system should be used.**

KEYWORDS: **Drug-resistant, hearing loss, ototoxicity, systematic review, tuberculosis**

The World Health Organization (WHO) estimates that there are 650,000 cases globally of multidrug-resistant (MDR) tuberculosis (TB) (*Mycobacterium tuberculosis* resistant to rifampicin and isoniazid) [1]. A small proportion of these cases are diagnosed and appropriately treated but with the imminent roll-out of newer molecular diagnostic tools [2, 3], a much larger proportion is likely to be treated. The treatment of drug-resistant (DR)-TB requires the use of second-line anti-TB medications many of which are associated with significant adverse events [4]. The injectable drugs, aminoglycosides and polypeptides are associated with a risk to renal function, hearing and the vestibular system. Nephrotoxicity is generally reversible but damage to the auditory and vestibular systems is usually permanent. The monitoring of hearing loss is important for two reasons. First, if detected early it may be possible to alter the regimen to stop or reduce the dose of the responsible drug, preventing progression of hearing loss to the point where it would impact on communication. Second, if significant hearing loss has developed and is detected, interventions can be implemented to assist in communication. These include hearing aids, cochlear implants or other hearing impaired tools, teaching and training. Despite the increasing literature on DR-TB over the last 20 yrs, few studies have investigated hearing loss in patients undergoing treatment. Existing studies have used varied case definitions, making comparisons between studies challenging.

Here we review how hearing is tested and assess the implications of testing in resource-limited settings, where the majority of patients with DR-TB are likely to be treated. We describe testing of young children who are not old enough to co-operate with the pure tone audiometry procedure. We systematically review the literature which has assessed hearing in patients undergoing treatment for DR-TB, as well as existing international guidelines. We discuss the different components of hearing loss and potential interventions upon identification of hearing loss. Finally we propose a standardisation in the classification of hearing loss for academic studies in adults and children treated for DR-TB.

THE PHYSIOLOGY OF HEARING AND BALANCE

Sounds, in the form of vibrations, impact on the pinna of the ear and are transmitted down the auditory channel to the tympanic membrane. The vibrations are transmitted through the auditory ossicles (the malleus, incus and stapes) onto the hair cells of the basilar membrane within the

AFFILIATIONS
*Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, Stellenbosch,
#Audiology Dept, Brooklyn Chest Hospital, Cape Town,
¶Tygerberg Children's Hospital, Tygerberg, South Africa, and
+Dept of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.

CORRESPONDENCE
J.A. Seddon
Desmond Tutu TB Centre, Dept of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Health Sciences
Stellenbosch University
PO Box 19063
Tygerberg
South Africa
E-mail: jseddon@sun.ac.za

Received:
March 14 2012
Accepted after revision:
May 20 2012
First published online:
June 14 2012

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

Chapter 11

Donald PR, van Helden PD (eds): Antituberculosis Chemotherapy.
Prog Respir Res. Basel, Karger, 2011, vol 40, pp 1–15

Second-Line Antituberculosis Drugs: Current Knowledge, Recent Research Findings and Controversies

H. Simon Schaaf[a,b] · James A. Seddon[a,c] · Jose A. Caminero[d,e]

[a]Department of Paediatrics and Child Health, Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, and [b]Tygerberg Children's Hospital, Tygerberg, South Africa; [c]London School of Hygiene and Tropical Medicine, London, UK; [d]Department of Pneumology, University Hospital of Gran Canaria Dr. Negrin, Las Palmas, Spain; [e]International Union against Tuberculosis and Lung Disease, Paris, France

Abstract

The treatment of drug-resistant tuberculosis (TB) necessitates the use of drugs that are poorly understood, less efficacious and often associated with more adverse effects than those used to treat drug-susceptible TB. Many of these second-line compounds were discovered over 50 years ago and were soon superseded by more effective and better tolerated drugs. However, in treating drug-resistant TB, we must re-evaluate these drugs as the available armamentarium of drugs is so limited. New medications, as well as established medications not previously used against TB, need to also be considered. As diagnostic techniques improve and more cases of drug-resistant TB are diagnosed, clinicians must be familiar with these second-line drugs to enable them to successfully manage patients. This article reviews the literature, often limited and sometimes elderly, regarding the second-line drugs. It also examines recent research findings and identifies areas of controversy and discussion. It comments on the laboratory, pharmacokinetic and pharmacodynamic properties of the drugs as well as discusses adverse effects.

Multidrug-resistant (MDR) and, more recently, extensively drug-resistant (XDR) tuberculosis (TB) has necessitated the use of second-line anti-TB agents. Worldwide, 440,000 new cases of MDR-TB were estimated to have occurred in 2008, 5.4% of which were XDR-TB cases [1]. The majority of these patients are not diagnosed, and only a small proportion is treated with appropriate second-line anti-TB regimens. There is currently a strong move to roll out diagnostic facilities to identify MDR-TB cases in high TB burden countries with limited resources. However, if the diagnosis of MDR-TB is improved, treatment must be available, and for treatment to be available, it is imperative that there should be a good knowledge of the agents used. Therefore this overview does not discuss treatment options for MDR/XDR-TB but rather presents the individual agents or drug groups that are used as second-line drugs with the emphasis on recent findings and controversies.

The World Health Organization (WHO) divides the agents for MDR/XDR-TB treatment into 5 major groups (table 1). Although the current WHO guidelines classify the injectable agents as group 2 and the fluoroquinolones as group 3, there are good reasons to argue that, due to the efficacy of the agents, the severity of adverse effects and also cost, the fluoroquinolones should rather be group 2 and the injectables group 3 [3]. In this overview we will discuss only second-line agents excluding the fluoroquinolones, as these are discussed in a separate paper (i.e. we will discuss groups 2, 4 and 5). A summary is provided in table 2.

The Injectable Antituberculosis Agents: Aminoglycosides and Cyclic Polypeptides

The current WHO group 2 agents include streptomycin, kanamycin, amikacin and capreomycin (viomycin, another cyclic polypeptide, is not currently included) [2]. In many regions with MDR-TB, resistance surveillance shows high rates (>50%) of streptomycin resistance, precluding its use in routine MDR-TB management. For this reason kanamycin

Tuberculosis 92 (2012) 9–17

Contents lists available at SciVerse ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

Paediatric use of second-line anti-tuberculosis agents: A review

James A. Seddon [a,b,*], Anneke C. Hesseling [a], Ben J. Marais [c], Helen McIlleron [d], Charles A. Peloquin [e], Peter R. Donald [f], H. Simon Schaaf [a,f]

[a] Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, South Africa
[b] Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
[c] Sydney Institute for Emerging Infectious Diseases and Biosecurity (SEIB) and the Children's Hospital at Westmead, University of Sydney, Australia
[d] Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, South Africa
[e] College of Pharmacy, and the Emerging Pathogens Institute, University of Florida, USA
[f] Tygerberg Children's Hospital, Tygerberg, South Africa

ARTICLE INFO

Article history:
Received 29 August 2011
Received in revised form
25 September 2011
Accepted 1 November 2011

Keywords:
Tuberculosis
Children
Drug-resistant
Second-line
Pharmacokinetics

SUMMARY

Childhood multidrug-resistant tuberculosis (MDR-TB) is an emerging global epidemic. With the imminent roll-out of rapid molecular diagnostic tests, more children are likely to be identified and require treatment. As MDR-TB is resistant to the most effective first-line drugs, clinicians will have to rely on second-line medications which are less effective and often associated with more pronounced adverse effects than first-line therapy. Despite the fact that most of these agents were discovered many years ago, robust information is lacking regarding their pharmacokinetic and pharmacodynamic properties, adverse effects and drug interactions, especially in children. Children differ from adults in the way that drugs are administered, the manner in which they are metabolised and in the adverse effects experienced. The interaction of these drugs with human immunodeficiency virus infection and antiretroviral therapy is also poorly documented. This article reviews the available second-line drugs currently used in the treatment of MDR-TB in children and discusses medication properties and adverse effects while potential interactions with antiretroviral therapy are explored.

1. Introduction

It is rarely emphasized that multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) also affect children and that paediatric drug-resistant TB can be viewed as an emerging global epidemic.[1] MDR-TB is defined as *Mycobacterium tuberculosis (M. tuberculosis)* resistant to the most potent first-line anti-TB medications, isoniazid and rifampicin, while XDR-TB has additional resistance to the most active second-line agents, injectable drugs (aminoglycosides and/or cyclic polypeptides) and fluoroquinolones. There were an estimated 440,000 cases globally of MDR-TB during 2009.[2] Given the fact that childhood TB represents at least 10–20% of the total cases in areas with poor epidemic control,[3–5] this translates into a minimum global estimate of around 40,000 paediatric cases of MDR-TB per year. Accurate reporting and optimal management of these cases are challenging, due to the difficulty in confirming the diagnosis, limited awareness and experience in dealing with these patients, the complexity and duration of treatment, and the limited availability of adequate drugs and child-friendly formulations. In addition, in settings with a high burden of MDR-TB and human immunodeficiency virus (HIV), up to 40% of children with MDR-TB are also HIV-infected.[6] These children are at risk of multiple opportunistic infections, have specific nutritional and metabolic requirements and absorb medications in a different manner to those HIV-uninfected. The combination of MDR-TB and HIV can have serious psychological effects. Both conditions are stigmatised and are perceived to carry poor prognosis. HIV-infected children are also treated with antiretroviral therapy (ART) medications which have the potential to interact with the second-line anti-TB drugs. Few studies have examined the management of children with MDR-TB. Those that have are small and focus mainly on outcomes with little attention to the careful documentation of the challenges of treatment.[7–19] With the imminent roll-out of more rapid, molecular diagnostic tests to identify MDR-TB,[20,21] case detection, including that of children, is likely to rise. In order to manage children with MDR-TB

* Corresponding author. Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505, South Africa. Tel.: +27 722470795/213789177; fax: +21 219389792.
E-mail addresses: jseddon@sun.ac.za, james.seddon@doctors.org.uk (J.A. Seddon), annekeh@sun.ac.za (A.C. Hesseling), benm1@chw.edu.au (B.J. Marais), helen.mcilleron@uct.ac.za (H. McIlleron), peloquin@cop.ufl.edu (C.A. Peloquin), prd@sun.ac.za (P.R. Donald), hss@sun.ac.za (H.S. Schaaf).

1472-9792/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tube.2011.11.001

Correspondence

Retooling Existing Tuberculosis Drugs for Children

To the Editor—The article by Dooley and colleagues [1], on behalf of the efficacy subgroup of Research Excellence to Stop TB Resistance (RESIST-TB), is timely and much needed. New tuberculosis drugs are indeed likely to be several years away from widespread use, necessitating our continued reliance on existing drugs. Because combinations of 3 or more new drugs are even further away, the first new agents licensed will need to be protected by multidrug regimens of existing medications. Regimens of existing drugs require optimization of dose, treatment duration, and treatment combinations. This paper serves as a call to action to address these research priorities.

Despite the much-needed perspective provided, we note with concern the absence of any discussion regarding the existing, albeit limited, evidence base for pediatric use of existing tuberculosis drugs and the retooling of dosages and regimens necessary to optimize treatment in children. Furthermore, no research priorities are identified for the investigation of existing pediatric drugs. Children with tuberculosis differ from adults in many respects: the spectrum of disease manifested, the way medications are administered, the manner in which drugs are absorbed, and also the adverse effects experienced. Young children tend to metabolize drugs more rapidly than adults [2], resulting in lower serum concentrations following like-for-like dosing. Specific forms of tuberculosis, such as tuberculous meningitis, are more common in children; specific drug properties, for example, cerebrospinal fluid penetration should therefore be considered.

The discussion regarding retooling of existing tuberculosis drugs should stimulate consideration of the appropriate timing to include children in drug trials of both novel and existing agents. Given the paucibacillary nature of most forms of pediatric tuberculosis, at least equal efficacy can be expected for the treatment of drug-resistant disease in children compared to adults. However, the high frequency of adverse drug effects, for example, thyroid toxicity in developing children [3], make the urgent evaluation of shorter and less toxic combination regimens mandatory.

Knowledge of the pharmacokinetics of existing and novel drugs in children, drug-drug interactions, and the development of child-friendly formulations are also priorities. The effect of human immunodeficiency virus (HIV) coinfection and the interaction between tuberculosis drugs and antiretroviral therapy is a further important pediatric consideration, given the high prevalence of pediatric HIV infection in settings where drug-resistant tuberculosis is increasing [4].

There is an increasing awareness of the importance of including children in clinical research on new and existing drugs [5]. Regulatory authorities have now made pediatric evaluation of novel drugs a prerequisite for regulatory approval in Europe and the United States [6–7]. However, major gaps remain in our knowledge of existing tuberculosis drugs and drug regimens in children and for the retooling of these drugs with new drug candidates. As researchers, international policymakers, implementers, and civil society we should be advocating for the needs of children, the most vulnerable members of our society.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

James A Seddon,[1,2] Anneke C Hesseling,[1] and H. Simon Schaaf[1]

[1]Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa; and [2]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, United Kingdom

References

1. Dooley KE, Mitnick C, Degroote MA, et al. Old drugs, new purpose: retooling existing drugs for optimized treatment of resistant tuberculosis. Clin Infect Dis **2012**; 55: 572–81.
2. McIlleron H, Willemse M, Werely CJ, et al. Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines. Clin Infect Dis **2009**; 48: 1547–53.
3. Thee S, Zollner EW, Willemse M, Hesseling AC, Magdorf K, Schaaf HS. Abnormal thyroid function tests in children on ethionamide treatment (short communication). Int J Tuberc Lung Dis **2011**; 15:1191–3.
4. Seddon JA, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. Int J Tuberc Lung Dis **2012**; 16:928–33.
5. Burman WJ, Cotton MF, Gibb DM, Walker AS, Vernon AA, Donald PR. Ensuring the involvement of children in the evaluation of new tuberculosis treatment regimens. PLoS Med **2008**; 5:e176.

INT J TUBERC LUNG DIS 16(7):928–933

http://dx.doi.org/10.5588/ijtld.11.0679
E-published ahead of print 8 May 2012

The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa

J. A. Seddon,*† A. C. Hesseling,* B. J. Marais,‡§ A. Jordaan,¶ T. Victor,¶ H. S. Schaaf*#

*Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa; †Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK; ‡The Children's Hospital at Westmead and §The Sydney Institute for Emerging Infectious Diseases and Biosecurity, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia; ¶Division of Molecular Biology and Human Genetics, Department of Biomedical Science; Faculty of Health Sciences, Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis, Research/Medical Research Council Centre for Molecular and Cellular Biology, Stellenbosch University, Tygerberg, #Tygerberg Children's Hospital, Cape Town, South Africa

SUMMARY

SETTING: Tygerberg Children's Hospital, Cape Town, South Africa.

OBJECTIVE: To determine the prevalence and trend of drug resistance and human immunodeficiency virus (HIV) co-infection among children with culture-confirmed tuberculosis (TB).

METHOD: Prospective surveillance from March 2007 to February 2009, compared to three previous surveys (1994–1998, 2003–2005, 2005–2007). Drug susceptibility testing (DST) against isoniazid (INH) and rifampicin (RMP) was performed using genotypic and phenotypic testing. If multidrug-resistant TB (MDR-TB) was detected, further DST against ethambutol (EMB) and second-line drugs was performed.

RESULTS: A total of 294 children with a median age of 26 months (range 3 days–13 years) were diagnosed with culture-confirmed TB. DST results were available for 292 (99.3%); 41 (14%) were INH-resistant, including 26 (8.9%) with MDR-TB. Four children (1.4%) had RMP monoresistance. EMB resistance was present in 12/24 (50%) MDR-TB cases tested. Two isolates were resistant to ofloxacin; none had extensively drug-resistant TB. Of those tested, 29% (63/217) were HIV-infected. Any resistance to RMP increased between 1994 and 2009 ($P < 0.001$), as did RMP monoresistance ($P = 0.009$) and MDR-TB ($P < 0.001$). Sensitivity was 87.5% and specificity 100% for genotypic compared to phenotypic testing for INH resistance.

CONCLUSIONS: RMP, and consequently multidrug, resistance is increasing among children with TB in this setting. EMB resistance is common among children with resistance to RMP and INH.

KEY WORDS: paediatric; DST; resistant; surveillance; TB

THE WORLD HEALTH Organization (WHO) estimated that there were 440 000 new cases of multidrug-resistant tuberculosis (MDR-TB; i.e., *Mycobacterium tuberculosis* resistant to isoniazid [INH] and rifampicin [RMP]) worldwide in 2008.[1] Children with drug-resistant TB usually have transmitted resistance, whereby the child is infected by an organism with established resistance.[2–5] This contrasts with adults, in whom drug resistance is a result of both transmission and acquisition, the latter due to a susceptible organism developing resistance due to inadequate treatment.[6] Children rarely have acquired resistance, as paediatric TB is usually paucibacillary; with small organism loads, it is unlikely that resistant mutants will occur and be selected. This is supported by studies comparing the genetic DNA fingerprint (restriction fragment length polymorphism [RFLP]), as well as the drug susceptibility testing (DST) patterns of organisms from children with drug-resistant TB together with the likely source case.[7] Both RFLP and DST are generally concordant in such cases, implying transmission of resistance from adults to children.[8] As paediatric MDR-TB cases represent recent infection with a drug-resistant strain, DST patterns in children, particularly among young children, provide important information regarding current transmission patterns in a community or setting, facilitating individual case management, surveillance and public health planning.

Traditionally, DST has been determined by phenotypic methods whereby bacilli are grown in the presence of an antibiotic. If more than a certain percentage (usually 1% or more) of bacilli grow in comparison to a control without antibiotic, the bacilli are classified as being resistant. These tests are reliable but are

Correspondence to: James Alexander Seddon, Paediatrics and Child Health, Desmond Tutu TB Centre, PO Box 19063, Tygerberg, Cape Town, Western Cape 7505, South Africa. Tel: (+27) 21 938 9177. Fax: (+27) 21 938 9719. e-mail: jseddon@sun.ac.za

Article submitted 29 September 2011. Final version accepted 15 January 2012.

Public Health Action

ADVANCE RELEASE XX AUGUST 2012

International Union Against Tuberculosis and Lung Disease
Health solutions for the poor

Decentralised care for the management of child contacts of multidrug-resistant tuberculosis

J. A. Seddon,[1,2] A. C. Hesseling,[1] R. Dunbar,[1] H. Cox,[3] J. Hughes,[3] K. Fielding,[4] P. Godfrey-Faussett,[2] H. S. Schaaf[1,5]

http://dx.doi.org/10.5588/pha.12.0023

Setting: Cape Town, South Africa.
Objective: To determine the number of multidrug-resistant tuberculosis (MDR-TB) child contacts routinely identified by health services and whether a model of decentralised care improves access.
Methods: All MDR-TB source cases registered in Cape Town from April 2010 to March 2011 were included. All child contacts assessed at hospital and outreach clinics were recorded from May 2010 to June 2011. Electronic probabilistic matching was used to match source cases with potential child contacts; the number of children accessing decentralised (Khayelitsha) and hospital-based care was compared.
Results: Of 1221 MDR-TB source cases identified, 189 (15.5%) were registered in Khayelitsha; 31 (16.4%) had at least one child contact assessed. In contrast, 95 (9.2%) of the 1032 source cases diagnosed in the other Cape Town subdistricts (hospital-based care) had at least one child contact assessed ($P = 0.003$). Children in Khayelitsha were seen at a median of 71 days (interquartile range [IQR] 37–121 days) after source case diagnosis compared to 90 days (IQR 56–132 days) in other subdistricts ($P = 0.15$).
Conclusion: Although decentralised care led to an increased number of child contacts being evaluated, both models led to the assessment of a small number of potential child MDR-TB contacts, with considerable delay in assessment.

According to World Health Organization (WHO) estimates, there were 650 000 prevalent cases of multidrug-resistant tuberculosis (MDR-TB, defined as TB resistant to rifampicin [RMP] and isoniazid [INH]) worldwide in 2010.[1,2] Patients with drug-resistant TB (DR-TB) live and interact with numerous other people, termed DR-TB contacts. Although the majority of individuals infected with *Mycobacterium tuberculosis* will never develop TB disease,[3] young children aged <5 years[4,5] and individuals with impaired immunity (e.g., human immunodeficiency virus [HIV] infected) are at high risk of developing disease following exposure and infection.[6,7]

A key TB control strategy is to identify the contacts of newly diagnosed TB cases and screen them for TB disease, enabling early treatment. If contacts are well but are at high risk of development of disease, they can be considered for preventive treatment.[8] INH given daily for 6 months reduces the risk of progression from infection to disease in child contacts of drug-susceptible TB.[9,10] Although evidence regarding preventive treatment regimens for child contacts of MDR-TB patients is limited, exposed children should be identified and screened for MDR-TB disease and followed up for at least 1 year.[11]

The WHO and the majority of national TB programmes advise that children aged <5 years and HIV-infected children in contact with an infectious case of DR-TB should be identified and seen by a specialist experienced in the management of paediatric DR-TB.[2,12,13] In many settings with a high burden of DR-TB, contact tracing is poorly implemented, while specialists with appropriate experience are few and usually based in academic referral centres. This results in a long travel distance for patients, which may be expensive and time-consuming. Such obstacles may result in a failure to access appropriate health services.

Khayelitsha, a peri-urban township and health subdistrict, is located on the outskirts of the City of Cape Town, South Africa. Médecins Sans Frontières has been working in partnership with the local health authorities since 2007 to pilot a decentralised model of care for patients with DR-TB. This patient-centred, community-based approach is aimed at increasing DR-TB case detection, improving treatment outcomes and reducing DR-TB transmission.[14]

One component of the programme includes active follow-up of child contacts. In December 2008, a specialist paediatric monthly outreach service was established to manage child contacts of DR-TB patients. In the remaining seven subdistricts of the City of Cape Town, the traditional, hospital-based system of care was continued, including the identification of child contacts by local services and routine referral to the hospital-based specialist service.

The aim of this study was to determine the number of MDR-TB child contacts identified and whether decentralised care offers improved access compared to hospital-based care. We also aimed to determine whether decentralised care was associated with more rapid identification of child contacts.

STUDY POPULATION AND METHODS

Setting and population

The study was based in the City of Cape Town health district, one of six health districts in the Western Cape Province of South Africa, with a population of 3.4 million. The district comprises eight subdistricts, including Khayelitsha, which has a population of approximately 500 000.[15] Khayelitsha is a poor subdistrict with a predominantly Xhosa-speaking, ethnically black

AFFILIATIONS
1 Desmond Tutu TB Centre Faculty of Health Science Stellenbosch University, South Africa
2 Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK
3 Médecins Sans Frontière Cape Town, South Africa
4 Department of Infectious Diseases Epidemiology, Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK
5 Tygerberg Children's Hospital, Tygerberg, South Africa

CORRESPONDENCE
James Seddon
Desmond Tutu TB Centre
Department of Paediatrics and Child Health
Clinical Building
Room 0085
Faculty of Health Sciences
Stellenbosch University
PO Box 19063
Tygerberg, South Africa
Tel: (+27) 722 470 795
(+27) 21 378 9177
Fax: (+27) 21 938 9792
e-mail: jseddon@sun.ac.za

ACKNOWLEDGEMENTS
The authors thank K. Zimri and Z. Mramba for their assistance. This research was supported by a United States Agency for International Development (USAID) Cooperative Agreement (TREAT TB-Agreement No. GHN-A-00-08-00004-00; JA and HSS), the Sir Halley Steward Trust (JAS), the South African Medical Research Council (HSS) and the National Research Foundation of South Africa (HSS). The contents are the responsibility of the authors and do not necessarily reflect the views of the funders.

KEY WORDS
tuberculosis; child; paediatric; drug-resistant; decentralised; access; care

Received 19 June 2012
Accepted 3 August 2012

Public Health Action

ADVANCE RELEASE XX AUGUST 2012

International Union Against Tuberculosis and Lung Disease
Health solutions for the poor

Why do child contacts of multidrug-resistant tuberculosis not come to the assessment clinic?

K. Zimri,[1] A. C. Hesseling,[1] P. Godfrey-Faussett,[2] H. S. Schaaf,[1,3] J. A. Seddon[1,2]

http://dx.doi.org/10.5588/pha.12.0024

Background: Local policy advises that children exposed to multidrug-resistant tuberculosis (MDR-TB) should be assessed in a specialist clinic. Many children, however, are not brought for assessment.

Methods: Focus group discussion was used to design appropriate questionnaires. From 1 September 2011, the first 50 children referred to the specialist paediatric MDR-TB clinic, Cape Town, South Africa, and who attended their clinic appointment, were recruited. The first 50 children who were referred but who did not attend were concurrently identified, traced and recruited. Differences in group characteristics were compared.

Results: The median age of the children was 35 months: 48 (48%) were boys, 4 (4%) were human immunodeficiency virus infected and 47 (47%) were of coloured ethnicity. Factors significantly associated with non-attendance at the DR-TB clinic were: coloured ethnicity (OR 2.82, 95%CI 1.21–6.59, $P = 0.01$), the mother being the source case (OR 3.78, 95%CI 1.29–11.1, $P = 0.02$), having a smoker resident in the house (OR 2.37, 95%CI 1.01–5.57, $P = 0.04$), the time ($P = 0.002$) and cost ($P = 0.03$) required to get to the specialist clinic, and fear of infection whilst waiting to be seen (OR 2.45, 95%CI 1.07–5.60, $P = 0.03$).

Conclusions: Reasons for non-attendance at paediatric MDR-TB clinic appointments are complex and are influenced by demographic, social, logistical and cultural factors.

The World Health Organization (WHO) and other agencies recommend that child contacts of multidrug-resistant tuberculosis (MDR-TB) should be assessed for TB disease and, if well, followed up for a period of at least 2 years.[1–11] The rationale is that if child contacts are found to have MDR-TB disease, treatment can be initiated rapidly. If they do not have disease, they are followed to detect incident TB disease. Children at the highest risk of disease progression following infection are the young (aged <5 years)[12,13] and the human immunodeficiency virus (HIV) infected.[14] The policy regarding preventive treatment of child contacts of MDR-TB patients is debatable, with little evidence to inform practice.[15] A wide variety of advice is provided by different agencies, but in the Western Cape Province of South Africa the policy is to give ethambutol, ofloxacin and high-dose isoniazid (INH) daily for 6 months.

In the paediatric TB literature, few studies have quantified the proportion of eligible child contacts brought for assessment following exposure to a case of infectious, drug-susceptible TB.[16–20] Few studies have examined reasons for non-attendance. Children may not be identified, or they may be identified but then not brought to clinic appointments. In other health care contexts, the reasons for failure to attend paediatric clinic appointments are complex, but include logistic and financial aspects, parents' educational status and the attitudes of the parents towards the child, including perceptions regarding the importance of the disease.[21,22] The attrition for child TB contacts appears to occur at every step in the identification and referral cascade.[19]

According to WHO estimates, there were 650000 prevalent cases of MDR-TB worldwide in 2010.[23] MDR-TB is defined as TB resistant at least rifampicin and INH.[3] Not all of the estimated adult cases are currently diagnosed, but with the imminent roll-out of new molecular diagnostic tests, the proportion diagnosed is likely to rise.[24] As each MDR-TB source case interacts with multiple children,[25] a large number of children are exposed each year. The management of child contacts of MDR-TB differs from that of drug-susceptible TB, as in most programmes they are managed by a clinician with specialist knowledge and experience in paediatric MDR-TB.[1,6] This can have further logistic and financial implications, as this service is frequently only available in academic centres, potentially leading to long delays in obtaining appointments, together with implications for travel and incurred cost to the family. Furthermore, MDR-TB may be perceived as more dangerous and more difficult to manage, possibly further affecting clinic attendance. We aimed to determine potential reasons for clinic non-attendance among child contacts of MDR-TB cases.

METHODS

Setting

The TB notification rate in the Western Cape Province of South Africa was 976 per 100000 in 2009.[26] Of children with culture-confirmed TB during 2007–2009 at the Tygerberg Children's Hospital (TCH), 8.9% were diagnosed with MDR-TB.[27] Local policy is that, following the diagnosis of MDR-TB in an adult, a home visit is performed. HIV-infected children and children aged <5 years who have been in contact with the MDR-TB source case are referred to their local clinic (roughly 100 exist in the City of Cape Town Health district), where they are assessed by the local clinic team before referral to the regional paediatric drug-resistant (DR) TB clinic. This DR-TB clinic takes place at TCH, a large provincial, academic hospital and, as an outreach service, is

AFFILIATIONS
1 Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, South Africa
2 Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK
3 Tygerberg Children's Hospital, Tygerberg, South Africa

CORRESPONDENCE
James Seddon
Desmond Tutu TB Centre
Department of Paediatrics and Child Health
Clinical Building
Room 0085
Faculty of Health Sciences
Stellenbosch University
PO Box 19063
Tygerberg, South Africa
Tel: (+27) 722 470 795
(+27) 21 378 9177
Fax: (+27) 21 938 9792
e-mail: jseddon@sun.ac.za

ACKNOWLEDGEMENTS
The authors thank Z Mramba and H Finlayson for their assistance. This research was supported by a United States Agency for International Development (USAID) Cooperative Agreement (TREAT TB–Agreement No. GHN-A-00-08-00004-00; JAS and HSS), the Sir Halley Steward Trust (JAS), the South African Medical Research Council (HSS) and the National Research Foundation of South Africa (HSS). The contents are the responsibility of the author(s) and do not necessarily reflect the views of the funders.

KEY WORDS
paediatric; referral; appointment; attendance

Received 19 June 2012
Accepted 29 July 2012

all symptoms in patients receiving oseltamivir treatment. Fever and cough were reduced by 2 and 3 days, respectively, a higher reduction than that reported by systematic reviews of seasonal influenza.[6] A study of children 1–3 years with seasonal Influenza A H1N1 detected a median reduction of illness of 3.5 days and an average fever resolution time of 1.8 days following oseltamivir treatment within 24 hours; they proposed that the observed differences from previous reports were related to earlier treatment.[7] This explanation may be valid to the current study, because 62.5% of our patients were treated within 24 hours.

The World Health Organization reported a current estimated SAR in household contacts of H1N1 of 22–33% and a seasonal influenza SAR of 5–15%.[8] Epidemiological field studies in several American states indicated that the SAR in household contacts for acute respiratory illness was 18–19% and 8–12% for ILI.[9] These differences in SARs may be influenced by different community control measures such as hand washing, school closures, quarantine, public recommendations, use of masks, isolation of the index cases or number of households contacts by index cases.

The overall SAR for our population was 15%, but this rate significantly differed for household contacts based on whether oseltamivir prophylaxis was received (10.9% treated versus 37.8% untreated). The use of neuraminidase inhibitors to limit the spread of influenza is a key component of containment strategies and the prevention of infections in people at risk of complications.

Oseltamivir was well tolerated in this series; only mild gastrointestinal symptoms were observed in 12% of patients with oseltamivir treatment and in only 6% of contacts with prophylaxis. That may be explained by the double dose used for treatment, but a possible bias is that the household contacts were nearly all adults. No neuropsychiatric side effects were reported in our series as reported in United Kingdom and Japanese studies; nevertheless, several recent reviews have demonstrated that oseltamivir is not associated with an increased risk for those events.[10]

REFERENCES

1. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med*. 2010;362:1708–1719.
2. Centers for Disease Control and Prevention. 2009 H1N1 early outbreak and disease characteristics. October 27, 2009. Available at: http://www.cdc.gov/h1n1flu/surveillanceqa.htm. Accessed November 3, 2009.
3. World Health Organization. Collaborating Centre, Atlanta, CDC. CDC Realtime RTPCR (rRTPCR) protocol for detection and characterization of swine influenza A (H1N1), revision 2, October 2009. Available at: http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html. Accessed November 11, 2009.
4. Nougairède A, Ninove L, Zandotti C, et al. Novel virus influenza A (H1N1sw) in South-Eastern France, April-August 2009. *PLoS ONE*. 2010;5:e9214.
5. Fraser C, Donnelly CA, Cauchemez S, et al.; WHO Rapid Pandemic Assessment Collaboration. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science*. 2009;324:1557–1561.
6. Shun-Shin M, Thompson M, Heneghan C, et al. Neuraminidase inhibitors for treatment and prophylaxis of influenza in children: systematic review and meta-analysis of randomised controlled trials. *BMJ*. 2009;339:b3172.
7. Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1-3 years of age: a randomized controlled trial. *Clin Infect Dis*. 2010;51:887–894.
8. World Health Organization (WHO). New influenza A (H1N1) virus: global epidemiological situation, June 2009. *Wkly Epidemiol Rec*. 2009;84:249–257.
9. Cauchemez S, Donnelly CA, Reed C, et al. Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States. *N Engl J Med*. 2009;361:2619–2627.
10. Centers for Disease Control and Prevention. Recommendations of the Advisory Committee on Immunization Practices (ACIP) Antiviral agents for the treatment and chemoprophylaxis of influenza. *MMWR*. 2011;60–124.

DISCORDANT DRUG SUSCEPTIBILITY FOR *MYCOBACTERIUM TUBERCULOSIS* WITHIN FAMILIES

James A. Seddon, MRCPCH,† Annemie M. Jordaan, Med Tech,‡ Thomas C. Victor, PhD,‡ and H. Simon Schaaf, MD*§*

Abstract: Children with presumed tuberculosis who are in contact with a multidrug-resistant source case should be treated according to the drug susceptibility of the source case's isolate. However, it is important to obtain a microbiologic diagnosis as it is possible for the child to have a different susceptibility profile to the source case. We present 2 such cases.

Key Words: tuberculosis, children, resistant, discordant

Accepted for publication March 20, 2012.

From the *Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa; †Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK; ‡Division of Molecular Biology and Human Genetics, Department of Biomedical Science; Faculty of Health Sciences, Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis, Research/Medical Research Council Centre for Molecular and Cellular Biology, Stellenbosch University, Tygerberg, South Africa; and §Tygerberg Children's Hospital, Tygerberg, South Africa.

This work was supported by a grant (GHN-A-00-08-00004-00) from TREAT TB, USAID (J.A.S. and H.S.S.), the Sir Halley Steward Trust (J.A.S.), the South African Medical Research Council (H.S.S.) and the National Research Foundation of South Africa (H.S.S.). The funding agencies had no role in the design or conduct of the study, in the collection, management, analysis or interpretation of the data or in the preparation, review or approval of the manuscript. The authors have no conflicts of interest to disclose.

Address for correspondence: James A. Seddon, MRCPCH, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, South Africa. E-mail: jseddon@sun.ac.za

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

DOI: 10.1097/INF.0b013e3182567e20

Children with tuberculosis (TB) usually have paucibacillary disease and consequently microbiologic diagnosis can be challenging. The diagnosis is usually made presumptively on the basis of suggestive symptoms, signs and radiologic changes.[1] Access to microbiologic culture and drug susceptibility testing (DST), whether phenotypic or genotypic, is often limited where the burden of TB is highest. Children with presumed TB who are in contact with a multidrug-resistant TB (MDR-TB; *Mycobacterium tuberculosis* resistant to both isoniazid and rifampin) source case should be treated according to the DST pattern of the source case's isolate.[1,2] As it is assumed that children will have the same strain and DST as the identified source case,[3] this general strategy is usually valid. However, it is important to obtain a microbiologic diagnosis as it is possible for the child to have a different DST to the source case. The implications for the management of the child are significant. We present 2 children who developed TB following contact with parents who had MDR-TB. The study was approved by Stellenbosch University Ethics Committee.

CASE 1

A 50-month-old girl presented with fever, weight loss, cough and contact with her father who had previously been diagnosed with MDR-TB, susceptible to ethambutol, ethionamide, ofloxacin and amikacin. She had received Bacille Calmette-Guérin immunization

Drug-Resistant Tuberculosis Transmission and Resistance Amplification within Families

James A. Seddon, Rob M. Warren, Donald A. Enarson, Nulda Beyers, and H. Simon Schaaf

Drug-resistant tuberculosis is caused by transmission of resistant strains of *Mycobacterium tuberculosis* and by acquisition of resistance through inadequate treatment. We investigated the clinical and molecular features of the disease in 2 families after drug-resistant tuberculosis was identified in 2 children. The findings demonstrate the potential for resistance to be transmitted and amplified within families.

The devastating effects of extensively drug-resistant tuberculosis (XDR TB) gained international attention after the 2006 outbreak in Tugela Ferry, South Africa. The evolution of the epidemic is the result of transmission of resistant strains and strain acquisition of resistance through inadequate treatment (*1*). Multidrug-resistant (MDR) TB is disease caused by *Mycobacterium tuberculosis* that is resistant to isoniazid and rifampin, and XDR TB is disease caused by *M. tuberculosis* that is additionally resistant to a fluoroquinolone and an injectable second-line anti-TB drug. Because children usually have transmitted resistance (*2*), they can be seen as the end of a sequence of transmission events. We describe investigations of 2 families after the identification of children with drug-resistant TB in terms of clinical features and molecular characteristics of the isolates.

The Study

This investigation was conducted in a suburban community of Cape Town, South Africa, where TB incidence was 978/100,000 population in 2009 (Health Systems Trust). Since 1994, microbiological samples from all patients treated for TB in this area have been sent to the research laboratory at Tygerberg Hospital, Stellenbosch University. From 2008 through 2010, two children from this community received a diagnosis of MDR TB.

Information was obtained from several sources to document the sequence of events that culminated in the development of MDR TB in each child. A home visit was made, and the family was interviewed after written informed consent was obtained. Family members were included if they either lived with or spent substantial amount of time with the child (*3*). Information on TB diagnosis, treatment, and outcome was obtained at interview. If a family member was identified as having had TB, family contacts of that person were included. Searches for case notes for those included were made at the local clinic, the academic hospitals, and the regional TB hospital responsible for drug-resistant TB management. Also, the local clinic TB register was consulted. The investigation was approved by the Stellenbosch University Ethics Committee.

Sputum samples from the 2 families were identified, and isolates were genotyped by spoligotyping (*4*) and IS*6110* DNA fingerprinting (*5*). Strains were identified according to distinct IS*6110* banding patterns by using Gelcompar II (Applied Maths, Sint-Martens-Latem, Belgium) or characteristic spoligotype pattern (*6*). Mutations conferring resistance to isoniazid, rifampin, ethambutol, pyrazinamide, ofloxacin, and amikacin were determined by DNA sequencing of the *inhA* promoter, *katG*, *rpoB*, *embB*, *pncA*, *gyrA*, and *rrs* genes, respectively (*7*).

A 19-month-old girl (A3) received a diagnosis of TB in March 2008 after a 6-month course of preventive therapy with isoniazid. She was brought for assessment with a 2-weeek history of cough, respiratory distress, and fever. She had contact with a patient with pre–XDR TB (MDR TB resistant to either a fluoroquinolone or a second-line injectable drug), and therefore the following antimicrobial drugs were administered: capreomycin, ethionamide, ethambutol, *para*-aminosalicylic acid, terizidone, clarithromycin, and high-dose isoniazid. Gastric aspirate samples were sent to the National Health Laboratory Service; *M. tuberculosis* grew in culture and was resistant to rifampin, isoniazid, and ofloxacin and susceptible to amikacin and ethionamide. She received treatment for 18 months from the time of her first negative culture (the first 6 months included the injectable medication) and recovered.

Patient 1's family consisted of 18 persons (Figure 1). The husband of her aunt (A2) had drug-resistant TB. He cared for the girl on a daily basis. He had received treatment initially for drug-susceptible TB; this was changed to MDR TB therapy when resistance to rifampin and isoniazid was determined and then to XDR TB treatment when resistance

Author affiliations: London School of Hygiene and Tropical Medicine, London, UK (J.A. Seddon); Stellenbosch University, Tygerberg, South Africa (J.A. Seddon, R.M. Warren, N. Beyers, H.S. Schaaf,); International Union Against Tuberculosis and Lung Disease, Paris, France (D.A. Enarson); and Tygerberg Children's Hospital, Tygerberg (H.S. Schaaf)

DOI: http://dx.doi.org/10.3201/eid1808.111650

MAJOR ARTICLE

Culture-Confirmed Multidrug-Resistant Tuberculosis in Children: Clinical Features, Treatment, and Outcome

James A. Seddon,[1,2] Anneke C. Hesseling,[1] Marianne Willemse,[3] Peter R. Donald,[1] and H. Simon Schaaf[1,4]

[1]Desmond Tutu Tuberculosis Centre, Faculty of Health Sciences, Stellenbosch University, South Africa; [2]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, United Kingdom; [3]Brooklyn Hospital for Chest Diseases, Cape Town, and [4]Tygerberg Children's Hospital, South Africa

Background. Multidrug-resistant (MDR) tuberculosis in children is frequently associated with delayed diagnosis and treatment. There is limited evidence regarding the management and outcome of children with MDR-tuberculosis.

Methods. All children <15 years of age with a diagnosis of culture-confirmed MDR-tuberculosis were included in this retrospective cohort study from 1 January 2003 to 31 December 2008, with follow-up documented until 31 May 2011. We identified children from Brooklyn Hospital for Chest Diseases and Tygerberg Children's Hospital, Western Cape Province, South Africa. Treatment outcomes were defined as 2-month sputum-culture conversion, treatment episode outcome, and survival.

Results. A total of 111 children (median age, 50 months) were included. The diagnosis was delayed in children who had no identified MDR-tuberculosis index case (median delay, 123 vs 58 days; $P < .001$). Sixty-two percent of patients (53 of 85) were sputum-smear positive, and 43% of patients (43 of 100) were human immunodeficiency virus (HIV) infected. Overall, 82% had favorable treatment outcomes; total mortality was 12%. Malnutrition was associated with failure to culture-convert at 2 months (odds ratio [OR], 4.49 [95% confidence interval {CI}, 1.32–15.2]; $P = .02$) and death (OR, 15.0 [95% CI, 1.17–192.5]; $P = .04$) in multivariate analysis. HIV coinfection (OR, 24.7 [95% CI, 1.79–341.1]; $P = .02$) and the presence of extrapulmonary tuberculosis (OR, 37.8 [95% CI, 2.78–513.4]; $P = .006$) predicted death.

Conclusions. Despite advanced disease at presentation and a high prevalence of human immunodeficiency virus coinfection, children with MDR-tuberculosis can be treated successfully, using individualized treatment under routine conditions.

Multidrug-resistant (MDR) tuberculosis is caused by *Mycobacterium tuberculosis* resistant to isoniazid and rifampin. The World Health Organization (WHO) estimated that there were 440 000 new MDR-tuberculosis cases worldwide during 2008 [1]. Treatment outcomes are generally poor in adults, with favorable outcomes reported in only 60% of those receiving treatment [2]. Even though childhood tuberculosis makes up 15%–20% of the global tuberculosis burden [3], MDR-tuberculosis is poorly studied in children, the literature including mainly case reports or small case series [4–16].

The diagnosis of tuberculosis in young children is challenging and often delayed [17]. Symptoms and signs may be nonspecific, especially in children <3 years of age and in children infected with human immunodeficiency virus (HIV) [18]. Because of the paucibacillary nature of childhood tuberculosis, a microbiological diagnosis is typically made in only 20%–40% of cases with radiological evidence of intrathoracic disease [19]. Because drug susceptibility testing (DST) is only possible following bacteriological confirmation, confirmed MDR-tuberculosis in children is infrequent. In

Received 12 June 2011; accepted 15 September 2011.

Correspondence: James Seddon, MBBS, MA, MRCPCH, DTM&H, Department of Paediatrics and Child Health, Desmond Tutu Tuberculosis Centre, Clinical Building, Rm 0085, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, South Africa (jseddon@sun.ac.za).

Clinical Infectious Diseases

1058-4838/2011/00-0001$14.00
DOI: 10.1093/cid/cir772

Impact of Drug Resistance on Clinical Outcome in Children With Tuberculous Meningitis

James A. Seddon, MRCPCH,† Douwe H. Visser, MD,‡ Margaux Bartens,‡ Annemie M. Jordaan, MTech,§ Thomas C. Victor, PhD,§ A. Marceline van Furth, PhD,‡ Johan F. Schoeman, MD,¶ and H. Simon Schaaf, MD*¶*

Background: Tuberculous meningitis (TBM) is associated with delayed diagnosis and poor outcome in children. This study investigated the impact of drug resistance on clinical outcome in children with TBM.

Methods: All children (0–13 years) were included if admitted to Tygerberg Children's Hospital, Cape Town, South Africa, from January 2003 to April 2009 with a diagnosis of either confirmed TBM, or probable TBM with mycobacterial isolation from a site other than cerebrospinal fluid. Mycobacterial samples underwent drug susceptibility testing to rifampin and isoniazid. Children were treated with isoniazid, rifampin, pyrazinamide and ethionamide according to local guidelines.

Results: One hundred twenty-three children were included; 13% (16 of 123) had any form of drug resistance, and 4% (5 of 123) had multidrug-resistant tuberculosis. Time from start of symptoms to appropriate treatment was longer in children with any drug resistance (median: 31 days versus 9 days; $P = 0.001$). In multivariable analysis, young age ($P = 0.013$) and multidrug-resistant tuberculosis (adjusted odds ratio: 12.4 [95% confidence interval: 1.17–132.3]; $P = 0.037$) remained risk factors for unfavorable outcome, and multidrug-resistant tuberculosis remained a risk for death (adjusted odds ratio: 63.9 [95% confidence interval: 4.84–843.2]; $P = 0.002$). We did not detect any difference in outcome between those with isolates resistant to only isoniazid and those with fully susceptible strains (adjusted odds ratio: 0.22 [confidence interval: 0.03–1.87]; $P = 0.17$).

Conclusion: Multidrug-resistant TBM in children has poor clinical outcome and is associated with death. We did not find any difference in the outcomes between children with isoniazid monoresistant TBM and those with drug-susceptible TBM. One explanation could be the local treatment regimen. Further investigation of this regimen is indicated.

Key Words: pediatric, meningitis, tuberculosis, children, resistance, outcome

(*The Pediatr Infect Dis J* 2012;31: 711–716)

Accepted for publication March 1, 2012.

From the *Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa; †Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; ‡Department of Paediatrics, VU University Medical Centre, Amsterdam, the Netherlands; §Division of Molecular Biology and Human Genetics, Department of Biomedical Science, Faculty of Health Sciences, Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis, Research/Medical Research Council Centre for Molecular and Cellular Biology, Stellenbosch University, Tygerberg; and the ¶Tygerberg Children's Hospital, Cape Town, South Africa.

Supported by a grant (GHN-A-00-08-00004-00) from TREAT TB, USAID (JAS and HSS), the Sir Halley Steward Trust (JAS), the South African Medical Research Council (HSS) and the National Research Foundation of South Africa (HSS). The authors have no other funding or conflicts of interest to disclose.

Address for correspondence: James A. Seddon, MRCPCH, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, South Africa. E-mail: jseddon@sun.ac.za.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/12/3107-711
DOI: 10.1097/INF.0b013e318253aef8

Tuberculous meningitis (TBM) is a severe form of tuberculosis (TB) and frequently occurs in early childhood.[1] Hematogenous spread of bacilli from a primary pulmonary focus leads to the development of a Rich focus in the brain. Rupture of this caseous granuloma into the subarachnoid space causes the clinical features of TBM.[2,3] This usually starts insidiously with a prodromal period of nonspecific symptoms but as the disease progresses, neck stiffness, loss of consciousness, motor paresis and convulsions invariably follow. The diagnosis is often delayed and only considered after irreversible neurologic damage has already occurred.[1,4] Untreated, the condition is almost universally fatal with a median time to death of 19.5 days.[5] Even for those treated, TBM is associated with high rates of mortality and morbidity; about 80% of children with advanced disease at diagnosis (TBM stage II and III) will develop neurologic sequelae.[1,4] TBM is the most common cause of bacterial meningitis in the Western Cape Province (WCP) of South Africa.[6]

The World Health Organization (WHO) estimated that there were 440,000 new cases of multidrug-resistant (MDR) TB globally during 2008.[7] MDR-TB is caused by *Mycobacterium tuberculosis* resistant to both isoniazid and rifampin. Extensively drug-resistant TB is additionally resistant to a fluoroquinolone and an injectable second-line anti-TB medication. As TB in children is usually paucibacillary, microbiologic diagnosis occurs in only 20–40% of cases with evidence of disease.[8] As drug susceptibility testing (DST) requires a microbiologic diagnosis, the diagnosis of MDR-TB in children is often made presumptively. This is based on signs, symptoms and radiology suggestive of TB in the context of either an MDR-TB source case or treatment failing in a child being treated with a first-line regimen. As an MDR-TB source case is not always identified, most children with MDR-TB are initially treated with a first-line regimen until their culture and DST results are available, an MDR-TB source case is identified or treatment is found to be failing. The initiation of appropriate treatment with second-line drugs is therefore often delayed in children with MDR-TB.[9,10]

MDR-TBM has very poor outcome[11–14] but there are few data regarding children. The relationship between the *M. tuberculosis* strain and clinical phenotype has been explored in both adults and children with TBM[15–17] with conflicting results. The relationship between strain type and drug resistance pattern is complex, but a strong association exists between drug resistance and the Beijing genotype.[18–20] The aim of this study is to analyze whether a relationship exists between the drug susceptibility pattern of the infecting *M. tuberculosis* organism and the clinical outcome of TBM in children and to determine whether this relationship is influenced by the genotype of the strain.

PATIENTS AND METHODS

Setting

Tygerberg Children's Hospital (TCH), in the WCP, South Africa, provides specialized care to half of the province's 1.2 million children. The WCP had a TB notification rate of 976 per 100,000 in 2009,[21] and among all children with routinely diagnosed

Multidrug-Resistant Tuberculosis of the Spine in Children—Characteristics from a High Burden Setting

by James A. Seddon,[1,2] Peter R. Donald,[3] Gert J. Vlok,[4,5] *and* H. Simon Schaaf[2,3]

[1]*Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, 7505, South Africa*

[2]*Department of Clinical Research, Faculty of Infectious and Tropical Medicine, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK*

[3]*Tygerberg Children's Hospital, Cape Town, Tygerberg, 7505, South Africa*

[4]*Division of Orthopaedic Surgery, Faculty of Health Sciences, Stellenbosch University, Tygerberg, 7505, South Africa*

[5]*Tygerberg Hospital, Cape Town, Tygerberg, 7505, South Africa*

Correspondence: James A. Seddon, Desmond Tutu TB Centre, PO Box 19063, Francie Van Zyl Drive, Tygerberg, 7505, Cape Town, South Africa. Tel: +27 21 938 9177; Fax: +27 21 938 9719; E-mail: <jseddon@sun.ac.za>.

Summary

Background: Few studies have described children with spinal multidrug-resistant tuberculosis (MDR-TB). Treatment involves surgery and medical care with long courses of drug therapy.

Methods: Hospital and laboratory records at Brooklyn Chest and Tygerberg Children's Hospitals, Cape Town, South Africa, were analysed (January 2004 until December 2010) searching for children treated for MDR spinal TB.

Results: Of the 11 children identified, 4 were excluded. Of the 7 remaining, 5 were boys; median age: 8 years, median delay to treatment initiation: 36 weeks. Among them one child died, five have completed treatment and one is near the end of therapy. Medications were well-tolerated and although two of the surviving children have spinal deformity, none have significant neurological deficit.

Conclusions: The diagnosis of spinal MDR-TB is often delayed in children, frequently leading to advanced disease and severe vertebral damage. Children tolerate therapy well and, once identified, it is a condition that can be treated successfully.

Introduction

Spinal tuberculosis (TB) in children can be a debilitating disease with potential long-term neurological sequelae. Treatment involves a combination of surgical and medical care with long courses of drug therapy. Multidrug-resistant (MDR)-TB occurs when *Mycobacterium tuberculosis* is resistant to rifampicin and isoniazid. It was estimated that there were 440 000 new cases of MDR-TB globally during 2008 [1] and in many high-incidence settings childhood TB makes up 15–20% of the total burden [2–4]. Paediatric MDR-TB is difficult to diagnose and the initiation of effective therapy is often delayed [5]. The drugs used are less effective and more toxic than the first-line medications; treatment is therefore longer and with frequent adverse effects. Few paediatric spinal MDR-TB studies have been published.

Acknowledgements

The authors would like to thank Dr Marianne Willemse, Mrs Klassina Zimri and Miss Zingiwe Mramba. J.A.S., H.S.S. and P.R.D. participated in the design of the study. J.A.S. and H.S.S. identified the patients and collected the data. G.J.V. provided advice and suggestions regarding the surgical aspects of the study. J.A.S. drafted the initial manuscript after which all authors gave critical input. All authors read and approved the final manuscript.

Funding

This work was supported by a grant (GHN-A-00-08-00004-00) from TREAT TB, USAID (to J.A.S. and H.S.S.), the Sir Halley Steward Trust (to J.A.S.), the South African Medical Research Council (to H.S.S.) and the National Research Foundation of South Africa (to H.S.S. and P.R.D.). The funding agencies had no role in the design or conduct of the study, in the collection, management, analysis or interpretation of the data, or in the preparation, review or approval of the manuscript.

doi:10.1093/tropej/fmr104

Journal of Infection (2012) xx, 1–10

ELSEVIER

BIAA British Infection Association

www.elsevierhealth.com/journals/jinf

Hearing loss in children treated for multidrug-resistant tuberculosis

James A. Seddon [a,b,*], Stephanie Thee [c], Kayleen Jacobs [d], Adam Ebrahim [d], Anneke C. Hesseling [a], H. Simon Schaaf [a,e]

[a] *Desmond Tutu TB Centre, Faculty of Health Sciences, Stellenbosch University, South Africa*
[b] *Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK*
[c] *Department of Paediatric Pneumology and Immunology, Charité, Universitätsmedizin, Berlin, Germany*
[d] *Audiology Department, Brooklyn Chest Hospital, Cape Town, South Africa*
[e] *Tygerberg Children's Hospital, Tygerberg, South Africa*

Accepted 2 September 2012
Available online ■ ■ ■

KEYWORDS
Hearing;
Audiology;
Tuberculosis;
Multidrug-resistant;
Resistant;
Ototoxicity;
Children;
Paediatric

Summary *Objective:* The aminoglycosides and polypeptides are vital drugs for the management of multidrug-resistant (MDR) tuberculosis (TB). Both classes of drug cause hearing loss. We aimed to determine the extent of hearing loss in children treated for MDR-TB.
Methods: In this retrospective study, children (<15 years) admitted to Brooklyn Chest Hospital, Cape Town, South Africa, from January 2009 until December 2010, were included if treated for MDR-TB with injectable drugs. Hearing was assessed and classified using audiometry and otoacoustic emissions.
Results: Ninety-four children were included (median age: 43 months). Of 93 tested, 28 (30%) were HIV-infected. Twenty-three (24%) children had hearing loss. Culture-confirmed, as opposed to presumed, diagnosis of TB was a risk factor for hearing loss (OR: 4.12; 95% CI: 1.13–15.0; $p = 0.02$). Seven of 11 (64%) children classified as having hearing loss using audiometry had progression of hearing loss after finishing the injectable drug.
Conclusions: Hearing loss is common in children treated for MDR-TB. Alternative drugs are required for the treatment of paediatric MDR-TB.

Abbreviations: MDR, multidrug-resistant; DR, drug-resistant; TB, tuberculosis; WHO, World Health Organization; PTA, pure tone audiometry; OAE, otoacoustic emission; DPOAE, distortion product otoacoustic emission; BCH, Brooklyn Chest Hospital; IM, intramuscular; ASHA, American Speech and Hearing Association; OR, odds ratio; CI, confidence intervals; IQR, inter-quartile range.

* Corresponding author. Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Clinical Building, Room 0085, Faculty of Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg, South Africa. Tel.: +27 21 378 9177; fax: +27 21 938 9792.
E-mail address: jseddon@sun.ac.za (J.A. Seddon).

0163-4453/$36
http://dx.doi.org/10.1016/j.jinf.2012.09.002

Please cite this article in press as: Seddon JA, et al., Hearing loss in children treated for multidrug-resistant tuberculosis, J Infect (2012), http://dx.doi.org/10.1016/j.jinf.2012.09.002

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2011, p. 5560–5567 Vol. 55, No. 12
0066-4804/11/$12.00 doi:10.1128/AAC.05429-11

Pharmacokinetics of Isoniazid, Rifampin, and Pyrazinamide in Children Younger than Two Years of Age with Tuberculosis: Evidence for Implementation of Revised World Health Organization Recommendations▽

S. Thee,[1,2] J. A. Seddon,[2,3] P. R. Donald,[2] H. I. Seifart,[4] C. J. Werely,[5] A. C. Hesseling,[2] B. Rosenkranz,[4] S. Roll,[6] K. Magdorf,[1] and H. S. Schaaf[2]*

Department of Paediatric Pneumology and Immunology, Charité, Universitätsmedizin Berlin, Germany[1]; Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa[2]; Clinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom[3]; Division of Pharmacology, Stellenbosch University, Tygerberg, South Africa[4]; Division of Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa[5]; and Institute for Social Medicine, Epidemiology and Health Economics, Charité, Universitätsmedizin, Berlin, Germany[6]

Received 31 July 2011/Returned for modification 7 September 2011/Accepted 27 September 2011

The World Health Organization (WHO) recently issued revised first-line antituberculosis (anti-TB) drug dosage recommendations for children. No pharmacokinetic studies for these revised dosages are available for children <2 years. The aim of the study was to document the pharmacokinetics of the first-line anti-TB agents in children <2 years of age comparing previous and revised WHO dosages of isoniazid (INH; 5 versus 10 mg/kg/day), rifampin (RMP; 10 versus 15 mg/kg/day), and pyrazinamide (PZA; 25 versus 35 mg/kg/day) and to investigate the effects of clinical covariates, including HIV coinfection, nutritional status, age, gender, and type of tuberculosis (TB), and the effect of NAT2 acetylator status. Serum INH, PZA, and RMP levels were prospectively assessed in 20 children <2 years of age treated for TB following the previous and the revised WHO dosage recommendations. Samples were taken prior to dosing and at 0.5, 1.5, 3, and 5 h following dosing. The maximum drug concentration in serum (C_{max}), the time to C_{max} (t_{max}), and the area under the concentration-time curve (AUC) were calculated. Eleven children had pulmonary and 9 had extrapulmonary TB. Five were HIV infected. The mean C_{max} (μg/ml) following the administration of previous/revised dosages were as follows: INH, 3.19/8.11; RMP, 6.36/11.69; PZA, 29.94/47.11. The mean AUC (μg · h/ml) were as follows: INH, 8.09/20.36; RMP, 17.78/36.95; PZA, 118.0/175.2. The mean C_{max} and AUC differed significantly between doses. There was no difference in the t_{max} values achieved. Children less than 2 years of age achieve target concentrations of first-line anti-TB agents using revised WHO dosage recommendations. Our data provided supportive evidence for the implementation of the revised WHO guidelines for first-line anti-TB therapy in young children.

Isoniazid (INH), rifampin (RMP), and pyrazinamide (PZA) are routinely used to treat tuberculosis (TB) in children (23, 44). Recommendations for pediatric dosages are based on a small number of pharmacokinetic studies, few of which included children younger than 2 years of age. During early life, children experience significant changes in the relative sizes of their body compartments and their ability to absorb, metabolize, and excrete drugs (5, 17). These changes are greatest within the first 2 years of life (4). Most published studies on first-line anti-TB drugs in children have not analyzed differences between older and younger children or the effect of HIV coinfection. The pharmacokinetics of INH are further complicated by genetic polymorphisms of *N*-acetyltransferase type 2 (NAT2) in the metabolic pathway of INH, which influences INH concentrations (18, 26, 46).

In the absence of pharmacodynamic data for children and therefore data that demonstrate an association between serum drug concentration and clinical outcome, optimal anti-TB therapy should aim to produce the targeted serum drug concentrations that have been determined in adult pharmacokinetic and pharmacodynamic studies. For INH, the proposed optimal maximum serum drug concentration (C_{max}) for therapy is 3 to 5 μg/ml (15, 27). Target serum RMP concentrations in adults after a standard oral dose of 600 mg are in the range of 8 to 24 μg/ml; serum RMP concentrations below 8 μg/ml are considered low, and those below 4 μg/ml are considered very low (28, 29). There is more uncertainty regarding the optimal therapeutic serum PZA concentration. In adults, serum PZA concentrations are targeted at 20 to 60 μg/ml (11, 28). However, in a recent study of adults, poor treatment outcome of pulmonary TB was associated with serum PZA concentrations of <35 μg/ml (8).

Optimal anti-TB therapy is important in all children but particularly in young children (<2 years of age) and those HIV infected, where there is a high risk of progression to severe

* Corresponding author. Mailing address: Department of Paediatrics and Child Health, P.O. Box 19063, Tygerberg 7505, South Africa. Phone: 27.21.9389112. Fax: 27.21.9389138. E-mail: hss@sun.ac.za.
▽ Published ahead of print on 3 October 2011.

INT J TUBERC LUNG DIS 16(12):1588–1593

http://dx.doi.org/10.5588/ijtld.12.0322
E-published ahead of print 2 October 2012

Linezolid-containing regimens for the treatment of drug-resistant tuberculosis in South African children

P. C. Rose,* U. M. Hallbauer,†‡ J. A. Seddon,*§ A. C. Hesseling,* H. S. Schaaf*¶

*Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Cape Town, Tygerberg, †Department of Paediatrics and Child Health, Faculty of Health Sciences, Free State University, Bloemfontein, ‡Department of Paediatrics, Pelonomi Hospital, Bloemfontein, South Africa; §Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK; ¶Tygerberg Children's Hospital, Cape Town, Tygerberg, South Africa

SUMMARY

BACKGROUND: Treatment options for drug-resistant tuberculosis (DR-TB) are limited. Linezolid has been successfully used to treat DR-TB in adults, but there are few case reports of its use in children for TB. The reported rate of adverse events in adults is high.

METHODS: We conducted a retrospective review of children with DR-TB treated with linezolid-containing regimens from February 2007 to March 2012 at two South African hospitals.

RESULTS: Seven children (three human immunodeficiency virus [HIV] infected) received a linezolid-containing regimen. All had culture-confirmed DR-TB; five had previously failed second-line anti-tuberculosis treatment. Four children were cured and three were still receiving anti-tuberculosis treatment, but had culture converted. None of the non-HIV-infected children experienced adverse events while receiving linezolid. Three HIV-infected children had adverse events, one of which was life-threatening; linezolid was permanently discontinued in this case. Adverse events included lactic acidosis ($n = 1$), pancreatitis ($n = 2$), peripheral neuropathy ($n = 1$) and asymptomatic bone marrow hypoplasia ($n = 1$).

CONCLUSION: Linezolid-containing regimens can be effective in treating children with DR-TB even after failing second-line treatment. Adverse events should be monitored, especially in combination with medications that have similar adverse effects. Linezolid remains costly, and a reduced dosage and duration may result in fewer adverse events and lower cost.

KEY WORDS: paediatric; TB; long-term; linezolid

THE NUMBER OF PEOPLE with drug-resistant tuberculosis (DR-TB) is increasing worldwide; the World Health Organization (WHO) estimated that there were approximately 650 000 prevalent cases of multidrug-resistant TB (MDR-TB) globally in 2010.[1] The treatment of MDR-TB (i.e., *Mycobacterium tuberculosis* resistant to at least isoniazid [INH] and rifampicin [RMP]) and extensively drug-resistant TB (XDR-TB; i.e., MDR-TB with additional resistance to the fluoroquinolones and at least one second-line injectable agent) is complicated by limited treatment options, long treatment duration and a high risk of adverse events.[2]

Linezolid is an oxazolidinone antibiotic used primarily in the treatment of DR gram-positive bacterial infections, with both in vitro and in vivo activity against *M. tuberculosis*. It inhibits protein synthesis at an early stage of translation by binding to the 23S rRNA. This unique mechanism of action means that cross-resistance with other anti-tuberculosis drugs is unlikely.[3] It is listed by the WHO as an agent for the treatment of both MDR-TB and XDR-TB, categorised under Group five, indicating unclear efficacy.[4]

A number of case series have reported good treatment outcomes in adults with MDR-TB and XDR-TB treated with linezolid-containing regimens, despite significant adverse events, in particular neuropathies and myelosuppression.[5–7] Four case reports of linezolid use in children with MDR-TB and XDR-TB describe the outcomes for a total of seven children treated with linezolid-containing regimens.[8–11] Three children experienced adverse events. Linezolid was stopped in one patient who developed lactic acidosis, and the dosage was reduced in another two children, one of whom developed anaemia and neuropathy and the other an urticarial rash. All children had a favourable outcome, either cure or clinical response, and were asymptomatic at treatment completion. Two reviews have described the use of linezolid in children, mainly documenting short-course treatment

Correspondence to: Penelope C Rose, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Cape Town, PO Box 19063, Tygerberg 7505, South Africa. Tel: (+27) 21 938 9177/9173. Fax: (+27) 21 938 9719. e-mail: pcrose@sun.ac.za

Article submitted 1 May 2012. Final version accepted 1 July 2012.

References

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA.* 1999;**282**:677-686.
2. World Health Organization, Geneva, Switzerland. Global Tuberculosis Control Report - surveillance, planning, financing. 2008.
3. Dye C. Global epidemiology of tuberculosis. *Lancet.* 2006;**367**:938-940.
4. Mandalakas AM, Detjen AK, Hesseling AC, Benedetti A, Menzies D. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis.* 2011;**15**:1018-1032.
5. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. *Lancet.* 2005;**365**:130-134.
6. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR. Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med.* 2006;**173**:1078-1090.
7. Du Preez K, Schaaf HS, Dunbar R, et al. Incomplete registration and reporting of culture-confirmed childhood tuberculosis diagnosed in hospital. *Public Health Action.* 2011;**1**:19-24.
8. Petrini B, Hoffner S. Drug-resistant and multidrug-resistant tubercle bacilli. *Int J Antimicrob Agents.* 1999;**13**:93-97.
9. Pablos-Mendez A, Raviglione MC, Laszlo A, et al. Global surveillance for antituberculosis-drug resistance, 1994-1997. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *N Engl J Med.* 1998;**338**:1641-1649.
10. Cohn DL, Bustreo F, Raviglione MC. Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. International Union Against Tuberculosis and Lung Disease. *Clin Infect Dis.* 1997;**24 Suppl 1**:S121-130.
11. Mitnick CD, Shin SS, Seung KJ, et al. Comprehensive treatment of extensively drug-resistant tuberculosis. *N Engl J Med.* 2008;**359**:563-574.
12. Keshavjee S, Gelmanova IY, Farmer PE, et al. Treatment of extensively drug-resistant tuberculosis in Tomsk, Russia: a retrospective cohort study. *Lancet.* 2008;**372**:1403-1409.
13. World Health Organization, Geneva, Switzerland. Global tuberculosis control. *WHO/HTM/TB/2011.16.* 2011.
14. World Health Organization, Geneva, Switzerland. Anti-tuberculosis drug resistance in the world - report no. 4. 2008; **(WHO/HTM/TB/2008.394)**.
15. Department of Health, Republic of South Africa. Management of Drug-resistant Tuberculosis. Policy Guidelines. Available at: http://www.doh.gov.za/docs/policy/2011/policy_TB.pdf (accessed 17 July 2012). 2011.
16. World Health Organization, Geneva, Switzerland. Guidance for National Tuberculosis Programmes on the management of tuberculosis in children. *WHO/HTM/TB/2006.371, WHO/FCH/CAH/2006.7.* 2006.
17. van Rie A, Beyers N, Gie RP, Kunneke M, Zietsman L, Donald PR. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Arch Dis Child.* 1999;**80**:433-437.
18. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of community-based surveillance data. *Int J Tuberc Lung Dis.* 2006;**10**:259-263.
19. Lin PL, Flynn JL. Understanding latent tuberculosis: a moving target. *J Immunol.* 2010;**185**:15-22.
20. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med.* 2000;**161**:1376-1395.

21. Schaaf HS, Gie RP, Kennedy M, Beyers N, Hesseling PB, Donald PR. Evaluation of young children in contact with adult multidrug-resistant pulmonary tuberculosis: a 30-month follow-up. *Pediatrics.* 2002;**109**:765-771.
22. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis.* 2006;**10**:1192-1204.
23. Graham NM, Nelson KE, Solomon L, et al. Prevalence of tuberculin positivity and skin test anergy in HIV-1-seropositive and -seronegative intravenous drug users. *JAMA.* 1992;**267**:369-373.
24. Hesseling AC, Mandalakas AM, Kirchner HL, et al. Highly discordant T cell responses in individuals with recent exposure to household tuberculosis. *Thorax.* 2009;**64**:840-846.
25. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med.* 2008;**149**:177-184.
26. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. *Pediatr Infect Dis J.* 2011.
27. Huang YW, Shen GH, Lee JJ, Yang WT. Latent tuberculosis infection among close contacts of multidrug-resistant tuberculosis patients in central Taiwan. *Int J Tuberc Lung Dis.* 2010;**14**:1430-1435.
28. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. *MMWR Recomm Rep.* 2010;**59**:1-25.
29. European Centre for Disease Prevention and Control, Stockholm, Sweden. Use of interferon-gamma release assays in support of TB diagnosis. 2011.
30. World Health Organization, Geneva, Switzerland. Strategic and technical advisory group for tuberculosis (STAG-TB). Report of the tenth meeting. 2010.
31. Rieder HL. Epidemiologic Basis of Tuberculosis Control. First Edition. *International Union Against Tuberculosis and Lung Disease.* 1999.
32. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol.* 1974;**99**:131-138.
33. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in guinea-pigs of South Indian and British tubercle bacilli. *Tubercle.* 1960;**41**:1-22.
34. Middlebrook G, Cohn ML. Some observations on the pathogenicity of isoniazid-resistant variants of tubercle bacilli. *Science.* 1953;**118**:297-299.
35. Barroso E, Mota R, Pinheiro V, Campelo C, Rodrigues J. Occurence of active tuberculosis in households inhabited by patients with susceptible and mulidrug-resistant tuberculosis. *Jornal Brasileiro de Pneumologia.* 2004;**30**:401-408.
36. Snider DE, Jr., Kelly GD, Cauthen GM, Thompson NJ, Kilburn JO. Infection and disease among contacts of tuberculosis cases with drug-resistant and drug-susceptible bacilli. *Am Rev Respir Dis.* 1985;**132**:125-132.
37. Teixeira L, Perkins MD, Johnson JL, et al. Infection and disease among household contacts of patients with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2001;**5**:321-328.
38. Risk of tuberculosis among contacts of isoniazid-resistant and isoniazid-susceptible cases. *Int J Tuberc Lung Dis.* 2011;**15**:782-788.
39. Johnson R, Warren RM, van der Spuy GD, et al. Drug-resistant tuberculosis epidemic in the Western Cape driven by a virulent Beijing genotype strain. *Int J Tuberc Lung Dis.* 2010;**14**:119-121.
40. Hanekom M, van der Spuy GD, Streicher E, et al. A recently evolved sublineage of the Mycobacterium tuberculosis Beijing strain family is associated with an increased ability to spread and cause disease. *J Clin Microbiol.* 2007;**45**:1483-1490.
41. Thwaites G, Caws M, Chau TT, et al. Relationship between Mycobacterium tuberculosis genotype and the clinical phenotype of pulmonary and meningeal tuberculosis. *J Clin Microbiol.* 2008;**46**:1363-1368.
42. Gessner BD, Weiss NS, Nolan CM. Risk factors for pediatric tuberculosis infection and disease after household exposure to adult index cases in Alaska. *J Pediatr.* 1998;**132**:509-513.
43. Singh M, Mynak ML, Kumar L, Mathew JL, Jindal SK. Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis. *Arch Dis Child.* 2005;**90**:624-628.

44. Shaw JB, Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. *Am Rev Tuberc.* 1954;**69**:724-732.
45. Kenyon TA, Creek T, Laserson K, et al. Risk factors for transmission of Mycobacterium tuberculosis from HIV-infected tuberculosis patients, Botswana. *Int J Tuberc Lung Dis.* 2002;**6**:843-850.
46. Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence. *Lancet Infect Dis.* 2003;**3**:288-296.
47. Cruciani M, Malena M, Bosco O, Gatti G, Serpelloni G. The impact of human immunodeficiency virus type 1 on infectiousness of tuberculosis: A meta-analysis. *Clin Infect Dis.* 2001;**33**:1922-1930.
48. Escombe AR, Moore DA, Gilman RH, et al. The infectiousness of tuberculosis patients coinfected with HIV. *PLoS Med.* 2008;**5**:e188.
49. Lienhardt C, Fielding K, Sillah J, et al. Risk factors for tuberculosis infection in sub-Saharan Africa: a contact study in The Gambia. *Am J Respir Crit Care Med.* 2003;**168**:448-455.
50. Lienhardt C, Sillah J, Fielding K, et al. Risk factors for tuberculosis infection in children in contact with infectious tuberculosis cases in the Gambia, West Africa. *Pediatrics.* 2003;**111**:e608-614.
51. Lienhardt C. From exposure to disease: the role of environmental factors in susceptibility to and development of tuberculosis. *Epidemiol Rev.* 2001;**23**:288-301.
52. Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: The effects of chemotherapy. *Tubercle.* 1976;**57**:275-299.
53. Andersen S, Geser A. The distribution of tuberculous infection among households in African communities. *Bull World Health Organ.* 1960;**22**:39-60.
54. Narain R, Nair SS, Rao GR, Chandrasekhar P. Distribution of tuberculous infection and disease among households in a rural community. *Bull World Health Organ.* 1966;**34**:639-654.
55. den Boon S, Verver S, Marais BJ, et al. Association between passive smoking and infection with Mycobacterium tuberculosis in children. *Pediatrics.* 2007;**119**:734-739.
56. Wood R, Johnstone-Robertson S, Uys P, et al. Tuberculosis transmission to young children in a South African community: modeling household and community infection risks. *Clin Infect Dis.* 2010;**51**:401-408.
57. Loudon RG, Spohn SK. Cough frequency and infectivity in patients with pulmonary tuberculosis. *Am Rev Respir Dis.* 1969;**99**:109-111.
58. Schaaf HS, Shean K, Donald PR. Culture-confirmed multidrug-resistant tuberculosis: diagnostic delay, clinical features, and outcome. *Arch Dis Child.* 2003;**88**:1106-1111.
59. Nathanson E, Lambregts-van Weezenbeek C, Rich ML, et al. Multidrug-resistant tuberculosis management in resource-limited settings. *Emerg Infect Dis.* 2006;**12**:1389-1397.
60. Marais BJ, Gie RP, Schaaf HS, et al. The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis.* 2004;**8**:278-285.
61. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis.* 2004;**8**:392-402.
62. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med.* 1989;**320**:545-550.
63. Hesseling AC, Cotton MF, Jennings T, et al. High incidence of tuberculosis among HIV-infected infants: evidence from a South African population-based study highlights the need for improved tuberculosis control strategies. *Clin Infect Dis.* 2009;**48**:108-114.
64. Schaaf HS, Van Rie A, Gie RP, et al. Transmission of multidrug-resistant tuberculosis. *Pediatr Infect Dis J.* 2000;**19**:695-699.
65. Kritski AL, Marques MJ, Rabahi MF, et al. Transmission of tuberculosis to close contacts of patients with multidrug-resistant tuberculosis. *Am J Respir Crit Care Med.* 1996;**153**:331-335.
66. Schaaf HS, Michaelis IA, Richardson M, et al. Adult-to-child transmission of tuberculosis: household or community contact? *Int J Tuberc Lung Dis.* 2003;**7**:426-431.
67. Bayona J, Chavez-Pachas AM, Palacios E, Llaro K, Sapag R, Becerra MC. Contact investigations as a means of detection and timely treatment of persons with infectious multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2003;**7**:S501-509.
68. Steiner P, Rao M, Mitchell M, Steiner M. Primary drug-resistant tuberculosis in children. Correlation of drug-susceptibility patterns of matched patient and source case strains of Mycobacterium tuberculosis. *Am J Dis Child.* 1985;**139**:780-782.

69. Verver S, Warren RM, Munch Z, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet.* 2004;**363**:212-214.
70. Madico G, Gilman RH, Checkley W, et al. Community infection ratio as an indicator for tuberculosis control. *Lancet.* 1995;**345**:416-419.
71. van Rie A, Victor TC, Richardson M, et al. Reinfection and mixed infection cause changing Mycobacterium tuberculosis drug-resistance patterns. *Am J Respir Crit Care Med.* 2005;**172**:636-642.
72. Warren RM, Victor TC, Streicher EM, et al. Patients with active tuberculosis often have different strains in the same sputum specimen. *Am J Respir Crit Care Med.* 2004;**169**:610-614.
73. Wootton SH, Gonzalez BE, Pawlak R, et al. Epidemiology of pediatric tuberculosis using traditional and molecular techniques: Houston, Texas. *Pediatrics.* 2005;**116**:1141-1147.
74. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. *Int J Tuberc Lung Dis.* 2009;**13**:1320-1330.
75. Caws M, Thwaites G, Stepniewska K, et al. Beijing genotype of Mycobacterium tuberculosis is significantly associated with human immunodeficiency virus infection and multidrug resistance in cases of tuberculous meningitis. *J Clin Microbiol.* 2006;**44**:3934-3939.
76. Maree F, Hesseling AC, Schaaf HS, et al. Absence of an association between Mycobacterium tuberculosis genotype and clinical features in children with tuberculous meningitis. *Pediatr Infect Dis J.* 2007;**26**:13-18.
77. Hesseling AC, Marais BJ, Kirchner HL, et al. Mycobacterial genotype is associated with disease phenotype in children. *Int J Tuberc Lung Dis.* 2010;**14**:1252-1258.
78. Kong Y, Cave MD, Zhang L, et al. Association between Mycobacterium tuberculosis Beijing/W lineage strain infection and extrathoracic tuberculosis: Insights from epidemiologic and clinical characterization of the three principal genetic groups of M. tuberculosis clinical isolates. *J Clin Microbiol.* 2007;**45**:409-414.
79. Johnson R, Warren R, Strauss OJ, et al. An outbreak of drug-resistant tuberculosis caused by a Beijing strain in the western Cape, South Africa. *Int J Tuberc Lung Dis.* 2006;**10**:1412-1414.
80. Marais BJ, Victor TC, Hesseling AC, et al. Beijing and Haarlem genotypes are overrepresented among children with drug-resistant tuberculosis in the Western Cape Province of South Africa. *J Clin Microbiol.* 2006;**44**:3539-3543.
81. Nodieva A, Jansone I, Broka L, Pole I, Skenders G, Baumanis V. Recent nosocomial transmission and genotypes of multidrug-resistant Mycobacterium tuberculosis. *Int J Tuberc Lung Dis.* 2010;**14**:427-433.
82. Marais BJ, Gie RP, Hesseling AH, Beyers N. Adult-type pulmonary tuberculosis in children 10-14 years of age. *Pediatr Infect Dis J.* 2005;**24**:743-744.
83. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc.* 1970;**26**:28-106.
84. Gray DM, Zar H, Cotton M. Impact of tuberculosis preventive therapy on tuberculosis and mortality in HIV-infected children. *Cochrane Database Syst Rev.* 2009:CD006418.
85. Smieja MJ, Marchetti CA, Cook DJ, Smaill FM. Isoniazid for preventing tuberculosis in non-HIV infected persons. *Cochrane Database Syst Rev.* 2000:CD001363.
86. Hsu KH. Thirty years after isoniazid. Its impact on tuberculosis in children and adolescents. *JAMA.* 1984;**251**:1283-1285.
87. Seddon JA, Godfrey-Faussett P, Hesseling AC, Gie RP, Beyers N, Schaaf HS. Management of children exposed to multidrug-resistant Mycobacterium tuberculosis. *Lancet Infect Dis.* 2012;**12**:469-479.
88. Sneag DB, Schaaf HS, Cotton MF, Zar HJ. Failure of chemoprophylaxis with standard antituberculosis agents in child contacts of multidrug-resistant tuberculosis cases. *Pediatr Infect Dis J.* 2007;**26**:1142-1146.
89. Tochon M, Bosdure E, Salles M, et al. Management of young children in contact with an adult with drug-resistant tuberculosis, France, 2004-2008. *Int J Tuberc Lung Dis.* 2011;**15**:326-330.
90. Schaaf HS, Victor TC, Engelke E, et al. Minimal inhibitory concentration of isoniazid in isoniazid-resistant Mycobacterium tuberculosis isolates from children. *Eur J Clin Microbiol Infect Dis.* 2007;**26**:203-205.
91. Schaaf HS, Victor TC, Venter A, et al. Ethionamide cross- and co-resistance in children with isoniazid-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2009;**13**:1355-1359.
92. Muller B, Streicher EM, Hoek KG, et al. inhA promoter mutations: a gateway to extensively drug-resistant tuberculosis in South Africa? *Int J Tuberc Lung Dis.* 2011;**15**:344-351.

93. Centres for Disease Control, Atlanta, Georgia, USA. Management of persons exposed to multidrug-resistant tuberculosis. *MMWR Recomm Rep.* 1992;**41**:61-71.
94. European Centre for Disease Prevention and Control. Management of contacts of MDR TB and XDR TB patients. Available at: http://ecdc.europa.eu/en/publications/Publications/201203-Guidance-MDR-TB-contacts.pdf (accessed 15 May 2012). 2012.
95. Graham SM, Gie RP, Schaaf HS, Coulter JB, Espinal MA, Beyers N. Childhood tuberculosis: clinical research needs. *Int J Tuberc Lung Dis.* 2004;**8**:648-657.
96. Cobelens FG, Heldal E, Kimerling ME, et al. Scaling up programmatic management of drug-resistant tuberculosis: a prioritized research agenda. *PLoS medicine.* 2008;**5**:e150.
97. Scano F, Vitoria M, Burman W, Harries AD, Gilks CF, Havlir D. Management of HIV-infected patients with MDR- and XDR-TB in resource-limited settings. *Int J Tuberc Lung Dis.* 2008;**12**:1370-1375.
98. RESIST-TB. Report on the RESIST-TB Protocol Workshop, 5-6 October. *Available at: http://www.resisttb.org/uploads/RESIST-TB_Protocol_Workshop_summary.pdf (accessed 29-5-11).* 2009.
99. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. *Clin Infect Dis.* 2006;**42**:e69-71.
100. World Health Organization, Geneva, Switzerland. Guidelines for the programmatic management of drug-resistant tuberculosis. 2011 update.**WHO/HTM/TB/2011.6**.
101. Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Enarson DA, Beyers N. Radiographic signs and symptoms in children treated for tuberculosis: possible implications for symptom-based screening in resource-limited settings. *Pediatr Infect Dis J.* 2006;**25**:237-240.
102. Schaaf HS, Marais BJ. Management of multidrug-resistant tuberculosis in children: a survival guide for paediatricians. *Paediatr Respir Rev.* 2011;**12**:31-38.
103. Al-Dabbagh M, Lapphra K, McGloin R, et al. Drug-resistant tuberculosis: pediatric guidelines. *Pediatr Infect Dis J.* 2011;**30**:501-505.
104. Burman WJ, Cotton MF, Gibb DM, Walker AS, Vernon AA, Donald PR. Ensuring the involvement of children in the evaluation of new tuberculosis treatment regimens. *PLoS Med.* 2008;**5**:e176.
105. Schaaf HS, Willemse M, Cilliers K, et al. Rifampin pharmacokinetics in children, with and without human immunodeficiency virus infection, hospitalized for the management of severe forms of tuberculosis. *BMC Med.* 2009;**7**:19.
106. Schaaf HS, Parkin DP, Seifart HI, et al. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. *Arch Dis Child.* 2005;**90**:614-618.
107. McIlleron H, Willemse M, Werely CJ, et al. Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines. *Clin Infect Dis.* 2009;**48**:1547-1553.
108. Holdiness MR. Clinical pharmacokinetics of the antituberculosis drugs. *Clin Pharmacokinet.* 1984;**9**:511-544.
109. Peloquin CA, Nitta AT, Burman WJ, et al. Low antituberculosis drug concentrations in patients with AIDS. *Ann Pharmacother.* 1996;**30**:919-925.
110. Graham SM, Bell DJ, Nyirongo S, Hartkoorn R, Ward SA, Molyneux EM. Low levels of pyrazinamide and ethambutol in children with tuberculosis and impact of age, nutritional status, and human immunodeficiency virus infection. *Antimicrob Agents Chemother.* 2006;**50**:407-413.
111. Roy V, Gupta D, Gupta P, Sethi GR, Mishra TK. Pharmacokinetics of isoniazid in moderately malnourished children with tuberculosis. *Int J Tuberc Lung Dis.* 2010;**14**:374-376.
112. Donald PR, Parkin DP, Seifart HI, et al. The influence of dose and N-acetyltransferase-2 (NAT2) genotype and phenotype on the pharmacokinetics and pharmacodynamics of isoniazid. *Eur J Clin Pharmacol.* 2007;**63**:633-639.
113. World Health Organization, Geneva, Switzerland. Rapid Advice. Treatment of tuberculosis in children. *WHO/HTM/TB/2010.13.* 2010.
114. Lonnroth K, Williams BG, Stadlin S, Jaramillo E, Dye C. Alcohol use as a risk factor for tuberculosis - a systematic review. *BMC Public Health.* 2008;**8**:289.
115. Franke MF, Appleton SC, Bayona J, et al. Risk factors and mortality associated with default from multidrug-resistant tuberculosis treatment. *Clin Infect Dis.* 2008;**46**:1844-1851.
116. Johnston JC, Shahidi NC, Sadatsafavi M, Fitzgerald JM. Treatment outcomes of multidrug-resistant tuberculosis: a systematic review and meta-analysis. *PLoS One.* 2009;**4**:e6914.
117. SouthAfrica.info. South Africa's population. Available at: http://www.southafrica.info/about/people/population.htm (accessed 20 August 2012). 2011.

118. South African Health Review 2010. Health and related indicators. Available at: http://www.hst.org.za/sites/default/files/sahr10_21.pdf (accessed 17 April 2012).
119. South African HIV & AIDS Statistics. Available at: http://www.avert.org/safricastats.htm (accessed 24 August 2011).
120. City of Cape Town - Census 2001 - Khayelitsha 2001. Available at: http://www.capetown.gov.za/en/stats/2001census/Documents/Khayelitsha.htm (accessed 15 May 2012).
121. Médecins Sans Frontières, Western Cape Province Department of Health, City of Cape Town Department of Health, University of Cape Town Centre for Infectious Disease Epidemiology and Research. Providing HIV/TB Care At The Primary Health Care Level. Khayelitsha Annual Activity Report 2008-2009. Available at: http://www.health-e.org.za/documents/c110af046ff02d6d4d032755b5ca7e06.pdf (accessed 22 July 2012).
122. Medecins Sans Frontieres. Scaling up diagnosis and treatment of drug-resistant tuberculosis in Khayelitsha, South Africa: an integrated, community-based approach. Available at http://www.msf.org.za/publication/scaling-diagnosis-and-treatment-drug-resistant-tuberculosis-khayelitsha-south-africa (accessed on 5.9.11). 2011.
123. Blumberg HM, Leonard MK, Jr., Jasmer RM. Update on the treatment of tuberculosis and latent tuberculosis infection. *JAMA.* 2005;**293**:2776-2784.
124. Leung CC, Rieder HL, Lange C, Yew WW. Treatment of latent infection with m. tuberculosis: update 2010. *Eur Respir J.* 2010.
125. World Health Organization, Geneva, Switzerland. Chapter 4: childhood contact screening and management. *Int J Tuberc Lung Dis.* 2007;**11**:12-15.
126. Halsey NA, Coberly JS, Desormeaux J, et al. Randomised trial of isoniazid versus rifampicin and pyrazinamide for prevention of tuberculosis in HIV-1 infection. *Lancet.* 1998;**351**:786-792.
127. Mwinga A, Hosp M, Godfrey-Faussett P, et al. Twice weekly tuberculosis preventive therapy in HIV infection in Zambia. *AIDS.* 1998;**12**:2447-2457.
128. Ormerod LP. Reduced incidence of tuberculosis by prophylactic chemotherapy in subjects showing strong reactions to tuberculin testing. *Arch Dis Child.* 1987;**62**:1005-1008.
129. Hong Kong Chest Service, Tuberculosis Research Centre Madras, British Medical Research Council. A double-blind placebo-controlled clinical trial of three antituberculosis chemoprophylaxis regimens in patients with silicosis in Hong Kong. . *Am Rev Respir Dis.* 1992;**145**:36-41.
130. CDC/ATA/IDSA. Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med.* 2000;**161**:S221-247.
131. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. International Union Against Tuberculosis Committee on Prophylaxis. *Bull World Health Organ.* 1982;**60**:555-564.
132. Zar HJ, Cotton MF, Strauss S, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ.* 2007;**334**:136.
133. Madhi SA, Nachman S, Violari A, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med.* 2011;**365**:21-31.
134. World Health Organization, Geneva, Switzerland. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource constrained settings. *ISBN 978 92 4 150070 8.* 2010.
135. Fraser A, Paul M, Attamna A, Leibovici L. Drugs for preventing tuberculosis in people at risk of multiple-drug-resistant pulmonary tuberculosis. *Cochrane database of systematic reviews (Online).* 2006:CD005435.
136. Bamrah S, Brostrom R, Setik L, Fred D, Kawamura M, Mase S. An ounce of prevention: treating MDR-TB contacts in a resource-limited setting. *Abstract. International Union of Tuberculosis and Lung Disease Conference, Berlin, Germany 11-15 November.* 2010;**FA-1--656-14**:S180.
137. National Institute for Health and Clinical Excellence. Tuberculosis. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. *NICE clinical guideline 117.* 2011.
138. Department of Health, Republic of South Africa. Management of Drug-resistant Tuberculosis. Policy Guidelines. 2010.

139. Passannante MR, Gallagher CT, Reichman LB. Preventive therapy for contacts of multidrug-resistant tuberculosis. A Delphi survey. *Chest.* 1994;**106**:431-434.
140. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, ed. Red Book: Report of the Committee on Infectious Diseases. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics. 2009:680-701.
141. Partners in Health. The Partners in Health Guide to the Medical Management of Multidrug-Resistant Tuberculosis. 2003.
142. Francis J Curry National Tuberculosis Center. Drug-Resistant Tuberculosis: A Survival Guide for Clinicians, Second Edition. 2008.
143. Attamna A, Chemtob D, Attamna S, et al. Risk of tuberculosis in close contacts of patients with multidrug resistant tuberculosis: A nationwide cohort. *Thorax.* 2009;**64**:271.
144. Feja K, McNelley E, Tran CS, Burzynski J, Saiman L. Management of pediatric multidrug-resistant tuberculosis and latent tuberculosis infections in New York City from 1995 to 2003. *Pediatr Infect Dis J.* 2008;**27**:907-912.
145. Sasaki Y, Yamagishi F, Yagi T. The current situation of contacts examination and chemoprophylaxis for persons exposed to multi-drug resistant tuberculosis in ordinance-designated cities in Japan. *Kekkaku.* 2005;**80**:637-642.
146. Papastavros T, Dolovich LR, Holbrook A, Whitehead L, Loeb M. Adverse events associated with pyrazinamide and levofloxacin in the treatment of latent multidrug-resistant tuberculosis. *CMAJ.* 2002;**167**:131-136.
147. Cain KP, Nelson LJ, Cegielski JP. Global policies and practices for managing persons exposed to multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2010;**14**:269-274.
148. Pineiro Perez R, Mellado Pena MJ, Mendez Echevarria A, et al. Exposure to multiresisant tuberculosis: Study and follow-up of nine children. *Anales de Pediatria.* 2008;**68**:490-495.
149. Chopra I, Brennan P. Molecular action of anti-mycobacterial agents. *Tuber Lung Dis.* 1997;**78**:89-98.
150. Loebstein R, Koren G. Clinical pharmacology and therapeutic drug monitoring in neonates and children. *Pediatr Rev.* 1998;**19**:423-428.
151. Rey E, Gendrel D, Treluyer JM, et al. Isoniazid pharmacokinetics in children according to acetylator phenotype. *Fundam Clin Pharmacol.* 2001;**15**:355-359.
152. Sirgel FA, Donald PR, Odhiambo J, et al. A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. *J Antimicrob Chemother.* 2000;**45**:859-870.
153. Balcells ME, Thomas SL, Godfrey-Faussett P, Grant AD. Isoniazid preventive therapy and risk for resistant tuberculosis. *Emerg Infect Dis.* 2006;**12**:744-751.
154. Banerjee A, Dubnau E, Quemard A, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. *Science.* 1994;**263**:227-230.
155. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. *Int J Tuberc Lung Dis.* 2000;**4**:796-806.
156. Ijaz K, Jereb JA, Lambert LA, et al. Severe or fatal liver injury in 50 patients in the United States taking rifampin and pyrazinamide for latent tuberculosis infection. *Clin Infect Dis.* 2006;**42**:346-355.
157. Younossian AB, Rochat T, Ketterer JP, Wacker J, Janssens JP. High hepatotoxicity of pyrazinamide and ethambutol for treatment of latent tuberculosis. *Eur Respir J.* 2005;**26**:462-464.
158. American Thoracic Society/Centres for Disease Control. Update: adverse event data and revised American Thoracic Society/CDC recommendations against the use of rifampin and pyrazinamide for treatment of latent tuberculosis infection--United States, 2003. *MMWR.* 2003;**52**:735-739.
159. le Bourgeois M, de Blic J, Paupe J, Scheinmann P. Good tolerance of pyrazinamide in children with pulmonary tuberculosis. *Arch Dis Child.* 1989;**64**:177-178.
160. Sanchez-Albisua I, Vidal ML, Joya-Verde G, del Castillo F, de Jose MI, Garcia-Hortelano J. Tolerance of pyrazinamide in short course chemotherapy for pulmonary tuberculosis in children. *Pediatr Infect Dis J.* 1997;**16**:760-763.
161. Hoek KG, Schaaf HS, Gey van Pittius NC, van Helden PD, Warren RM. Resistance to pyrazinamide and ethambutol compromises MDR/XDR-TB treatment. *S Afr Med J.* 2009;**99**:785-787.
162. Mphahlele M, Syre H, Valvatne H, et al. Pyrazinamide resistance among South African multidrug-resistant Mycobacterium tuberculosis isolates. *Journal of clinical microbiology.* 2008;**46**:3459-3464.

163. Yu MC, Suo J, Lin TP, Luh KT. In vitro activity of ofloxacin against Mycobacterium tuberculosis. *J Formos Med Assoc.* 1997;**96**:13-16.

164. Ingham B, Brentnall D, Dale E, McFadzean J. Arthropathy induced by antibacerial fused N-Alkyl-4-pyridone-3-carboxylic acids. *Toxicology Letters.* 1977;**1**:21-26.

165. Burkhardt JE, Walterspiel JN, Schaad UB. Quinolone arthropathy in animals versus children. *Clin Infect Dis.* 1997;**25**:1196-1204.

166. Ginsburg AS, Grosset JH, Bishai WR. Fluoroquinolones, tuberculosis, and resistance. *Lancet Infect Dis.* 2003;**3**:432-442.

167. World Health Organization, Geneva, Switzerland. Ethambutol efficacy and toxicity: literature review and recommendations for daily and intermittent dosage in children. *(WHO/HTM/TB/2006.365; WHO/FCH/CAH/2006.3).* 2006.

168. Victor TC, Warren R, Butt JL, et al. Genome and MIC stability in Mycobacterium tuberculosis and indications for continuation of use of isoniazid in multidrug-resistant tuberculosis. *J Med Microbiol.* 1997;**46**:847-857.

169. Diacon AH, Pym A, Grobusch M, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med.* 2009;**360**:2397-2405.

170. Stevens JP, Daniel TM. Chemoprophylaxis of multidrug-resistant tuberculous infection in HIV-uninfected individuals using ciprofloxacin and pyrazinamide. A decision analysis. *Chest.* 1995;**108**:712-717.

171. Parsyan AE, Saukkonen J, Barry MA, Sharnprapai S, Horsburgh CR, Jr. Predictors of failure to complete treatment for latent tuberculosis infection. *J Infect.* 2007;**54**:262-266.

172. Bock NN, Metzger BS, Tapia JR, Blumberg HM. A tuberculin screening and isoniazid preventive therapy program in an inner-city population. *Am J Respir Crit Care Med.* 1999;**159**:295-300.

173. van Zyl S, Marais BJ, Hesseling AC, Gie RP, Beyers N, Schaaf HS. Adherence to anti-tuberculosis chemoprophylaxis and treatment in children. *Int J Tuberc Lung Dis.* 2006;**10**:13-18.

174. Barker RD, Millard FJ, Nthangeni ME. Unpaid community volunteers--effective providers of directly observed therapy (DOT) in rural South Africa. *S Afr Med J.* 2002;**92**:291-294.

175. Economist. Taken your medicine? *The Economist Technology Quarterly (June 6th).* 2009:7-8.

176. Marais BJ, van Zyl S, Schaaf HS, van Aardt M, Gie RP, Beyers N. Adherence to isoniazid preventive chemotherapy: a prospective community based study. *Arch Dis Child.* 2006;**91**:762-765.

177. Coyne KM, Pozniak AL, Lamorde M, Boffito M. Pharmacology of second-line antituberculosis drugs and potential for interactions with antiretroviral agents. *AIDS.* 2009;**23**:437-446.

178. Peloquin CA, Berning SE, Huitt GA, Iseman MD. AIDS and TB drug absorption. *Int J Tuberc Lung Dis.* 1999;**3**:1143-1144.

179. World Health Organization, Geneva, Switzerland. Antiretroviral therapy of HIV infection in infants and children: towards universal access. Recommendations for a public health approach. 2006.

180. Welch SB, Gibb D. When should children with HIV infection be started on antiretroviral therapy? *PLoS Med.* 2008;**5**:e73.

181. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med.* 2008;**359**:2233-2244.

182. World Health Organization, Geneva, Switzerland. Guidelines on co-trimoxazole prophylaxis for HIV-related infections among children, adolescents and adults. Recommendations for a public health approach. 2006

183. International Network of the Study of HIV-associated IRIS. Case definition: consensus criteria for diagnosis of paediatric TB IRIS. 2008 (http://www.inshi.umn.edu/definitions/Peds_TB_IRIS/home.html - accessed on 29.5.11).

184. Boulware DR, Callens S, Pahwa S. Pediatric HIV immune reconstitution inflammatory syndrome. *Curr Opin HIV AIDS.* 2008;**3**:461-467.

185. Zampoli M, Kilborn T, Eley B. Tuberculosis during early antiretroviral-induced immune reconstitution in HIV-infected children. *Int J Tuberc Lung Dis.* 2007;**11**:417-423.

186. Muller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect Dis.* 2010;**10**:251-261.

187. Becerra MC, Appleton SC, Franke MF, et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet.* 2011;**377**:147-152.

188. Resch SC, Salomon JA, Murray M, Weinstein MC. Cost-effectiveness of treating multidrug-resistant tuberculosis. *PLoS Med.* 2006;**3**:e241.
189. Tupasi TE, Gupta R, Quelapio MI, et al. Feasibility and cost-effectiveness of treating multidrug-resistant tuberculosis: a cohort study in the Philippines. *PLoS Med.* 2006;**3**:e352.
190. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *Lancet Infect Dis.* 2009;**9**:153-161.
191. Fairlie L, Beylis NC, Reubenson G, Moore DP, Madhi SA. High prevalence of childhood multi-drug resistant tuberculosis in Johannesburg, South Africa: a cross sectional study. *BMC infectious diseases.* 2011;**11**:28.
192. Seddon JA, Hesseling AC, Willemse M, Donald PR, Schaaf HS. Culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment, and outcome. *Clin Infect Dis.* 2012;**54**:157-166.
193. Padayatchi N, Bamber S, Dawood H, Bobat R. Multidrug-resistant tuberculous meningitis in children in Durban, South Africa. *Pediatr Infect Dis J.* 2006;**25**:147-150.
194. Schluger NW, Lawrence RM, McGuiness G, Park M, Rom WN. Multidrug-resistant tuberculosis in children: two cases and a review of the literature. *Pediatr Pulmonol.* 1996;**21**:138-142.
195. Suessmuth S, Bange FC, Gappa M. Multidrug resistant tuberculosis in a 6 year old child. *Paediatr Respir Rev.* 2007;**8**:265-268.
196. Kjollerstrom P, Brito MJ, Gouveia C, Ferreira G, Varandas L. Linezolid in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis in paediatric patients: Experience of a paediatric infectious diseases unit. *Scand J Infect Dis.* 2011.
197. World Health Organization, Geneva, Switzerland. Guidlelines for the programmatic management of drug-resistant tuberculosis - Emergency update. *WHO/HTM/TB/2008.402.* 2008.
198. Wright CA, Hesseling AC, Bamford C, Burgess SM, Warren R, Marais BJ. Fine-needle aspiration biopsy: a first-line diagnostic procedure in paediatric tuberculosis suspects with peripheral lymphadenopathy? *Int J Tuberc Lung Dis.* 2009;**13**:1373-1379.
199. Oberhelman RA, Soto-Castellares G, Gilman RH, et al. Diagnostic approaches for paediatric tuberculosis by use of different specimen types, culture methods, and PCR: a prospective case-control study. *Lancet Infect Dis.* 2010;**10**:612-620.
200. Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. *Clin Infect Dis.* 2012.
201. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. *Paediatr Respir Rev.* 2011;**12**:16-21.
202. Goussard P, Gie RP, Kling S, et al. The diagnostic value and safety of transbronchial needle aspiration biopsy in children with mediastinal lymphadenopathy. *Pediatr Pulmonol.* 2010;**45**:1173-1179.
203. Seddon JA, Hesseling AC, Marais BJ, et al. Paediatric use of second-line anti-tuberculosis agents: A review. *Tuberculosis (Edinb).* 2012;**92**:9-17.
204. Drobac PC, Mukherjee JS, Joseph JK, et al. Community-based therapy for children with multidrug-resistant tuberculosis. *Pediatrics.* 2006;**117**:2022-2029.
205. Mendez Echevarria A, Baquero Artigao F, Garcia Miguel MJ, et al. Multidrug-resistant tuberculosis in the pediatric age group. *Anales de Pediatria.* 2007;**67**:206-211.
206. Swanson DS, Starke JR. Drug-resistant tuberculosis in pediatrics. *Pediatr Clin North Am.* 1995;**42**:553-581.
207. Ren Y, Nuttall JJ, Egbers C, et al. Effect of rifampicin on lopinavir pharmacokinetics in HIV-infected children with tuberculosis. *J Acquir Immune Defic Syndr.* 2008;**47**:566-569.
208. Louw GE, Warren RM, Gey van Pittius NC, et al. Rifampicin Reduces Susceptibility to Ofloxacin in Rifampicin-resistant Mycobacterium tuberculosis through Efflux. *Am J Respir Crit Care Med.* 2011;**184**:269-276.
209. Weiner M, Burman W, Luo CC, et al. Effects of rifampin and multidrug resistance gene polymorphism on concentrations of moxifloxacin. *Antimicrob Agents Chemother.* 2007;**51**:2861-2866.
210. Frydenberg AR, Graham SM. Toxicity of first-line drugs for treatment of tuberculosis in children: review. *Trop Med Int Health.* 2009;**14**:1329-1337.
211. Johnson R, Streicher EM, Louw GE, Warren RM, van Helden PD, Victor TC. Drug resistance in Mycobacterium tuberculosis. *Curr Issues Mol Biol.* 2006;**8**:97-111.
212. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB. Tuberculosis drug resistance mutation database. *PLoS Med.* 2009;**6**:e2.

213. Hu Y, Coates AR, Mitchison DA. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of Mycobacterium tuberculosis. *Antimicrob Agents Chemother.* 2003;**47**:653-657.
214. Handbook of Anti-Tuberculosis Agents. *Tuberculosis (Edinb).* 2008;**88**:1-169.
215. Rodriguez JC, Cebrian L, Lopez M, Ruiz M, Jimenez I, Royo G. Mutant prevention concentration: comparison of fluoroquinolones and linezolid with Mycobacterium tuberculosis. *J Antimicrob Chemother.* 2004;**53**:441-444.
216. Cox H, Ford N. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis [Review article]. *Int J Tuberc Lung Dis.* 2012.
217. Van Deun A, Maug AK, Salim MA, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. *Am J Respir Crit Care Med.* 2010;**182**:684-692.
218. Gler MT, Skripconoka V, Sanchez-Garavito E, et al. Delamanid for multidrug-resistant pulmonary tuberculosis. *N Engl J Med.* 2012;**366**:2151-2160.
219. Diacon AH, Dawson R, du Bois J, et al. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrob Agents Chemother.* 2012;**56**:3027-3031.
220. Diacon AH, Donald PR, Pym A, et al. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob Agents Chemother.* 2012;**56**:3271-3276.
221. Ettehad D, Schaaf HS, Seddon JA, Cooke GS, Ford N. Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012;**12**:449-456.
222. Leimane V, Ozere I. Challengest of managing a child with MDR-TB: 41st World Conference on Lung Health of the International Union Against Tuberculosis and Lung Diseae (The Union), Cancun, Mexico, 3-7 December. 2009.
223. Schaaf HS, Seddon JA, Caminero JA. Second-line antituberclosis drugs: current knowledge and controversies. *Prog Respir Res.* 2011;**40**:81-95.
224. Steiner P, Rao M, Victoria M, Steiner M. Primary isoniazid-resistant tuberculosis in children. Clinical features, strain resistance, treatment, and outcome in 26 children treated at Kings County Medical Center of Brooklyn between the years 1961 and 1972. *Am Rev Respir Dis.* 1974;**110**:306-311.
225. Dramowski A, Morsheimer MM, Jordaan AM, Victor TC, Donald PR, Schaaf HS. Rifampicin-monoresistant Mycobacterium tuberculosis disease among children in Cape Town, South Africa. *Int J Tuberc Lung Dis.* 2012;**16**:76-81.
226. Hazbon MH, Brimacombe M, Bobadilla del Valle M, et al. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. *Antimicrob Agents Chemother.* 2006;**50**:2640-2649.
227. Cilliers K, Labadarios D, Schaaf HS, et al. Pyridoxal-5-phosphate plasma concentrations in children receiving tuberculosis chemotherapy including isoniazid. *Acta Paediatr.* 2010;**99**:705-710.
228. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect.* 2009;**59**:167-187.
229. Prasad K, Singh MB. Corticosteroids for managing tuberculous meningitis. *Cochrane Database Syst Rev.* 2008:CD002244.
230. Schoeman JF, Van Zyl LE, Laubscher JA, Donald PR. Effect of corticosteroids on intracranial pressure, computed tomographic findings, and clinical outcome in young children with tuberculous meningitis. *Pediatrics.* 1997;**99**:226-231.
231. Goussard P, Gie R. Airway involvement in pulmonary tuberculosis. *Paediatr Respir Rev.* 2007;**8**:118-123.
232. du Plessis J, Goussard P, Andronikou S, Gie R, George R. Comparing three-dimensional volume-rendered CT images with fibreoptic tracheobronchoscopy in the evaluation of airway compression caused by tuberculous lymphadenopathy in children. *Pediatr Radiol.* 2009;**39**:694-702.
233. Maydell A, Goussard P, Andronikou S, Bezuidenhout F, Ackermann C, Gie R. Radiological changes post-lymph node enucleation for airway obstruction in children with pulmonary tuberculosis. *Eur J Cardiothorac Surg.* 2010;**38**:478-483.
234. Webb EA, Hesseling AC, Schaaf HS, et al. High prevalence of Mycobacterium tuberculosis infection and disease in children and adolescents with type 1 diabetes mellitus. *Int J Tuberc Lung Dis.* 2009;**13**:868-874.

235. World Health Organization, Geneva, Switzerland. Antiretroviral therapy for HIV infection in infancts and children: towards universal access. Recommendations for a public health approach. . 2010.
236. Torok ME, Yen NT, Chau TT, et al. Timing of Initiation of Antiretroviral Therapy in Human Immunodeficiency Virus (HIV)-Associated Tuberculous Meningitis. *Clin Infect Dis.* 2011;**52**:1374-1383.
237. Collins S, Dent N, Binns P, Bahwere P, Sadler K, Hallam A. Management of severe acute malnutrition in children. *Lancet.* 2006;**368**:1992-2000.
238. Pawar UM, Kundnani V, Agashe V, Nene A. Multidrug-resistant tuberculosis of the spine--is it the beginning of the end? A study of twenty-five culture proven multidrug-resistant tuberculosis spine patients. *Spine.* 2009;**34**:E806-810.
239. Seddon JA, Donald PR, Vlok GJ, Schaaf HS. Multidrug-Resistant Tuberculosis of the Spine in Children - Characteristics from a High Burden Setting. *J Trop Pediatr.* 2011.
240. Seddon JA, Visser DH, Bartens M, et al. Impact of drug resistance on clinical outcome in children with tuberculous meningitis. *Pediatr Infect Dis J.* 2012;**31**:711-716.
241. McCracken GH, Jr. Aminoglycoside toxicity in infants and children. *Am J Med.* 1986;**80**:172-178.
242. Stelmachowicz PG, Pittman AL, Hoover BM, Lewis DE, Moeller MP. The importance of high-frequency audibility in the speech and language development of children with hearing loss. *Arch Otolaryngol Head Neck Surg.* 2004;**130**:556-562.
243. Livingstone N, McPhillips M. Motor skill deficits in children with partial hearing. *Dev Med Child Neurol.* 2011.
244. Seddon JA, Godfrey-Faussett P, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in patients on treatment for drug-resistant tuberculosis Jun 27. [Epub ahead of print]. *Eur Respir J.* 2012.
245. Seddon JA, Thee S, Hesseling AC, Schaaf HS. Hearing and renal impairment in children treated for drug-resistant tuberculosis (abstract). *43rd Union World Conference on Lung Health, 13-17 November 2012, Kuala Lumpur, Malaysia.* 2012.
246. Dubo S. Psychiatric study of children with pulmonary tuberculosis. *Am J Orthopsychiatry.* 1950;**20**:520-528.
247. World Health Organization, Geneva, Switzerland. 2009 WHO child growth standards and the identification of severe acute malnutrition in infants and children: A Joint Statement by the World Health Organization and the United Nations Children's Fund. Available at: http://www.who.int/nutrition/publications/severemalnutrition/9789241598163_eng.pdf (accessed 11 July 2012).
248. American Speech-Language-Hearing Association. Audiologic Management of Inidividuals Receiving Cochleotoxic Drug Therapy (Guideline). 1994;**Available from http://www.asha.org/docs/pdf/GL1994-00003.pdf (accessed August 2012).**
249. Donald PR, Maher D, Maritz JS, Qazi S. Ethambutol dosage for the treatment of children: literature review and recommendations. *Int J Tuberc Lung Dis.* 2006;**10**:1318-1330.
250. Garazzino S, Tovo PA. Clinical experience with linezolid in infants and children. *J Antimicrob Chemother.* 2011;**66 Suppl 4**:iv23-iv41.
251. Clarke M, Dick J, Zwarenstein M, Lombard CJ, Diwan VK. Lay health worker intervention with choice of DOT superior to standard TB care for farm dwellers in South Africa: a cluster randomised control trial. *Int J Tuberc Lung Dis.* 2005;**9**:673-679.
252. Farmer P, Kim JY. Community based approaches to the control of multidrug resistant tuberculosis: introducing "DOTS-plus". *BMJ.* 1998;**317**:671-674.
253. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR. The clinical and radiological features of tuberculosis in adolescents. *Annals of tropical paediatrics.* 2000;**20**:5-10.
254. Schaaf HS, Marais BJ, Hesseling AC, Brittle W, Donald PR. Surveillance of antituberculosis drug resistance among children from the Western Cape Province of South Africa--an upward trend. *Am J Public Health.* 2009;**99**:1486-1490.
255. Schaaf HS, Marais BJ, Hesseling AC, Gie RP, Beyers N, Donald PR. Childhood drug-resistant tuberculosis in the Western Cape Province of South Africa. *Acta Paediatr.* 2006;**95**:523-528.
256. Matlow A, Robb M, Goldman C. Infection control and paediatric tuberculosis: A practical guide for the practicing paediatrician. *Paediatr Child Health.* 2003;**8**:624-626.
257. Centers for Disease Control and Prevention. Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings, 2005. MMWR 2005;54(No. RR-17).
258. World Health Organization, Geneva, Switzerland. WHO policy on TB infection control in health-care facilities, congregate settings and households. Available at:

http://whqlibdoc.who.int/publications/2009/9789241598323_eng.pdf (accessed 12 July 2012). 2009;**WHO/HTM/TB/2009.419**.

259. Granich RM, Oh P, Lewis B, Porco TC, Flood J. Multidrug resistance among persons with tuberculosis in California, 1994-2003. *JAMA.* 2005;**293**:2732-2739.

260. Pinon M, Scolfaro C, Bignamini E, et al. Two pediatric cases of multidrug-resistant tuberculosis treated with linezolid and moxifloxacin. *Pediatrics.* 2010;**126**:e1253-1256.

261. Smith KC, Seaworth BJ. Drug-resistant tuberculosis: controversies and challenges in pediatrics. *Expert review of anti-infective therapy.* 2005;**3**:995-1010.

262. Thee S, Zollner EW, Willemse M, C. HA, Magdorf K, Schaaf HS. Abnormal thyroid function tests in children on ethionamide treatement (short communication). *Int J Tuberc Lung Dis.* 2011;**15**:1191-1193.

263. van der Watt JJ, Harrison TB, Benatar M, Heckmann JM. Polyneuropathy, anti-tuberculosis treatment and the role of pyridoxine in the HIV/AIDS era: a systematic review. *Int J Tuberc Lung Dis.* 2011;**15**:722-728.

264. Vega P, Sweetland A, Acha J, et al. Psychiatric issues in the management of patients with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2004;**8**:749-759.

265. Ziganshina LE, Squire SB. Fluoroquinolones for treating tuberculosis. *Cochrane Database Syst Rev.* 2008.

266. Meissner HC, Townsend T, Wenman W, et al. Hematologic effects of linezolid in young children. *Pediatr Infect Dis J.* 2003;**22**:S186-192.

267. Allen BW, Mitchison DA, Chan YC, Yew WW, Allan WG, Girling DJ. Amikacin in the treatment of pulmonary tuberculosis. *Tubercle.* 1983;**64**:111-118.

268. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. *Lancet Infect Dis.* 2010;**10**:621-629.

269. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. *Antimicrob Agents Chemother.* 2005;**49**:3192-3197.

270. Jureen P, Angeby K, Sturegard E, et al. Wild-type MIC distributions for aminoglycoside and cyclic polypeptide antibiotics used for treatment of Mycobacterium tuberculosis infections. *J Clin Microbiol.* 2010;**48**:1853-1858.

271. Rastogi N, Labrousse V, Goh KS. In vitro activities of fourteen antimicrobial agents against drug susceptible and resistant clinical isolates of Mycobacterium tuberculosis and comparative intracellular activities against the virulent H37Rv strain in human macrophages. *Curr Microbiol.* 1996;**33**:167-175.

272. Treluyer JM, Merle Y, Tonnelier S, Rey E, Pons G. Nonparametric population pharmacokinetic analysis of amikacin in neonates, infants, and children. *Antimicrob Agents Chemother.* 2002;**46**:1381-1387.

273. Peloquin CA, Berning SE, Nitta AT, et al. Aminoglycoside toxicity: daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clin Infect Dis.* 2004;**38**:1538-1544.

274. Kam KM, Yip CW, Cheung TL, Tang HS, Leung OC, Chan MY. Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: correlation with ofloxacin susceptibility. *Microb Drug Resist.* 2006;**12**:7-11.

275. Schaefer HG, Stass H, Wedgwood J, et al. Pharmacokinetics of ciprofloxacin in pediatric cystic fibrosis patients. *Antimicrob Agents Chemother.* 1996;**40**:29-34.

276. Peltola H, Vaarala M, Renkonen OV, Neuvonen PJ. Pharmacokinetics of single-dose oral ciprofloxacin in infants and small children. *Antimicrob Agents Chemother.* 1992;**36**:1086-1090.

277. Bethell DB, Day NP, Dung NM, et al. Pharmacokinetics of oral and intravenous ofloxacin in children with multidrug-resistant typhoid fever. *Antimicrob Agents Chemother.* 1996;**40**:2167-2172.

278. Gendrel D, Chalumeau M, Moulin F, Raymond J. Fluoroquinolones in paediatrics: a risk for the patient or for the community? *Lancet Infect Dis.* 2003;**3**:537-546.

279. Capparelli EV, Reed MD, Bradley JS, et al. Pharmacokinetics of gatifloxacin in infants and children. *Antimicrob Agents Chemother.* 2005;**49**:1106-1112.

280. Chien S, Wells TG, Blumer JL, et al. Levofloxacin pharmacokinetics in children. *J Clin Pharmacol.* 2005;**45**:153-160.

281. Johnson JL, Hadad DJ, Boom WH, et al. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis.* 2006;**10**:605-612.

282. Auclair B, Nix DE, Adam RD, James GT, Peloquin CA. Pharmacokinetics of ethionamide administered under fasting conditions or with orange juice, food, or antacids. *Antimicrob Agents Chemother.* 2001;**45**:810-814.

283. Dover LG, Corsino PE, Daniels IR, et al. Crystal structure of the TetR/CamR family repressor Mycobacterium tuberculosis EthR implicated in ethionamide resistance. *J Mol Biol.* 2004;**340**:1095-1105.

284. Zhu M, Namdar R, Stambaugh JJ, et al. Population pharmacokinetics of ethionamide in patients with tuberculosis. *Tuberculosis (Edinb).* 2002;**82**:91-96.

285. Eule H. [Ethionamide concentration in the blood and urine of healthy persons and lung patients]. *Beitr Klin Erforsch Tuberk Lungenkr.* 1965;**132**:339-342.

286. Donald PR, Seifart HI. Cerebrospinal fluid concentrations of ethionamide in children with tuberculous meningitis. *J Pediatr.* 1989;**115**:483-486.

287. Thee S, Seifart HI, Rosenkranz B, et al. Pharmacokinetics of ethionamide in children. *Antimicrob Agents Chemother.* 2011;**55**:4594-4600.

288. Vora A. Terizidone (Drug Review). *J Assoc Physician India.* 2010;**58**:267-268.

289. Zitkova L, Tousek J. Pharmacokinetics of cycloserine and terizidone. A comparative study. *Chemotherapy.* 1974;**20**:18-28.

290. Zhu M, Nix DE, Adam RD, Childs JM, Peloquin CA. Pharmacokinetics of cycloserine under fasting conditions and with high-fat meal, orange juice, and antacids. *Pharmacotherapy.* 2001;**21**:891-897.

291. Nopponpunth V, Sirawaraporn W, Greene PJ, Santi DV. Cloning and expression of Mycobacterium tuberculosis and Mycobacterium leprae dihydropteroate synthase in Escherichia coli. *J Bacteriol.* 1999;**181**:6814-6821.

292. Peloquin CA, Berning SE, Huitt GA, Childs JM, Singleton MD, James GT. Once-daily and twice-daily dosing of p-aminosalicylic acid granules. *Am J Respir Crit Care Med.* 1999;**159**:932-934.

293. Soderhjelm L. Serum para-aminosalicylic acid following oral ingestion in children. *Tex Rep Biol Med.* 1949;**7**:471-479.

294. Clofazimine. *Tuberculosis (Edinb).* 2008;**88**:96-99.

295. Shen GH, Wu BD, Hu ST, Lin CF, Wu KM, Chen JH. High efficacy of clofazimine and its synergistic effect with amikacin against rapidly growing mycobacteria. *Int J Antimicrob Agents.* 2010;**35**:400-404.

296. Nix DE, Adam RD, Auclair B, Krueger TS, Godo PG, Peloquin CA. Pharmacokinetics and relative bioavailability of clofazimine in relation to food, orange juice and antacid. *Tuberculosis (Edinb).* 2004;**84**:365-373.

297. Jungbluth GL, Welshman IR, Hopkins NK. Linezolid pharmacokinetics in pediatric patients: an overview. *Pediatr Infect Dis J.* 2003;**22**:S153-157.

298. Chiappini E, Conti C, Galli L, de Martino M. Clinical efficacy and tolerability of linezolid in pediatric patients: a systematic review. *Clin Ther.* 2010;**32**:66-88.

299. Hachem R, Afif C, Gokaslan Z, Raad I. Successful treatment of vancomycin-resistant Enterococcus meningitis with linezolid. *Eur J Clin Microbiol Infect Dis.* 2001;**20**:432-434.

300. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med.* 2008;**178**:1180-1185.

301. Dauby N, Muylle I, Mouchet F, Sergysels R, Payen MC. Meropenem/Clavulanate and Linezolid Treatment for Extensively Drug-Resistant Tuberculosis. *Pediatr Infect Dis J.* 2011.

302. Schaaf HS, Willemse M, Donald PR. Long-term linezolid treatment in a young child with extensively drug-resistant tuberculosis. *Pediatr Infect Dis J.* 2009;**28**:748-750.

303. Ellard GA, Dickinson JM, Gammon PT, Mitchison DA. Serum concentrations and antituberculosis activity of thiacetazone. *Tubercle.* 1974;**55**:41-54.

304. Peloquin CA, Nitta AT, Berning SE, Iseman MD, James GT. Pharmacokinetic evaluation of thiacetazone. *Pharmacotherapy.* 1996;**16**:735-741.

305. Hugonnet JE, Tremblay LW, Boshoff HI, Barry CE, 3rd, Blanchard JS. Meropenem-clavulanate is effective against extensively drug-resistant Mycobacterium tuberculosis. *Science.* 2009;**323**:1215-1218.

306. Chambers HF, Moreau D, Yajko D, et al. Can penicillins and other beta-lactam antibiotics be used to treat tuberculosis? *Antimicrob Agents Chemother.* 1995;**39**:2620-2624.

307. Segura C, Salvado M, Collado I, Chaves J, Coira A. Contribution of beta-lactamases to beta-lactam susceptibilities of susceptible and multidrug-resistant Mycobacterium tuberculosis clinical isolates. *Antimicrob Agents Chemother.* 1998;**42**:1524-1526.

308. Abate G, Miorner H. Susceptibility of multidrug-resistant strains of Mycobacterium tuberculosis to amoxycillin in combination with clavulanic acid and ethambutol. *J Antimicrob Chemother.* 1998;**42**:735-740.

309. Gorzynski EA, Gutman SI, Allen W. Comparative antimycobacterial activities of difloxacin, temafloxacin, enoxacin, pefloxacin, reference fluoroquinolones, and a new macrolide, clarithromycin. *Antimicrob Agents Chemother.* 1989;**33**:591-592.

310. Luna-Herrera J, Reddy VM, Daneluzzi D, Gangadharam PR. Antituberculosis activity of clarithromycin. *Antimicrob Agents Chemother.* 1995;**39**:2692-2695.

311. Cavalieri SJ, Biehle JR, Sanders WE, Jr. Synergistic activities of clarithromycin and antituberculous drugs against multidrug-resistant Mycobacterium tuberculosis. *Antimicrob Agents Chemother.* 1995;**39**:1542-1545.

312. Mor N, Esfandiari A. Synergistic activities of clarithromycin and pyrazinamide against Mycobacterium tuberculosis in human macrophages. *Antimicrob Agents Chemother.* 1997;**41**:2035-2036.

313. Gan VN, Chu SY, Kusmiesz HT, Craft JC. Pharmacokinetics of a clarithromycin suspension in infants and children. *Antimicrob Agents Chemother.* 1992;**36**:2478-2480.

314. Donald PR. Childhood tuberculosis. *Curr Opin Pulm Med.* 2000;**6**:187-192.

315. Schaaf HS, Nelson LJ. Tuberculosis drug therapy in children. In: Schaaf HS, Zumla A, eds. *Tuberculosis. A comprehensive clinical reference*. Europe: Saunders Elsevier; 2009:627-638.

316. Donald PR, Schaaf HS. Old and new drugs for the treatment of tuberculosis in children. *Paediatr Respir Rev.* 2007;**8**:134-141.

317. World Health Organization, Geneva, Switzerland. Guidance for national tuberculosis programmes on the management of tuberculosis in children. Chapter 2: anti-tuberculosis treatment in children. *Int J Tuberc Lung Dis.* 2006;**10**:1205-1211.

318. Dheda K, Shean K, Zumla A, et al. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. *Lancet.* 2010;**375**:1798-1807.

319. Rao SC, Ahmed M, Hagan R. One dose per day compared to multiple doses per day of gentamicin for treatment of suspected or proven sepsis in neonates. *Cochrane Database Syst Rev.* 2006:CD005091.

320. Smyth AR, Bhatt J. Once-daily versus multiple-daily dosing with intravenous aminoglycosides for cystic fibrosis. *Cochrane Database Syst Rev.* 2010:CD002009.

321. Deutsch ES, Bartling V, Lawenda B, Schwegler J, Falkenstein K, Dunn S. Sensorineural hearing loss in children after liver transplantation. *Arch Otolaryngol Head Neck Surg.* 1998;**124**:529-533.

322. Selimoglu E. Aminoglycoside-induced ototoxicity. *Curr Pharm Des.* 2007;**13**:119-126.

323. Guthrie OW. Aminoglycoside induced ototoxicity. *Toxicology.* 2008;**249**:91-96.

324. Brummett RE, Fox KE. Aminoglycoside-induced hearing loss in humans. *Antimicrob Agents Chemother.* 1989;**33**:797-800.

325. Bardien S, de Jong G, Schaaf HS, Harris T, Fagan J, Petersen L. Aminoglycoside-induced hearing loss: South Africans at risk. *S Afr Med J.* 2009;**99**:440-441.

326. Bardien S, Human H, Harris T, et al. A rapid method for detection of five known mutations associated with aminoglycoside-induced deafness. *BMC Med Genet.* 2009;**10**:2.

327. Cortopassi G, Hutchin T. A molecular and cellular hypothesis for aminoglycoside-induced deafness. *Hear Res.* 1994;**78**:27-30.

328. Hutchin T, Haworth I, Higashi K, et al. A molecular basis for human hypersensitivity to aminoglycoside antibiotics. *Nucleic Acids Res.* 1993;**21**:4174-4179.

329. Hampel B, Hullmann R, Schmidt H. Ciprofloxacin in pediatrics: worldwide clinical experience based on compassionate use--safety report. *Pediatr Infect Dis J.* 1997;**16**:127-129.

330. Berning SE, Madsen L, Iseman MD, Peloquin CA. Long-term safety of ofloxacin and ciprofloxacin in the treatment of mycobacterial infections. *Am J Respir Crit Care Med.* 1995;**151**:2006-2009.

331. Kubin R. Safety and efficacy of ciprofloxacin in paediatric patients--review. *Infection.* 1993;**21**:413-421.

332. Davis R, Markham A, Balfour JA. Ciprofloxacin. An updated review of its pharmacology, therapeutic efficacy and tolerability. *Drugs.* 1996;**51**:1019-1074.

333. Schaad UB. Fluoroquinolone antibiotics in infants and children. *Infect Dis Clin North Am.* 2005;**19**:617-628.

334. Forsythe CT, Ernst ME. Do fluoroquinolones commonly cause arthropathy in children? *CJEM.* 2007;**9**:459-462.

335. Leibovitz E. The use of fluoroquinolones in children. *Curr Opin Pediatr.* 2006;**18**:64-70.

336. European Medicines Agency. Fluoroquinolones - Risk of QT interval prolongation. *Pharmacovigilance Working Party (PhVWP) Montly Report - December 2010 plenary meeting. EMA/CHMP/PhVWP/810358/2010.* **Available at:** http://www.ema.europa.eu/docs/en_GB/document_library/Report/2011/01/WC500100459.pdf **accessed on 20-9-11.**
337. Ridzon R, Meador J, Maxwell R, Higgins K, Weismuller P, Onorato IM. Asymptomatic hepatitis in persons who received alternative preventive therapy with pyrazinamide and ofloxacin. *Clin Infect Dis.* 1997;**24**:1264-1265.
338. Gupta DK. Acceptability of thioamides. I. Ethionamide. *J Postgrad Med.* 1977;**23**:175-180.
339. Gupta DK. Acceptability of thioamides. II. Prothionamide. *J Postgrad Med.* 1977;**23**:181-185.
340. Lees AW. Toxicity in Newly Diagnosed Cases of Pulmonary Tuberculosis Treated with Ethionamide. *Am Rev Respir Dis.* 1963;**88**:347-354.
341. Becks GP, Eggo MC, Burrow GN. Organic iodine inhibits deoxyribonucleic acid synthesis and growth in FRTL-5 thyroid cells. *Endocrinology.* 1988;**123**:545-551.
342. McDonnell ME, Braverman LE, Bernardo J. Hypothyroidism due to ethionamide. *N Engl J Med.* 2005;**352**:2757-2759.
343. Moulding T, Fraser R. Hypothyroidism related to ethionamide. *Am Rev Respir Dis.* 1970;**101**:90-94.
344. Marmorstein BL, Scheinhorn DJ. Effect of ethionamide on thyroid function. *Curr Ther Res Clin Exp.* 1976;**19**:483-486.
345. Drucker D, Eggo MC, Salit IE, Burrow GN. Ethionamide-induced goitrous hypothyroidism. *Annals of internal medicine.* 1984;**100**:837-839.
346. Hallbauer UM, Schaaf HS. Ethionamide-induced hypothyroidism in children. *South Afr J Epidemiol Infect.* 2011;**26**:161-163.
347. Swash M, Roberts AH, Murnaghan DJ. Reversible pellagra-like encephalopathy with ethionamide and cycloserine. *Tubercle.* 1972;**53**:132-136.
348. Gupta DK, Mital OP, Agarwala MC. Hepatic injury due to ethionamide (1314-TH) and prothionamide (1321-TH). *J Assoc Physicians India.* 1975;**23**:147-151.
349. Somner AR, Brace AA. Changes in serum transaminase due to prothionamide. *Tubercle.* 1967;**48**:137-143.
350. El-Khoury SA, Dunmore LA, Jr. Ethionamide and Hepatotoxicity: A Clinical Study. *Med Ann Dist Columbia.* 1964;**33**:15-17.
351. Lees AW. Jaundice Due to Ethionamide. *Br J Dis Chest.* 1963;**57**:158-161.
352. Phillips S, Tashman H. Ethionamide jaundice. *Am Rev Respir Dis.* 1963;**87**:896-898.
353. Conn HO, Binder HJ, Orr HD. Ethionamide-Induced Hepatitis. A Review with a Report of an Additional Case. *Am Rev Respir Dis.* 1964;**90**:542-552.
354. Phillips S, Trevathan RD. Serum glutamic oxaloacetic transaminase elevation and possible hepatotoxicity accompanying the administration of ethionamide. *Am Rev Respir Dis.* 1962;**86**:268-269.
355. Cameron SJ, Crompton GK. Severe hypoglycaemia in the course of treatment with streptomycin, isoniazid and ethionamide. *Tubercle.* 1967;**48**:307-310.
356. A comparison of the toxicity of prothionamide and ethionamide: a report from the research committee of the British Tuberculosis Association. *Tubercle.* 1968;**49**:125-135.
357. Yew WW, Wong CF, Wong PC, Lee J, Chau CH. Adverse neurological reactions in patients with multidrug-resistant pulmonary tuberculosis after coadministration of cycloserine and ofloxacin. *Clin Infect Dis.* 1993;**17**:288-289.
358. Walker WC, Murdoch JM. Cycloserine in the treatment of pulmonary tuberculosis; a report on toxicity. *Tubercle.* 1957;**38**:297-302.
359. Ritchie JA, Campbell AE, Cuthbert J, Bruce LG. Treatment of drug-resistant cases of pulmonary tuberculosis with cycloserine and pyrazinamide. *Tubercle.* 1958;**39**:289-295.
360. Akula SK, Aruna AS, Johnson JE, Anderson DS. Cycloserine-induced Stevens-Johnson syndrome in an AIDS patient with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 1997;**1**:187-190.
361. Kwon HM, Kim HK, Cho J, Hong YH, Nam H. Cycloserine-induced encephalopathy: evidence on brain MRI. *Eur J Neurol.* 2008;**15**:e60-61.
362. Weyer K. Annexure 2. DOTS-Plus for multidrug resistant tuberculosis patients in South Africa. Systematic evaluation of a standardised treatment regimen applied under tuberculosis control programme conditions. *http://www.sahealthinfo.org/tb/annexures1-4.pdf (accessed 30/10/10).* 2004.

363. Soumakis SA, Berg D, Harris HW. Hypothyroidism in a patient receiving treatment for multidrug-resistant tuberculosis. *Clin Infect Dis.* 1998;**27**:910-911.

364. Mizutani H, Horiba M, Shindoh J, Kimura T, Son M, Wakahara K. [Two cases of multi-drug-resistant pulmonary tuberculosis with para-aminosalicylic acid (PAS)-induced hypothyroidism]. *Kekkaku.* 2001;**76**:667-672.

365. Joules FE, Nassau E. Para-aminosalicylic acid; a clinical trial in 10 patients with pulmonary tuberculosis. *Tubercle.* 1949;**30**:98-102.

366. Co-operative controlled trial of a standard regimen of streptomycin, PAS and isoniazid and three alternative regimens of chemotherapy in Britain. A report from the British Medical Research Council. *Tubercle.* 1973;**54**:99-129.

367. Bower G. Skin Rash, Hepatitis, and Hemolytic Anemia Caused by Para-Aminosalicylic Acid. *Am Rev Respir Dis.* 1964;**89**:440-443.

368. Eisner EV, Kasper K. Immune thrombocytopenia due to a metabolite of para-aminosalicylic acid. *Am J Med.* 1972;**53**:790-796.

369. Feigin RD, Zarkowsky HF, Shearer W, Anderson DC. Thrombocytopenia following administration of para-aminosalicylic acid. *J Pediatr.* 1973;**83**:502-503.

370. Wurzel HA, Mayock RL. Thrombocytopenia induced by sodium para-aminosalicylic acid: report of a case. *J Am Med Assoc.* 1953;**153**:1094-1095.

371. Dandona P, Greenbury E, Beckett AG. Para-aminosalicylic acid-induced hypoglycaemia in a patient with diabetic nephropathy. *Postgraduate medical journal.* 1980;**56**:135-136.

372. Longstreth GF, Newcomer AD, Westbrook PR. Para-aminosalicylic acid-induced malabsorption. *Am J Dig Dis.* 1972;**17**:731-734.

373. Akhtar AJ, Crompton GK, Schonell ME. Para-aminosalicylic acid as a cause of intestinal malabsorption. *Tubercle.* 1968;**49**:328-331.

374. Nagaratnam N, Bhuvanendran N, Jiffry AJ, Ramachandra V. Lymphoma-like syndrome following para-aminosalicylic acid. *Postgraduate medical journal.* 1982;**58**:729-730.

375. McEvoy GK. AHFS drug information. *American Society of Health-System Pharmacists, Inc. Bethesda, MD.* 1996:399-401.

376. Kucers A, Bennett NM. *The use of antibiotics, 4th ed.* Philadelphia: Lippincott Williams & Wilkins; 1987.

377. Peloquin CA. Para-aminosalicylic acid. In: Yu VL, Edwards G, McKinnon PS, Peloquin CA, Morse GD, eds. *Antimicrobial Chemotherapy and Vaccines, 2nd edition, Volume II: Antimicrobial Agents*: Esun Technologies, LLC; 2005:551-558.

378. Wilson JW, Kelkar P, Frigas E. Para-aminosalicylic acid (PAS) desensitization review in a case of multidrug-resistant pulmonary tuberculosis. *Int J Tuberc Lung Dis.* 2003;**7**:493-497.

379. Kroger A, Pannikar V, Htoon MT, et al. International open trial of uniform multi-drug therapy regimen for 6 months for all types of leprosy patients: rationale, design and preliminary results. *Trop Med Int Health.* 2008;**13**:594-602.

380. Tan TQ. Update on the use of linezolid: a pediatric perspective. *Pediatr Infect Dis J.* 2004;**23**:955-956.

381. Kaplan SL. Use of linezolid in children. *Pediatr Infect Dis J.* 2002;**21**:870-872.

382. Saiman L, Goldfarb J, Kaplan SA, et al. Safety and tolerability of linezolid in children. *Pediatr Infect Dis J.* 2003;**22**:S193-200.

383. Condos R, Hadgiangelis N, Leibert E, Jacquette G, Harkin T, Rom WN. Case series report of a linezolid-containing regimen for extensively drug-resistant tuberculosis. *Chest.* 2008;**134**:187-192.

384. Anger HA, Dworkin F, Sharma S, Munsiff SS, Nilsen DM, Ahuja SD. Linezolid use for treatment of multidrug-resistant and extensively drug-resistant tuberculosis, New York City, 2000-06. *J Antimicrob Chemother.* 2010;**65**:775-783.

385. von der Lippe B, Sandven P, Brubakk O. Efficacy and safety of linezolid in multidrug resistant tuberculosis (MDR-TB)--a report of ten cases. *J Infect.* 2006;**52**:92-96.

386. Fortun J, Martin-Davila P, Navas E, et al. Linezolid for the treatment of multidrug-resistant tuberculosis. *J Antimicrob Chemother.* 2005;**56**:180-185.

387. Schecter GF, Scott C, True L, Raftery A, Flood J, Mase S. Linezolid in the treatment of multidrug-resistant tuberculosis. *Clin Infect Dis.* 2010;**50**:49-55.

388. Udwadia ZF, Sen T, Moharil G. Assessment of linezolid efficacy and safety in MDR- and XDR-TB: an Indian perspective. *Eur Respir J.* 2010;**35**:936-938; author reply 938-940.

389. Migliori GB, Eker B, Richardson MD, et al. A retrospective TBNET assessment of linezolid safety, tolerability and efficacy in multidrug-resistant tuberculosis. *Eur Respir J.* 2009;**34**:387-393.

390. Nunn P, Kibuga D, Gathua S, et al. Cutaneous hypersensitivity reactions due to thiacetazone in HIV-1 seropositive patients treated for tuberculosis. *Lancet.* 1991;**337**:627-630.
391. Okwera A, Whalen C, Byekwaso F, et al. Randomised trial of thiacetazone and rifampicin-containing regimens for pulmonary tuberculosis in HIV-infected Ugandans. The Makerere University-Case Western University Research Collaboration. *Lancet.* 1994;**344**:1323-1328.
392. Chintu C, Luo C, Bhat G, Raviglione M, DuPont H, Zumla A. Cutaneous hypersensitivity reactions due to thiacetazone in the treatment of tuberculosis in Zambian children infected with HIV-I. *Arch Dis Child.* 1993;**68**:665-668.
393. Miller AB. Thiacetazone toxicity: a general review. *Tubercle.* 1968;**49**:Suppl:54-56.
394. Pozniak A. Multidrug-resistant tuberculosis and HIV infection. *Ann N Y Acad Sci.* 2001;**953**:192-198.
395. Andrews JR, Shah NS, Gandhi N, Moll T, Friedland G. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *J Infect Dis.* 2007;**196 Suppl 3**:S482-490.
396. Di Perri G, Aguilar Marucco D, Mondo A, et al. Drug-drug interactions and tolerance in combining antituberculosis and antiretroviral therapy. *Expert Opin Drug Saf.* 2005;**4**:821-836.
397. McIlleron H, Khoo SH. Interactions between antituberculosis and antiretroviral agents. *Progress in Respiratory Research (in press).* 2011;**40**.
398. Seung KJ, Omatayo DB, Keshavjee S, Furin JJ, Farmer PE, Satti H. Early outcomes of MDR-TB treatment in a high HIV-prevalence setting in Southern Africa. *PLoS One.* 2009;**4**:e7186.
399. Huerga H, Arnold A, Chikwanha I, et al. MDR-TB treatment in Kenya: cohort description from the first program. *41st Union World Conference on Lung Health, 11-15 Nov 2010, Berlin. Abstract PC-100347-14.*
400. *The Sentinel Project on Pediatric Drug-Resistant Tuberculosis.* .**Available at:** www.sentinel-project.org. **(accessed 22.3.12)**
401. Mandalakas AM, Kirchner HL, Lombard C, et al. Well-quantified tuberculosis exposure is a reliable surrogate measure of tuberculosis infection. *Int J Tuberc Lung Dis.* 2012;**16**:1033-1039.
402. Davies PD. The natural history of tuberculosis in children. A study of child contacts in the Brompton Hospital Child Contact Clinic from 1930 to 1952. *Tubercle.* 1961;**42(Suppl)**:1-40.
403. Gedde-Dahl T. Tuberculous infection in the light of tuberculin matriculation. *Am J Hyg.* 1952;**56**:139-214.
404. Van Wyk SS, Mandalakas AM, Enarson DA, Gie RP, Beyers N, Hesseling AC. Tuberculosis contact investigation in a high-burden setting: house or household? *Int J Tuberc Lung Dis.* 2012;**16**:157-162.
405. Graham SM, Ahmed T, Amanullah F, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis.* 2012;**205 Suppl 2**:S199-208.
406. Clinical and Laboratory Standards Institute (CLSI). *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard - Second Edition*: CLSI document M24-A2 (ISBN 1-56238-746-4). Clinical and Laboratory Standards Insitutue, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2011.
407. World Health Organization, Geneva, Switzerland. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Policy statement. 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf (accessed August 2012).
408. Mukinda FK, Theron D, van der Spuy GD, et al. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. *Int J Tuberc Lung Dis.* 2012;**16**:196-202.
409. World Health Organization, Geneva, Switzerland. Provisional guidelines for the diagnosis and classification of the EPI target diseases for primary health care, surveillance and special studies. *EPI/GEN/83/4.* 1986.
410. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis.* 2010;**10**:803-812.
411. Wiseman CA, Gie RP, Starke JR, et al. A Proposed Comprehensive Classification of Tuberculosis Disease Severity in Children. *Pediatr Infect Dis J.* 2012.
412. World Health Organization, Geneva, Switzerland. International Statistical Classification of Diseases and Related Health Problems. 10th Revision. Available at: http://apps.who.int/classifications/icd10/browse/2010/en (accessed 15 March 2012). 2010.
413. Marais BJ, Gie RP, Schaaf HS, et al. A proposed radiological classification of childhood intra-thoracic tuberculosis. *Pediatr Radiol.* 2004;**34**:886-894.

414. Bloss E, Kuksa L, Holtz TH, et al. Adverse events related to multidrug-resistant tuberculosis treatment, Latvia, 2000-2004. *Int J Tuberc Lung Dis.* 2010;**14**:275-281.
415. Division of Microbiology and Infectious Diseases. Pediatric Toxicity Tables. November 2007 DRAFT. http://www.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/Documents/dmidpedtox.pdf. Accessed February 6, 2012.
416. Marra F, Marra CA, Moadebi S, et al. Levofloxacin treatment of active tuberculosis and the risk of adverse events. *Chest.* 2005;**128**:1406-1413.
417. Laserson KF, Thorpe LE, Leimane V, et al. Speaking the same language: treatment outcome definitions for multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2005;**9**:640-645.
418. Starke JR. Pediatric tuberculosis: time for a new approach. *Tuberculosis (Edinb).* 2003;**83**:208-212.
419. Chisti MJ, Tebruegge M, La Vincente S, Graham SM, Duke T. Pneumonia in severely malnourished children in developing countries - mortality risk, aetiology and validity of WHO clinical signs: a systematic review. *Trop Med Int Health.* 2009;**14**:1173-1189.
420. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int J Tuberc Lung Dis.* 2004;**8**:286-298.
421. Hesseling AC, Westra AE, Werschkull H, et al. Outcome of HIV infected children with culture confirmed tuberculosis. *Arch Dis Child.* 2005;**90**:1171-1174.
422. Soeters M, de Vries AM, Kimpen JL, Donald PR, Schaaf HS. Clinical features and outcome in children admitted to a TB hospital in the Western Cape--the influence of HIV infection and drug resistance. *S Afr Med J.* 2005;**95**:602-606.
423. Walters E, Cotton MF, Rabie H, Schaaf HS, Walters LO, Marais BJ. Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on anti-retroviral therapy. *BMC Pediatr.* 2008;**8**:1.
424. Orikiiriza J, Bakeera-Kitaka S, Musiime V, Mworozi EA, Mugyenyi P, Boulware DR. The clinical pattern, prevalence, and factors associated with immune reconstitution inflammatory syndrome in Ugandan children. *AIDS.* 2010;**24**:2009-2017.
425. American Speech-Language-Hearing Association. Guidelines for Audiologic Screening (Guideline). 1997;**Available at http://www.asha.org/docs/pdf/GL1997-00199.pdf (accessed August 2012)**.
426. American Speech-Language-Hearing Association. Audiologic Screening (Technical Report). 1994;**Available at http://www.asha.org/docs/pdf/TR1994-00238.pdf (accessed August 2012)**.
427. van Rie A, Warren R, Richardson M, et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N Engl J Med.* 1999;**341**:1174-1179.
428. Schaaf HS, Gie RP, Beyers N, Sirgel FA, de Klerk PJ, Donald PR. Primary drug-resistant tuberculosis in children. *Int J Tuberc Lung Dis.* 2000;**4**:1149-1155.
429. Kassa-Kelembho E, Bobossi-Serengbe G, Takeng EC, Nambea-Koisse TB, Yapou F, Talarmin A. Surveillance of drug-resistant childhood tuberculosis in Bangui, Central African Republic. *Int J Tuberc Lung Dis.* 2004;**8**:574-578.
430. Warren RM, Gey van Pittius NC, Barnard M, et al. Differentiation of Mycobacterium tuberculosis complex by PCR amplification of genomic regions of difference. *Int J Tuberc Lung Dis.* 2006;**10**:818-822.
431. NCCLS. *Susceptibility testing of mycobacteria, nocardiae and other aerobic actinomycetes: approved standard*: NCCLS document M24-A. 2003. NCCLS, 940 West Valley Road, Wayne, Pennysylvania, USA.
432. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. *Am J Respir Crit Care Med.* 2008;**177**:787-792.
433. Al-Dabbagh M, Lapphra K, McGloin R, et al. Drug-resistant Tuberculosis: Pediatric Guidelines. *Pediatr Infect Dis J.* 2011.
434. Louw GE, Warren RM, Donald PR, et al. Frequency and implications of pyrazinamide resistance in managing previously treated tuberculosis patients. *Int J Tuberc Lung Dis.* 2006;**10**:802-807.
435. Johnson R, Jordaan AM, Pretorius L, et al. Ethambutol resistance testing by mutation detection. *Int J Tuberc Lung Dis.* 2006;**10**:68-73.
436. Vilcheze C, Weisbrod TR, Chen B, et al. Altered NADH/NAD+ ratio mediates coresistance to isoniazid and ethionamide in mycobacteria. *Antimicrob Agents Chemother.* 2005;**49**:708-720.

437. Hesseling AC, Schaaf HS, Victor T, et al. Resistant Mycobacterium bovis Bacillus Calmette-Guerin disease: implications for management of Bacillus Calmette-Guerin Disease in human immunodeficiency virus-infected children. *Pediatr Infect Dis J.* 2004;**23**:476-479.
438. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *Lancet.* 2007;**369**:2042-2049.
439. Suarez-Garcia I, Rodriguez-Blanco A, Vidal-Perez JL, et al. Risk factors for multidrug-resistant tuberculosis in a tuberculosis unit in Madrid, Spain. *European Journal of Clinical Microbiology and Infectious Diseases.* 2009;**28**:325-330.
440. Marais BJ, Hesseling AC, Schaaf HS, Gie RP, van Helden PD, Warren RM. Mycobacterium tuberculosis transmission is not related to household genotype in a setting of high endemicity. *Journal of clinical microbiology.* 2009;**47**:1338-1343.
441. Health Systems Trust. Incidence of TB (all types) per 100000. 2009. [cited 2011 Feb 24] http://www.hst.org.za/healthstats/16/data.
442. Driver CR, Cordova IM, Munsiff SS. Targeting tuberculosis testing: the yield of source case investigations for young children with reactive tuberculin skin tests. *Public Health Rep.* 2002;**117**:366-372.
443. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J Clin Microbiol.* 1997;**35**:907-914.
444. van Embden JD, Cave MD, Crawford JT, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol.* 1993;**31**:406-409.
445. Streicher EM, Victor TC, van der Spuy G, et al. Spoligotype signatures in the Mycobacterium tuberculosis complex. *J Clin Microbiol.* 2007;**45**:237-240.
446. Streicher EM, Muller B, Chihota V, et al. Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa. *Infect Genet Evol.* 2011.
447. Furin JJ, Becerra MC, Shin SS, Kim JY, Bayona J, Farmer PE. Effect of administering short-course, standardized regimens in individuals infected with drug-resistant Mycobacterium tuberculosis strains. *Eur J Clin Microbiol Infect Dis.* 2000;**19**:132-136.
448. Warren RM, Streicher EM, Gey van Pittius NC, et al. The clinical relevance of Mycobacterial pharmacogenetics. *Tuberculosis (Edinb).* 2009;**89**:199-202.
449. World Health Organization, Geneva, Switzerland. Molecular Line Probe Assays for the rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy Statement. 2008:Available at: http://www.who.int/tb/features_archive/policy_statement.pdf (accessed August 2012).
450. Fair M, Cyr M, Allen AC, Wen SW, Guyon G, MacDonald RC. An assessment of the validity of a computer system for probabilistic record linkage of birth and infant death records in Canada. The Fetal and Infant Health Study Group. *Chronic Dis Can.* 2000;**21**:8-13.
451. Meray N, Reitsma JB, Ravelli AC, Bonsel GJ. Probabilistic record linkage is a valid and transparent tool to combine databases without a patient identification number. *J Clin Epidemiol.* 2007;**60**:883-891.
452. Claessens NJ, Gausi FF, Meijnen S, Weismuller MM, Salaniponi FM, Harries AD. Screening childhood contacts of patients with smear-positive pulmonary tuberculosis in Malawi. *Int J Tuberc Lung Dis.* 2002;**6**:362-364.
453. van Wyk SS, Reid AJ, Mandalakas AM, et al. Operational challenges in managing Isoniazid Preventive Therapy in child contacts: a high-burden setting perspective. *BMC Public Health.* 2011;**11**:544.
454. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Public Health.* 2008;**8**:15.
455. Sreeramareddy CT, Panduru KV, Menten J, Van den Ende J. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. *BMC infectious diseases.* 2009;**9**:91.
456. Finnie RK, Khoza LB, van den Borne B, Mabunda T, Abotchie P, Mullen PD. Factors associated with patient and health care system delay in diagnosis and treatment for TB in sub-Saharan African countries with high burdens of TB and HIV. *Trop Med Int Health.* 2011;**16**:394-411.
457. Nyirenda M, Sinfield R, Haves S, Molyneux EM, Graham SM. Poor attendance at a child TB contact clinic in Malawi. *Int J Tuberc Lung Dis.* 2006;**10**:585-587.
458. Zachariah R, Spielmann MP, Harries AD, et al. Passive versus active tuberculosis case finding and isoniazid preventive therapy among household contacts in a rural district of Malawi. *Int J Tuberc Lung Dis.* 2003;**7**:1033-1039.

459. Banu Rekha VV, Jagarajamma K, Wares F, Chandrasekaran V, Swaminathan S. Contact screening and chemoprophylaxis in India's Revised Tuberculosis Control Programme: a situational analysis. *Int J Tuberc Lung Dis.* 2009;**13**:1507-1512.
460. Andrews R, Morgan JD, Addy DP, McNeish AS. Understanding non-attendance in outpatient paediatric clinics. *Arch Dis Child.* 1990;**65**:192-195.
461. McClure RJ, Newell SJ, Edwards S. Patient characteristics affecting attendance at general outpatient clinics. *Arch Dis Child.* 1996;**74**:121-125.
462. Kitzinger J. Qualitative research. Introducing focus groups. *BMJ.* 1995;**311**:299-302.
463. Patton MQ. Qualitative research and evaluation methods. Third edition. *Sage Publications, Inc., Thousand Oaks, California, USA.* 2001.
464. Jones RG, Trivedi AN, Ayanian JZ. Factors influencing the effectiveness of interventions to reduce racial and ethnic disparities in health care. *Soc Sci Med.* 2010;**70**:337-341.
465. South African Advertising Research Foundation. Universal Living Standards Measure. Available at: http://www.saarf.co.za/LSM/lsms.asp (accessed 7th May 2012). 2004.
466. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet.* 2006;**368**:1575-1580.
467. Ethionamide. *Tuberculosis (Edinb).* 2008;**88**:106-108.
468. Donald PR, Schoeman JF, Van Zyl LE, De Villiers JN, Pretorius M, Springer P. Intensive short course chemotherapy in the management of tuberculous meningitis. *Int J Tuberc Lung Dis.* 1998;**2**:704-711.
469. Oyugi JH, Byakika-Tusiime J, Charlebois ED, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. *J Acquir Immune Defic Syndr.* 2004;**36**:1100-1102.
470. Vreeman RC, Wiehe SE, Pearce EC, Nyandiko WM. A systematic review of pediatric adherence to antiretroviral therapy in low- and middle-income countries. *Pediatr Infect Dis J.* 2008;**27**:686-691.
471. Simoni JM, Montgomery A, Martin E, New M, Demas PA, Rana S. Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management. *Pediatrics.* 2007;**119**:e1371-1383.
472. Giordano TP, Guzman D, Clark R, Charlebois ED, Bangsberg DR. Measuring adherence to antiretroviral therapy in a diverse population using a visual analogue scale. *HIV Clin Trials.* 2004;**5**:74-79.
473. DiMatteo MR, Giordani PJ, Lepper HS, Croghan TW. Patient adherence and medical treatment outcomes: a meta-analysis. *Med Care.* 2002;**40**:794-811.
474. Thomas TA, Shenoi SV, Heysell SK, et al. Extensively drug-resistant tuberculosis in children with human immunodeficiency virus in rural South Africa. *Int J Tuberc Lung Dis.* 2010;**14**:1244-1251.
475. Mukherjee JS, Joseph JK, Rich ML, et al. Clinical and programmatic considerations in the treatment of MDR-TB in children: a series of 16 patients from Lima, Peru. *Int J Tuberc Lung Dis.* 2003;**7**:637-644.
476. Beyers N, Gie RP, Schaaf HS, et al. Delay in the diagnosis, notification and initiation of treatment and compliance in children with tuberculosis. *Tuber Lung Dis.* 1994;**75**:260-265.
477. Marais BJ, Gie RP, Hesseling AC, et al. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. *Pediatrics.* 2006;**118**:e1350-1359.
478. Schaaf HS, Marais BJ, Whitelaw A, et al. Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. *BMC infectious diseases.* 2007;**7**:140.
479. Schaaf HS, Krook S, Hollemans DW, Warren RM, Donald PR, Hesseling AC. Recurrent culture-confirmed tuberculosis in human immunodeficiency virus-infected children. *Pediatr Infect Dis J.* 2005;**24**:685-691.
480. Chiang CY, Schaaf HS. Management of drug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2010;**14**:672-682.
481. Grant A, Gothard P, Thwaites G. Managing drug resistant tuberculosis. *BMJ.* 2008;**337**:a1110.
482. Schaaf HS, Donald PR. The epidemiology and management of drug-resistant tuberculosis in childhood. In: Spiegelburg DD, ed. *New topics in tuberculosis research.* New York: Nova Science Publishers, Inc.; 2007 71-102.
483. World Health Organization, Geneva, Switzerland. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. 2007; **(ISBN: 978 92 4 159562 9)**.

484. Hesseling AC, Walzl G, Enarson DA, et al. Baseline sputum time to detection predicts month two culture conversion and relapse in non-HIV-infected patients. *Int J Tuberc Lung Dis.* 2010;**14**:560-570.

485. Schaaf HS, Beyers N, Gie RP, et al. Respiratory tuberculosis in childhood: the diagnostic value of clinical features and special investigations. *Pediatr Infect Dis J.* 1995;**14**:189-194.

486. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Enarson DA, Beyers N. The spectrum of disease in children treated for tuberculosis in a highly endemic area. *Int J Tuberc Lung Dis.* 2006;**10**:732-738.

487. Donald PR, Schoeman JF. Tuberculous meningitis. *N Engl J Med.* 2004;**351**:1719-1720.

488. Donald PR, Schaaf HS, Schoeman JF. Tuberculous meningitis and miliary tuberculosis: the Rich focus revisited. *J Infect.* 2005;**50**:193-195.

489. Rich AR, McCordock HA. The pathogenesis of tuberculous meningitis. *Bull Johns Hopkins Hospital.* 1933;**52**:5-37.

490. van Well GT, Paes BF, Terwee CB, et al. Twenty years of pediatric tuberculous meningitis: a retrospective cohort study in the western cape of South Africa. *Pediatrics.* 2009;**123**:e1-8.

491. Lincoln EM. Tuberculous meningitis in children; with special reference to serous meningitis; tuberculous meningitis. *Am Rev Tuberc.* 1947;**56**:75-94.

492. Donald PR, Cotton MF, Hendricks MK, Schaaf HS, de Villiers JN, Willemse TE. Pediatric meningitis in the Western Cape Province of South Africa. *J Trop Pediatr.* 1996;**42**:256-261.

493. Patel VB, Padayatchi N, Bhigjee AI, et al. Multidrug-resistant tuberculous meningitis in KwaZulu-Natal, South Africa. *Clin Infect Dis.* 2004;**38**:851-856.

494. Thwaites GE, Lan NT, Dung NH, et al. Effect of antituberculosis drug resistance on response to treatment and outcome in adults with tuberculous meningitis. *J Infect Dis.* 2005;**192**:79-88.

495. Daikos GL, Cleary T, Rodriguez A, Fischl MA. Multidrug-resistant tuberculous meningitis in patients with AIDS. *Int J Tuberc Lung Dis.* 2003;**7**:394-398.

496. Sofia M, Maniscalco M, Honore N, et al. Familial outbreak of disseminated multidrug-resistant tuberculosis and meningitis. *Int J Tuberc Lung Dis.* 2001;**5**:551-558.

497. Thwaites GE, Chau TT, Caws M, et al. Isoniazid resistance, mycobacterial genotype and outcome in Vietnamese adults with tuberculous meningitis. *Int J Tuberc Lung Dis.* 2002;**6**:865-871.

498. Warren RM, Streicher EM, Sampson SL, et al. Microevolution of the direct repeat region of Mycobacterium tuberculosis: implications for interpretation of spoligotyping data. *J Clin Microbiol.* 2002;**40**:4457-4465.

499. Brudey K, Driscoll JR, Rigouts L, et al. Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 2006;**6**:23.

500. Thwaites GE, Duc Bang N, Huy Dung N, et al. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with Tuberculous meningitis. *J Infect Dis.* 2005;**192**:2134-2141.

501. Thwaites GE, Simmons CP, Than Ha Quyen N, et al. Pathophysiology and prognosis in vietnamese adults with tuberculous meningitis. *J Infect Dis.* 2003;**188**:1105-1115.

502. Donald PR. Cerebrospinal fluid concentrations of antituberculosis agents in adults and children. *Tuberculosis (Edinb).* 2010;**90**:279-292.

503. Nathanson E, Gupta R, Huamani P, et al. Adverse events in the treatment of multidrug-resistant tuberculosis: results from the DOTS-Plus initiative. *Int J Tuberc Lung Dis.* 2004;**8**:1382-1384.

504. Baquero-Artigao F, Garcia-Miguel MJ, Merino R, Garcia-Consuegra J, del Castillo F. Multidrug-resistant tuberculosis of the ankle: Case report. *Foot & Ankle International.* 2006;**27**:642-644.

505. Pavlopoulou ID, Theodoridou M, Daikos GL, et al. Drug-resistant tuberculous mastoiditis in 2 children. *Scand J Infect Dis.* 2000;**32**:436-438.

506. Rodriguez L, Weinberg B, Benaroya A, Gertner M. Multidrug-resistant tuberculous osteomyelitis. *Pediatr Infect Dis J.* 1997;**16**:1096-1096.

507. Inselman LS, DelaVega CE, Evans HE. Drug-resistant tuberculosis of the hip in a child. *New York State Journal of Medicine.* 1984;**84**:84-85.

508. Khan SA, Varshney MK, Hasan AS, Kumar A, Trikha V. Tuberculosis of the sternum. *J Bone Joint Surg Br.* 2007;**89**:817-820.

509. Polley P, Dunn R. Noncontiguous spinal tuberculosis: Incidence and management. *Eur Spine J.* 2009;**18**:1096-1101.

510. Sandher DS, Al-Jibury M, Paton RW, Ormerod LP. Bone and joint tuberculosis. *J Bone Joint Surg Br.* 2007;**89**:1379-1381.

511. Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. *Clin Infect Dis.* 2009;**49**:1350-1357.

512. Lindquist SW, Steinmetz BA, Starke JR. Multidrug-resistant tuberculosis of the first cervical vertebra in an immunocompetent adolescent. *Pediatr Infect Dis J.* 1997;**16**:333-336.

513. Hussey G, Kibel M, Parker N. Ciprofloxacin treatment of multiply drug-resistant extrapulmonary tuberculosis in a child. *Pediatr Infect Dis J.* 1992;**11**:408-409.

514. Agashe V, Shenai S, Mohrir G, et al. Osteoarticular tuberculosis--diagnostic solutions in a disease endemic region. *J Infect Dev Ctries.* 2009;**3**:511-516.

515. Bailey HL, Gabriel M, Hodgson AR, Shin JS. Tuberculosis of the spine in children. Operative findings and results in one hundred consecutive patients treated by removal of the lesion and anterior grafting. *J Bone Joint Surg Am.* 1972;**54**:1633-1657.

516. Luk KD. Tuberculosis of the spine in the new millennium. *Eur Spine J.* 1999;**8**:338-345.

517. Hodgson AR, Skinsnes OK, Leong CY. The pathogenesis of Pott's paraplegia. *J Bone Joint Surg Am.* 1967;**49**:1147-1156.

518. Leong JC. Tuberculosis of the spine. *J Bone Joint Surg Br.* 1993;**75**:173-175.

519. Hsu LC, Cheng CL, Leong JC. Pott's paraplegia of late onset. The cause of compression and results after anterior decompression. *J Bone Joint Surg Br.* 1988;**70**:534-538.

520. Hazra A, Laha B. Chemotherapy of osteoarticular tuberculosis. *Indian J Pharmacol.* 2005;**37**:5-12.

521. Ramachandran S, Clifton IJ, Collyns TA, Watson JP, Pearson SB. The treatment of spinal tuberculosis: a retrospective study. *Int J Tuberc Lung Dis.* 2005;**9**:541-544.

522. Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. Joint Tuberculosis Committee of the British Thoracic Society. *Thorax.* 1998;**53**:536-548.

523. ATS, CDC, IDSA. Treatmet of Tuberculosis. *MMRW June 20, 2003 / Vol. 52 / No. RR-11.* 2003;**52**.

524. American Academy of Pediatrics. Tuberculosis. In Pickering, LK ed. *Red Book: 2006 Report of the Committee on Infectious Dieseases* 2006;**26th Ed**:678-698.

525. Ge Z, Wang Z, Wei M. Measurement of the concentration of three antituberculosis drugs in the focus of spinal tuberculosis. *Eur Spine J.* 2008;**17**:1482-1487.

526. Meissner A, Borner K, Koeppe P. Concentrations of ofloxacin in human bone and in cartilage. *J Antimicrob Chemother.* 1990;**26 Suppl D**:69-74.

527. Tuli SM, Kumar K, Sen PC. Penetration of antitubercular drugs in clinical osteoarticular tubercular lesions. *Acta Orthop Scand.* 1977;**48**:362-368.

528. Liu P, Zhu Q, Jiang J. Distribution of Three Anti-tuberculous Drugs and Their Metabolites in Different Parts of Pathological Vertebrae with Spinal Tuberculosis. *Spine (Phila Pa 1976).* 2011;**36**:E1290-1295.

529. Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. *Lancet Infect Dis.* 2011;**11**:819-824.

530. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother.* 1999;**43**:1003-1012.

531. Moeller MP. Current state of knowledge: psychosocial development in children with hearing impairment. *Ear Hear.* 2007;**28**:729-739.

532. Moeller MP, Tomblin JB, Yoshinaga-Itano C, Connor CM, Jerger S. Current state of knowledge: language and literacy of children with hearing impairment. *Ear Hear.* 2007;**28**:740-753.

533. American Speech-Language-Hearing Association. Guidelines for the Audiologic Assessment of Children From Birth to 5 Years of Age. Available at: http://www.asha.org/docs/pdf/GL2004-00002.pdf (accessed August 2012). 2004.

534. Prayle A, Watson A, Fortnum H, Smyth A. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax.* 2010;**65**:654-658.

535. Seddon JA, Hesseling AC, Marais BJ, Jordaan A, Victor T, Schaaf HS. The evolving epidemic of drug-resistant tuberculosis among children in Cape Town, South Africa. *Int J Tuberc Lung Dis.* 2012;**16**:928-933.

536. Carhart R, Jerger JF. Preferred Methods for Clinical Determination of Pure-Tone Thresholds. *J Speech Hear Res.* 1959;**24**:330-345.

537. Goodman A. Reference zero levels for pure tone audiometer. *ASHA.* 1965;**7**:262-263.

538. Katz J, Medwetsky L, Burkard R, Hood LJ. *Handbook of Clinical Audiology*. Sixth ed: Lippincott Williams & Wilkins; 2009.

539. de Jager P, van Altena R. Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. *Int J Tuberc Lung Dis.* 2002;**6**:622-627.

540. Sturdy A, Goodman A, Jose RJ, et al. Multidrug-resistant tuberculosis (MDR-TB) treatment in the UK: a study of injectable use and toxicity in practice. *J Antimicrob Chemother.* 2011;**66**:1815-1820.

541. Bess FH, Dodd-Murphy J, Parker RA. Children with minimal sensorineural hearing loss: prevalence, educational performance, and functional status. *Ear Hear.* 1998;**19**:339-354.

542. Eisenberg LS. Current state of knowledge: speech recognition and production in children with hearing impairment. *Ear Hear.* 2007;**28**:766-772.

543. Jerger S. Current state of knowledge: perceptual processing by children with hearing impairment. *Ear Hear.* 2007;**28**:754-765.

544. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL. Language of early- and later-identified children with hearing loss. *Pediatrics.* 1998;**102**:1161-1171.

545. Moeller MP. Early intervention and language development in children who are deaf and hard of hearing. *Pediatrics.* 2000;**106**:E43.

546. Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. *Int J Tuberc Lung Dis.* 2004;**8**:636-647.

547. Walls T, Shingadia D. Global epidemiology of paediatric tuberculosis. *J Infect.* 2004;**48**:13-22.

548. Brent AJ, Anderson ST, Kampmann B. Childhood tuberculosis: out of sight, out of mind? *Trans R Soc Trop Med Hyg.* 2008;**102**:217-218.