UNDERSTANDING GRAM-NEGATIVE INFECTIONS AND ANTIMICROBIAL RESISTANCE IN ZIMBABWE

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Declaration of originality

I, Ioana Diana Olaru, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Ioana Diana Olaru Date: 29-AUG-2022

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Summary

Data on the burden of antimicrobial resistance (AMR) from sub-Saharan Africa are scarce. Although many countries have enrolled into the WHO Global Antimicrobial Resistance Use and Surveillance System (GLASS), less than 1% of contributing sites are from sub-Saharan Africa. In addition, data from both published research studies and surveillance may not be representative as reporting sites are usually large hospitals or referral laboratories. Patients encounter difficulties in accessing care and testing due to financial constraints while laboratories can be affected by stock outs of consumables, insufficient training, long turnaround times, and suboptimal quality of testing. The lack of diagnostics, safety netting for patients, and the high costs of care result in frequent overprescribing of antibiotics which leads in turn to further development of AMR.

This PhD explores some of these issues related to Gram-negative AMR and laboratory testing with a focus on Zimbabwe. I conducted a systematic review to determine the prevalence of AMR in sub-Saharan Africa in key Gram-negatives and a review investigating the association between HIV infection and AMR. I then conducted the ARGUS (Antimicrobial Resistance in Gram-negatives from Urinary Specimens) study, which recruited patients with suspected urinary tract infections from primary care clinics. Within the ARGUS study I aimed to i) determine the prevalence of resistance in community-acquired urinary tract infections ii) investigate the association between HIV infection and AMR; iii) identify mechanisms of resistance in *Escherichia coli* through sequencing; iv) assess the effect of AMR on patient outcomes; v) explore antibiotic prescribing and use in the outpatient setting; and vi) evaluate novel culture media.

This PhD showed that resistance to antibiotics recommended for the treatment of urinary tract infections by the national guidelines was common and patients who received ineffective antibiotics experienced less favourable outcomes. Patients with HIV infection were twice as likely to have infections with third-generation cephalosporin resistant *E. coli.* There was a large diversity of sequence types in *E. coli* and isolates frequently had resistance genes to multiple antibiotic classes. Around one in five patients had antibiotic exposure prior to accessing healthcare. Primary care providers acknowledged the problem of antibiotic overprescribing and the need for training on AMR. Novel chromogenic media evaluated in patients with urinary

tract infections and neonatal sepsis had a good performance and may be considered for surveillance in low-resource settings.

This work has highlighted the need for representative setting- and population- specific AMR data to inform treatment guidelines and improve patient care. Given the challenges in conducting laboratory testing, novel strategies for expanding use of laboratory diagnostics should be considered. However, sustained efforts are needed to acquire AMR data using high-quality testing and to use these data for informing treatment decisions. Reducing antibiotic use is challenging in this setting due to the economic situation, high burden of infections, diagnostic uncertainties and the need to provide care.

List of abbreviations

AMR: Antimicrobial Resistance

ARGUS: Antimicrobial Resistance in Gram-negatives from Urinary Specimens

CFU: Colony Forming Units

CLSI: Clinical and Laboratory Standards Institute

EDLIZ: Essential Drug List In Zimbabwe

ESBL: Extended-Spectrum Beta-Lactamases

EUCAST: European Committee on Antimicrobial Susceptibility Testing

ExPEC: Extraintestinal Pathogenic *Escherichia coli*

GAP-AMR: Global Action Plan on Antimicrobial Resistance

GLASS: Global Antimicrobial Resistance Surveillance System

HIV: Human Immunodeficiency Virus

LMIC: Low- and Middle-Income Countries

MLST: Multi-Locus Sequence Typing

MRCZ: Medical Research Council of Zimbabwe

PHC: Primary Health Clinics

PLWH: People Living With HIV

SDG: Sustained Development Goals

SLIPTA: Stepwise Laboratory Improvement Process Towards Accreditation

ST: Sequence Type

STI: Sexually Transmitted Infections

TAT: Turnaround Time

UTI: Urinary Tract Infection

WGS: Whole Genome Sequencing

WHO: World Health Organization

Table of contents

CHAPTER 1

BACKGROUND

1. INTRODUCTION

Antimicrobials have revolutionized modern medicine leading to important reductions in deaths and disability. Antimicrobial resistance (AMR) threatens to overturn these advances by rendering antimicrobial treatment ineffective and leading to increased patient mortality, morbidity and healthcare costs.[1,2] Recently the Global Research on AntiMicrobial Resistance (GRAM) project estimated for the first time the global burden of AMR for 23 pathogens and 88 pathogen-drug combinations. The study estimated that 4.95 million global deaths were associated with bacterial AMR in 2019 with 1.27 million directly attributed to AMR.[3] AMR is a global problem affecting all countries irrespective of income and geographical location,[4] and has been highlighted by the World Health Organization (WHO) as one of the major important public health threats of the 21st century.[5]

To address the problem of AMR, the WHO has introduced the Global Action Plan on AMR (GAP-AMR) that aims to ensure the successful treatment and prevention of infectious diseases by using effective and accessible medicines.[6] The GAP-AMR has five main objectives focusing on increasing awareness of AMR, strengthening surveillance and research, reducing the incidence of infections, optimizing antimicrobial drug use and supporting research and development in the field.[6] The Global AMR Surveillance System (GLASS) was designed to ensure standardized data collection and analysis and facilitate data sharing regionally and globally. For the purpose of AMR surveillance and comparability between different settings, GLASS focuses on key Gram-positive and Gram-negative pathogens isolated from priority samples such as blood and urine.[7] AMR surveillance has also been integrated into the Sustainable Development Goal (SDG) 3 “Ensuring healthy lives and promote well-being for all at all ages”. SDG 3 includes target 3.d “to strengthen the capacity of all countries, in particular developing countries, for early warning, risk reduction and management of national and global health risks”. One of the two indicators for this target is the “Percentage of bloodstream infections due to selected antimicrobial-resistant organisms” specifically third-

generation cephalosporin resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*.[8]

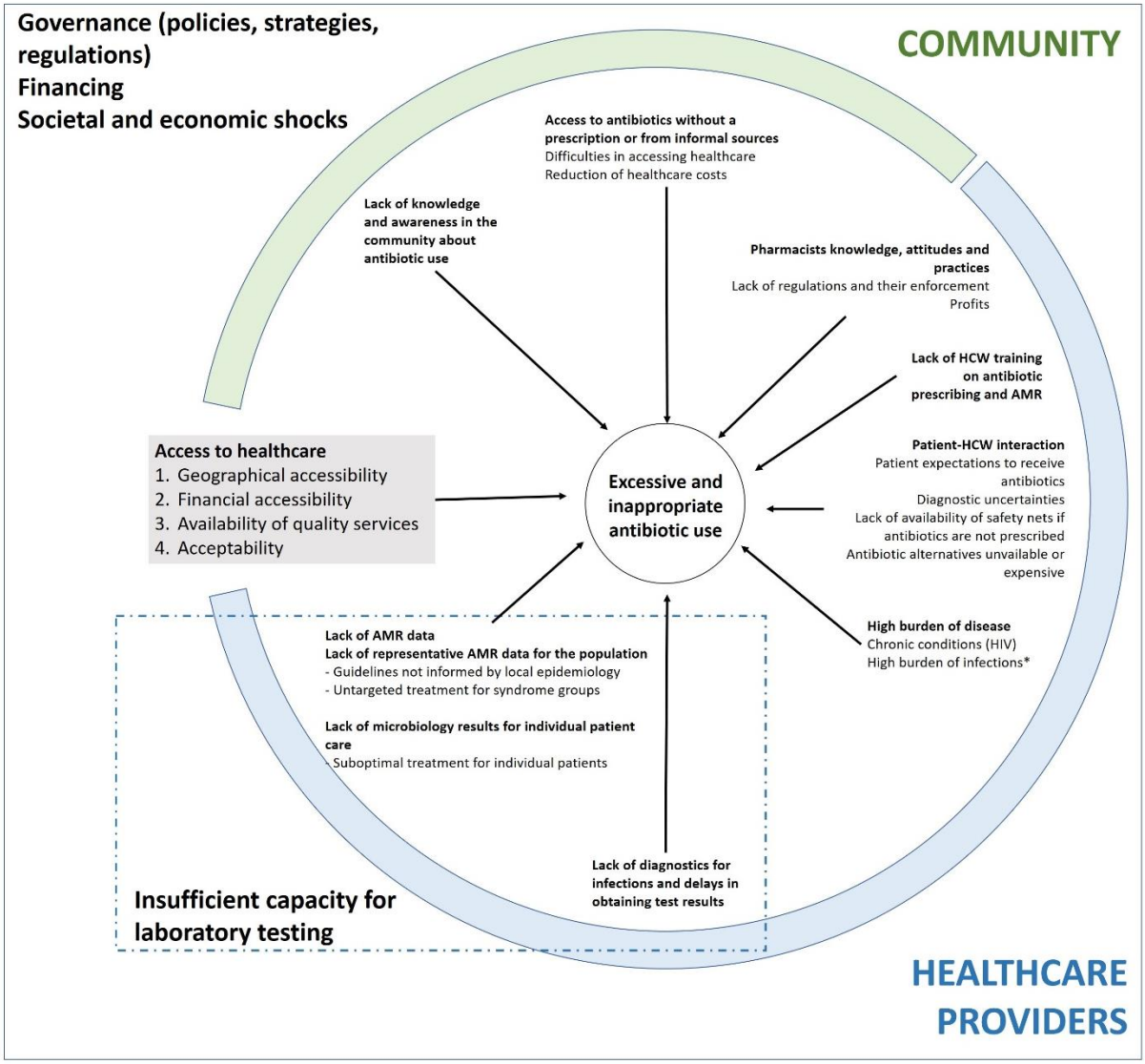
Several factors such as inappropriate antimicrobial use in humans and animals, insufficient or lacking infection control systems, lack of rapid diagnostics and vaccines, and the dissemination of successful bacterial clones harbouring resistance genes are widely recognised to drive the increase in AMR.[9] However, there are substantial differences between high- and low-resource settings in how these factors relate to AMR. Lack of investment in infrastructure and health services, poor governance and poverty lead to a high burden of infections, uncertainties around their diagnosis and optimal treatment, and overuse of antibiotics.[10,11] Lacking epidemiological data on causative organisms for infections and prevalence of resistance result in excessive and/or ineffective prescribing. In these settings antibiotics may be used both in the formal healthcare system and informally, without a prescription, as "quick fixes" for challenges arising from weak infrastructure, systems and governance combined with poverty.[10] While optimising antibiotic use is essential for preventing further development and transmission of resistance, different approaches may be needed in low and middle-income countries (LMICs) from those employed in high-income settings.

There are many structural and contextual factors leading to excessive antibiotic use (Figure 1). Healthcare system (including laboratory) strengthening will improve quality of healthcare overall and decrease antibiotic use and inappropriate prescribing. However, it requires medium- to long-term investment and concerted efforts from policymakers. More targeted interventions may be needed in the short-term to improve patient management. These interventions can be targeted at different levels within the framework and may include: (1) obtaining high-quality AMR burden data to inform local guidelines complemented by clinical information on risk factors for AMR; (2) development of simplified diagnostic methods aimed at increasing access to testing and enabling sentinel-site surveillance; (3) obtaining data on antibiotic use (both formally prescribed and over-the-counter) to understand how and where antibiotic use can be improved; (4) evaluating the training and educational needs of prescribers in relation to AMR and antibiotic use for designing tailored training programmes.

The US Centers for Disease Control and Prevention defines inappropriate antibiotic use as unnecessary use and inappropriate selection of the drug, dose or duration.[12] This definition may not be suitable for low-income settings where diagnostics are unavailable and guidelines for empiric antibiotic prescribing are rarely informed by country-specific AMR surveillance. Specifically, if epidemiological data on causative organisms for infections are limited and diagnostics cannot be accessed for individual patients, healthcare workers may find it

challenging to determine whether or not antibiotics are necessary. Antibiotic selection may also be inappropriate because of the very limited number of antibiotics which are available in pharmacies or affordable to patients. If treatment guidelines are not informed and updated to reflect local AMR burden, antibiotic prescribing in accordance to the guidelines may be ineffective for treatment. Further, the empiric selection of ineffective antibiotics cannot be corrected in the absence of microbiological results.

Figure 1. Conceptual framework representing the determinants of excessive and inappropriate antibiotic use in low-resource settings



Components of this framework have been adapted from Peters et al.[13] and Bitton et al.[14]

1.1. Availability of AMR data from sub-Saharan Africa

1.1.1. AMR surveillance data

Published research studies and routine surveillance play a key role in understanding the epidemiology of AMR. Although AMR surveillance is well established in most high income settings and global surveillance networks have been set up, few African countries contribute data to these networks.[15] According to the WHO-GLASS implementation report published in 2021, 94 countries across the five continents have enrolled into GLASS however, only 15 of the 47 countries in the African region reported data on the implementation of a national surveillance system and seven (Ethiopia, Madagascar, Mali, Mozambique, South Africa, Uganda and Zambia) provided AMR data. Overall, the majority of surveillance data reported to GLASS originated from sites located in high-income countries with sites in Africa representing 93/64747 (0.1%) in 2019 and 251/24721 (1%) in 2020 of the total number of reporting sites. Of the African sites, 208/251 (83%) were from South Africa reporting mainly on hospital data.[16] The limited data available and the focus on tertiary facilities mean that data from low-resource African settings may not be generalisable. This in turn limits their use for empirical treatment recommendations and routine patient care. In an effort to address the lack of data from low resource settings, WHO is planning to design and conduct surveys aimed at collecting representative and impactful AMR data within the GLASS initiative. These surveys will likely be initiated in the near future but no data are yet available. Because of differences between settings, site-specific surveillance data are needed to guide the choices of antibiotics in local guidelines.

Zimbabwe has enrolled into GLASS and has agreed an AMR National Action Plan[17] in 2017. The country has not yet reported any AMR surveillance data to GLASS.[16] Several laboratory strengthening initiatives supported by the Fleming Fund and the Multi-Partner Trust Fund are currently taking place in Zimbabwe. Establishing AMR surveillance systems is one of the aims of these initiatives.

1.1.2. AMR data from research studies

1.1.2.1. Systematic review and meta-analysis on the prevalence of resistance in Escherichia coli and Klebsiella pneumoniae in studies from sub-Saharan Africa

This section presents a systematic literature review of published studies from sub-Saharan Africa on the prevalence of AMR in the two most common Gram-negative pathogens which are associated with substantial mortality in sub-Saharan Africa and worldwide.[3] Some of the findings of this review were included as background information for the study protocol (Chapter 2). This section provides background to the study which was conducted for this PhD and highlights the lack of data on Gram-negative AMR in sub-Saharan Africa.

Background

AMR data collected as part of research studies could fill the gap in surveillance data. Availability of AMR data is crucial for guideline development. However, data used to inform treatment recommendations must be representative and of high quality. Comparison of AMR data across settings can be challenging due to differences in laboratory testing methods, in standards used for the interpretation of results, and in the quality of testing. Furthermore, the data from published research may originate preferentially from large hospitals and laboratories and therefore does not necessarily reflect population AMR prevalence. Routine testing may be limited to patients who can afford the costs of the tests, or who are not responding to treatment introducing selection bias.

In order to explore the data available from published studies from sub-Saharan Africa on AMR in key Gram-negative pathogens, I updated a previously published systematic review which included studies published up to January 2016.[4] The original review highlighted a lack of published studies on AMR for 23 of the 54 African countries with the vast majority of countries having fewer than five studies.[4] Beyond the low number of studies identified, the authors also expressed concerns regarding the quality of microbiology test results.[4]

The current systematic review was updated up to November 2019 and focuses on *E. coli* and *Klebsiella pneumoniae* which are the most common causes of Gram-negative infection and are considered priority pathogens by the WHO for AMR surveillance. These two pathogens were estimated to have caused 1.5 million deaths in 2019.[3] This systematic review aims to determine the prevalence of AMR of *E. coli* and *K. pneumoniae* isolated from priority specimens in

studies from sub-Saharan Africa with a focus on the quality of laboratory testing and the generalisability of findings.

Methods

This review is focused on priority pathogens (*E. coli* and *K. pneumoniae*) isolated from priority samples (blood or urine) as defined by the WHO for AMR surveillance.[18] Studies were included if they reported antibiotic susceptibility testing (AST) results for *E. coli* or *K. pneumonia*e isolated from urine or blood cultures from sub-Saharan African countries and were published between 2013-2019. Because of the rapid change in AMR prevalence, only studies published after 2013 were considered to ensure prevalence estimates reflected the current AMR epidemiology. Only studies which reported results stratified by organism and specimen type were included. No language restrictions were applied. The review followed the PRISMA guidelines and was registered on PROSPERO (CRD42020158561).

To reduce reporting bias, studies with less than 50 isolates were excluded ensuring precision of ±15% around a 30% prevalence estimate. Studies presenting data on pre-selected resistant isolates or specific patient populations recognized to be at increased risk for resistant infections were also excluded to minimise selection bias resulting in an over-estimation of resistance.

Eligible publications were identified using a search strategy (Table 1) adapted from a previously published systematic review by Tadesse et al. which evaluated prevalence of AMR in studies from Africa published between January 2013 and January 2016.[4] The search strategy was applied to MEDLINE, EMBASE, Web of Science and African Journals Online databases for articles published between January 2016 and November 2019. Studies published between 2013 and January 2016 were identified from the systematic review by Tadesse et al.[4] References of included studies and other reviews were manually searched for other potentially relevant publications.

Articles identified were imported into the bibliographic software manager Endnote X7 (Clarivate Analytics, Philadelphia, PA, USA). Titles and abstracts were then screened for eligibility. Full texts from potentially eligible articles were retrieved and inclusion and exclusion criteria were applied. Data were extracted using standardized tables. A random effects meta-analysis for proportions was performed using R v.4.1.1 (Foundation for Statistical Computing, Vienna, Austria) to obtain pool estimates of resistance prevalence that was grouped by drug tested and sample type. Variances in study specific prevalence were stabilised using the double arcsine method. Heterogeneity of studies was evaluated using the I^2 statistic. The quality of the studies was evaluated using a modified Newcastle-Ottawa Scale[19] (Table 2)

which included items on the representativeness of the population and of the samples, comparability between resistant and susceptible samples, how well the testing methods were described, and the use of quality control procedures.

Table 1. Search strategies used for the systematic review

Search strategy for EMBASE and OVID
('africa'/exp OR 'comoros' OR 'djibouti' OR 'madagascar' OR 'malawi' OR 'seychelles' OR 'cameroon' OR 'central african republic' OR 'chad' OR 'congo' OR 'DRC' OR 'equatorial guinea' OR 'atlantic islands' OR 'gabon' OR 'south sudan' OR 'sudan' OR 'botswana' OR 'lesotho' OR 'swaziland' OR 'eswatini' OR 'benin' OR 'burkina faso' OR 'cape verde' OR 'sao tome and principe' OR 'ghana' OR 'guinea' OR 'guinea-bissau' OR 'mauritania' OR 'mauritius' OR 'niger' OR 'senegal' OR 'sierra leone' OR 'togo' OR 'burundi*' OR 'eritrea*' OR 'ethiopia*' OR 'kenya*' OR 'mozambique*' OR 'rwanda*' OR 'somalia*' OR 'tanzania*' OR 'uganda*' OR 'zambia*' OR 'zimbabwe*' OR 'angola*' OR 'namibia*' OR 'south africa*' OR 'gambia*' OR 'liberia*' OR 'mali*' OR 'nigeria*' OR 'cote d'ivoire' OR 'ivory coast') AND
('anti-bacterial resistance' OR 'anti#bacterial resistance' OR 'antibiotic resistan*' OR 'antimicrobial resistan*' OR 'drug resistan*' OR 'drug#resistan*' OR 'multi-drug resistan*' OR 'multidrug resistan*' OR 'multiple drug resistan*' OR 'antibiotic susceptib*' OR 'antimicrobial* susceptib*' OR 'drug* susceptib*' OR 'multi-drug susceptib*' OR 'multidrug susceptib*' OR 'multiple-drug susceptib*' OR 'multiple drug* susceptib*' OR 'AMR' OR 'anti#microbial resistance' OR 'antibiotic resistance') AND
('E coli' OR 'Escherichia coli' OR 'Klebsiella' OR 'Enterobacteriaceae')
Search strategy for Web of Science
TS=("africa" OR "comoros" OR "Djibouti" OR "madagascar" OR "malawi" OR "seychelles" OR "cameroon" OR "central african republic" OR "chad" OR "congo" OR "DRC" OR "equatorial guinea" OR "atlantic islands" OR "gabon" OR "south sudan" OR "sudan" OR "botswana" OR "lesotho" OR "swaziland" OR "eswatini" OR "benin" OR "burkina faso" OR "cape verde" OR "sao tome and principe" OR "ghana" OR "guinea" OR "guinea-bissau" OR "mauritania" OR "mauritius" OR "niger" OR "senegal" OR "sierra leone" OR "togo" OR "burundi*" OR "eritrea*" OR "ethiopia*" OR "kenya*" OR "mozambique*" OR "rwanda*" OR "somalia*" OR "tanzania*" OR "uganda*" OR "zambia*" OR "zimbabwe*" OR "angola*" OR "namibia*" OR "south africa*" OR "gambia*" OR "liberia*" OR "mali*" OR "nigeria*" OR "cote d'ivoire" OR "ivory coast") AND
TS=(anti-bacterial resistance OR antibacterial resistance OR antibiotic resistan* OR antimicrobial resistan* OR drug resistan* OR drug-resistan* OR multi-drug resistan* OR multidrug resistan* OR multiple drug resistan* OR antibiotic susceptib* OR antimicrobial* susceptib* OR drug* susceptib* OR multi-drug susceptib* OR multidrug susceptib* OR multiple-drug susceptib* OR multiple drug susceptib* OR AMR OR anti-microbial resistance OR antibiotic resistance) AND
Search strategy for African Journals Online
(antimicrobial resistan* OR antibiotic resistan* OR AMR) AND (Escherichia coli OR "E. coli" OR Klebsiella OR Enterobacteriaceae)
2016-2019

Table 2. Quality assessment tool modified after the Newcastle Ottawa Scale

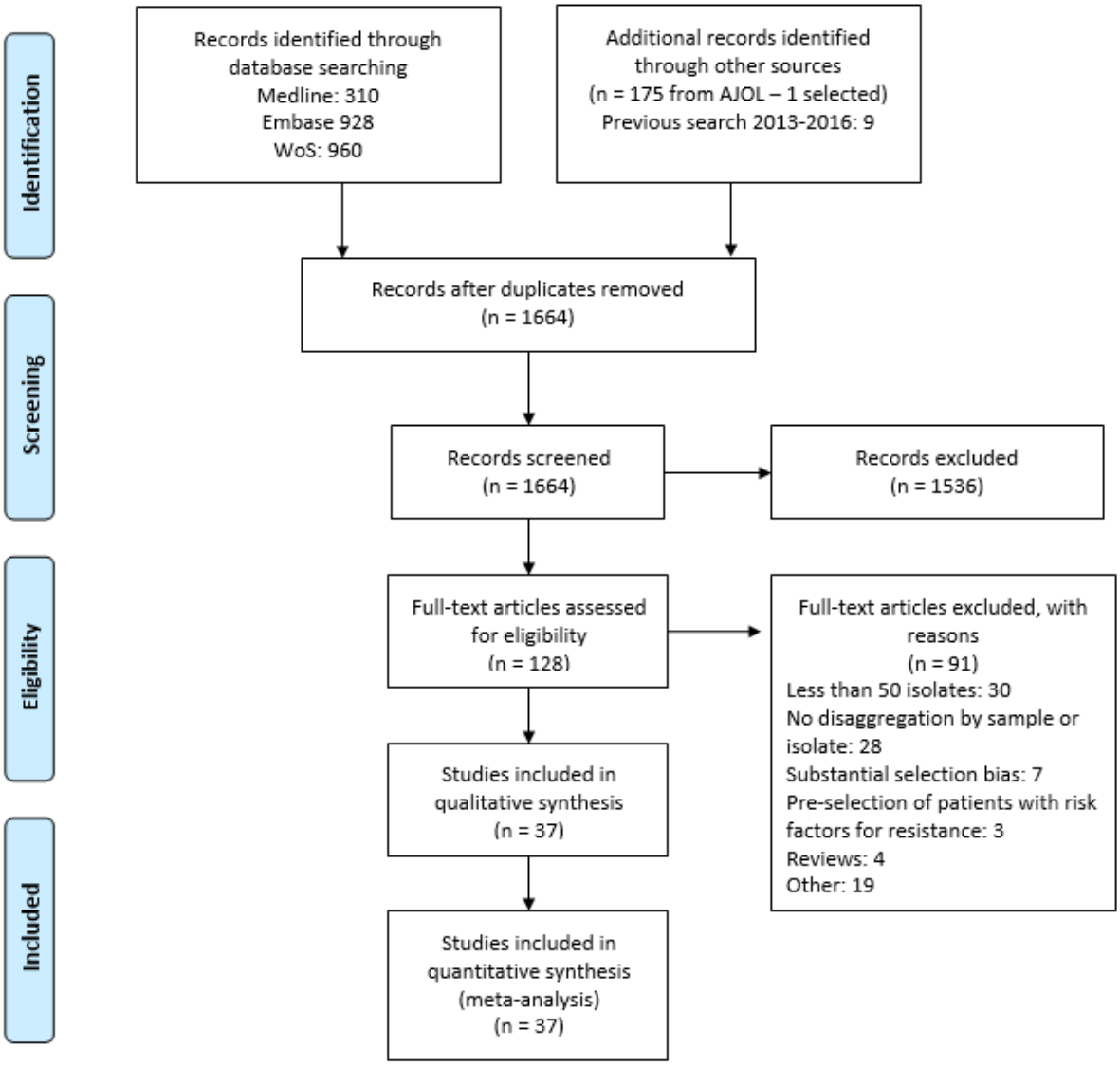
Selection
1. Representativeness of the target population: a. 3p-Truly representative of the average in the target population. * (all subjects or random sampling) b. 2p-Somewhat representative of the average in the target population. * (nonrandom sampling) c. 1p-Selected group of users. d. 0p-No description of the sampling strategy.
2. Representativeness of the samples: a. 3p-Truly representative of the average in the target population. * (all subjects or random sampling) b. 2p-Somewhat representative of the average in the target population. * (nonrandom sampling) c. 1p-Selected group of users. d. 0p-No description of the sampling strategy.
2. Sample size: a) 1p-Justified and satisfactory. b) 0p-Not justified.
3. Samples not tested: a) 2p-Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. * b) 1p-The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) 0p-No description of the response rate or the characteristics of the responders and the non-responders.
Comparability
1. The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled. a) 1p-The study controls for the most important factor (select one). b) 0p-The study control for any additional factor.
Outcome
1. Assessment of the outcome a) 2p-testing methods clearely described b) 1p-testing methods partially described c) 0p-testing methods not described
2. Quality of outcome ascertainment a) 2p-IQA and EQA described b) 1p-IQA or EQA described c) 0p-no description of either EQA or IQA
3. Statistical test: a) 1p-The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level. b) 0p-The statistical test is not appropriate, not described or incomplete.

Results

Characteristics of identified studies

Of the 1654 unique citations identified by the search, 128 were selected for full-text review and 28 were considered eligible. An additional 9 articles published between 2013 and 2016 were identified from the previously published systematic review. A total of 37 articles were selected for the qualitative and quantitative analysis (Figure 1; Chapter 2: Table 1). Countries with more than one study were Nigeria (n=8),[20-27] Ethiopia (n=6),[28-33] South Africa (n=5),[34-38] Kenya (n=4),[39-42] Tanzania (n=4) [43-46] and Ghana (n=3).[18,47,48] One study each was conducted in Malawi,[49] Chad,[50] Ivory Coast,[51] Madagascar,[52] Senegal,[53] and Zambia[54] (Chapter 2: Figure 1). One study reported AMR prevalence using data from 9 African countries.[55] Of the 37 eligible studies, 16 (43%) reported on isolates collected between 2010 and 2014[22,23,26,27,30-33,38,42,48,50,52-55] and 8 (22%)[18,21,24,29,37,43,45,46] reported on isolates collected after 2015. 19 of the studies collected data prospectively, while 16 extracted data from medical records or laboratory databases; two studies did not specify how data collection was performed.

Figure 1. PRISMA Flow Diagram for the included studies



Other: Unable to extract AST results: 6; not from sub-Saharan Africa: 1; not available as full text:2; not reporting on isolates causing infections: 4; very limited/ no AST results: 5; duplicated study: 1

Healthcare facilities and patient population

The majority of studies (n=22, 59%) reported data from a single-facility, usually a tertiary-care hospital (n=20, 54%). Only 6 (16%) studies were from district/regional hospitals, 2 (5%) from primary care clinics, 5 (14%) from a combination of higher- and lower-level facilities; for 4 (11%) studies, the type of healthcare facility was not specified.

Most studies were conducted in inpatients 12 (32%)[26,34-36,38,39,41,45,47-49,54] or in a combination of inpatients and outpatients in 15 (41%) studies[20-22,25,28,31,32,42-44,46,50,51,53,55]; 4 (11%) studies reported on data from outpatients only, and 6 (13%) studies did not specify the

setting.[18,29,30,33,37,52] Of the 4 studies in outpatients, 3 were in pregnant women attending antenatal clinics investigated for asymptomatic bacteriuria (2 studies[23,40]) or symptomatic urinary tract infections (UTIs, 1 study[21]), and one study reported on women with UTI symptoms attending a tertiary-care hospital as outpatients.[24] Of the 27 studies reporting on inpatients, only 9 (33%) described if infections were community- or hospital acquired, and in 4 community-acquired infections were predominant.[22,44,53,55]

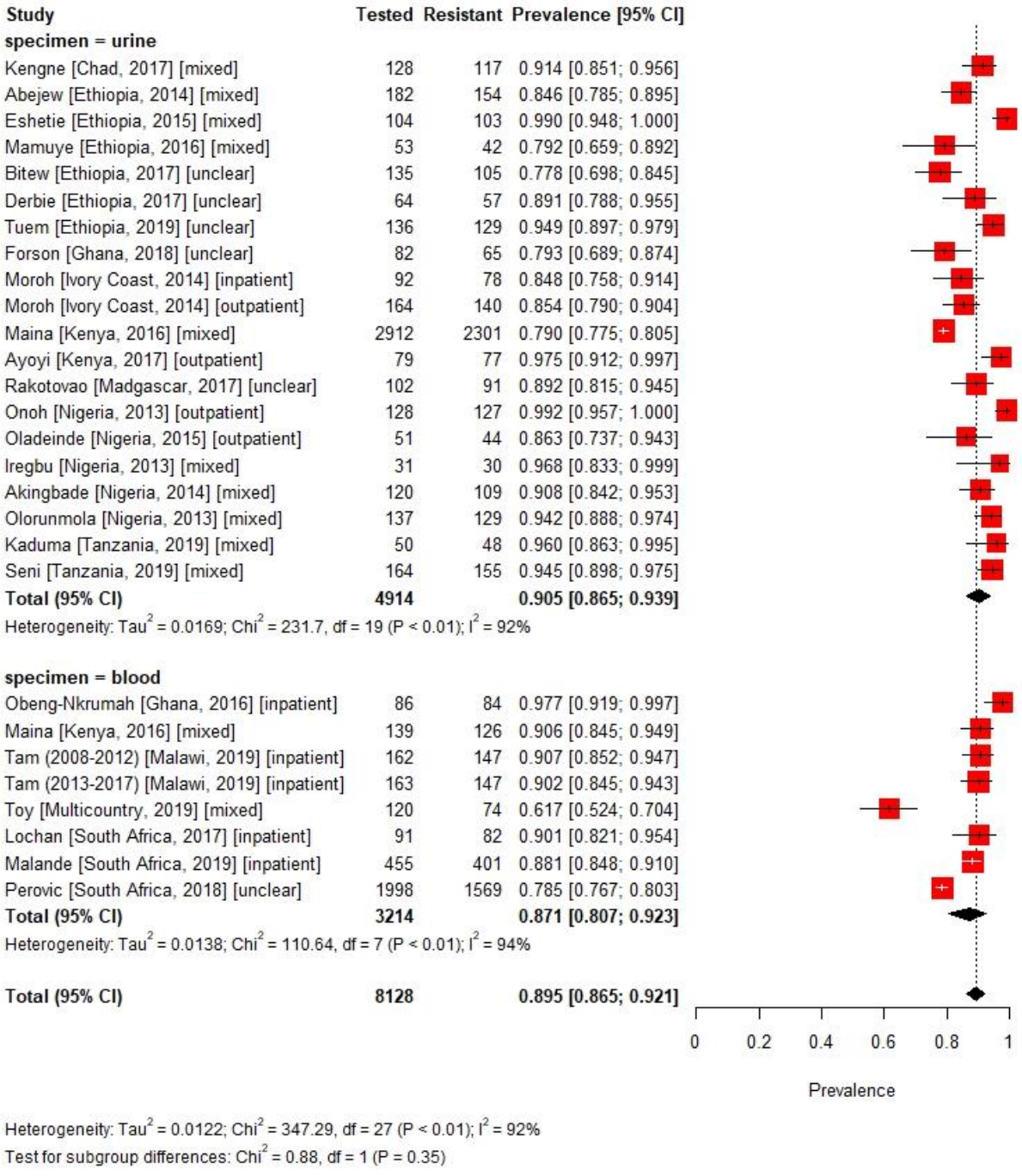
Samples, laboratories and testing methods

Samples were mostly processed in hospital laboratories in 15 (41%) studies, or in other types of laboratories (research, university and private laboratories, n=8); 14 (38%) studies did not specify the type of laboratory. Isolates were from urine (n=22 studies), blood (n=14 studies) and both urine and blood (n=1 study). Of 17,885 *E. coli* and *K. pneumoniae* isolates, for which information on the country of isolation was provided, 13,994 (78%) were from South Africa and Kenya. There were differences between the number of organisms isolated and the number tested for susceptibility. AST data were presented for 9646 of 10,643 (91%) *E. coli* and for 8446 of 9423 *K. pneumoniae* (90%) isolates. Seven studies presented AST data on fewer than 90% of the organisms isolated.[22,28,30,35,36,39,51]

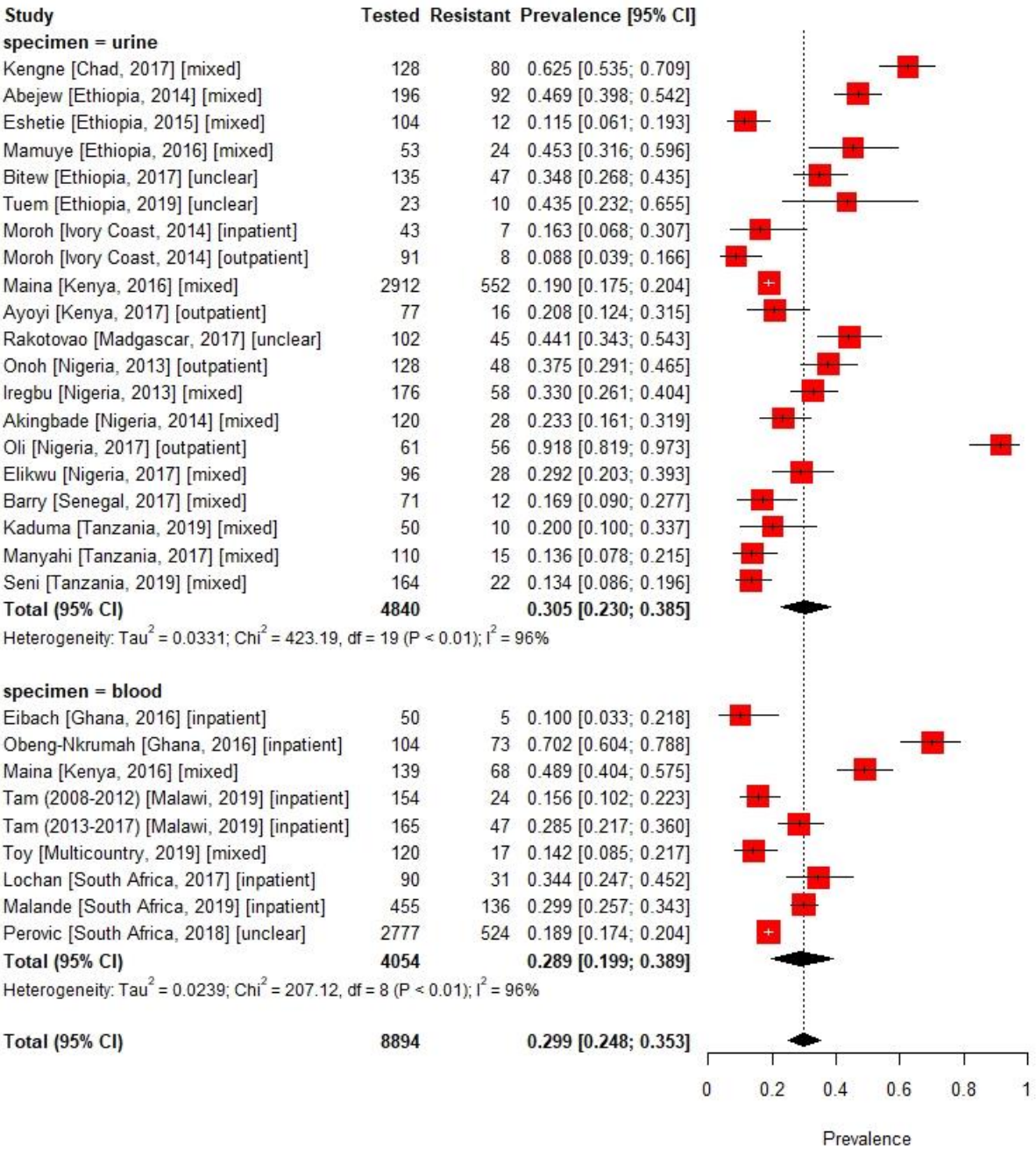
Of the 15 studies reporting on isolates from blood cultures, most used automated culture systems (12/15). AST was done using automated methods (n=11, 30%) and disk diffusion (n=24, 65%). Two (5%) studies did not specify the methods used for AST. Most laboratories (n=22) used the Clinical and Laboratory Standards Institute (CLSI) guidelines for interpreting AST results,[56] 8 studies used other national or international standards, while 7 studies failed to report the standards used. Only 12 studies described the implemented quality control procedures. Two of the laboratories reported being ISO-accredited and 3 had quality management systems in place.

Figure 2. Prevalence of *E. coli* resistance: ampicillin (Panel A), third generation cephalosporins (Panel B), ciprofloxacin (Panel C), and gentamicin (Panel D) of isolates from blood and urine samples

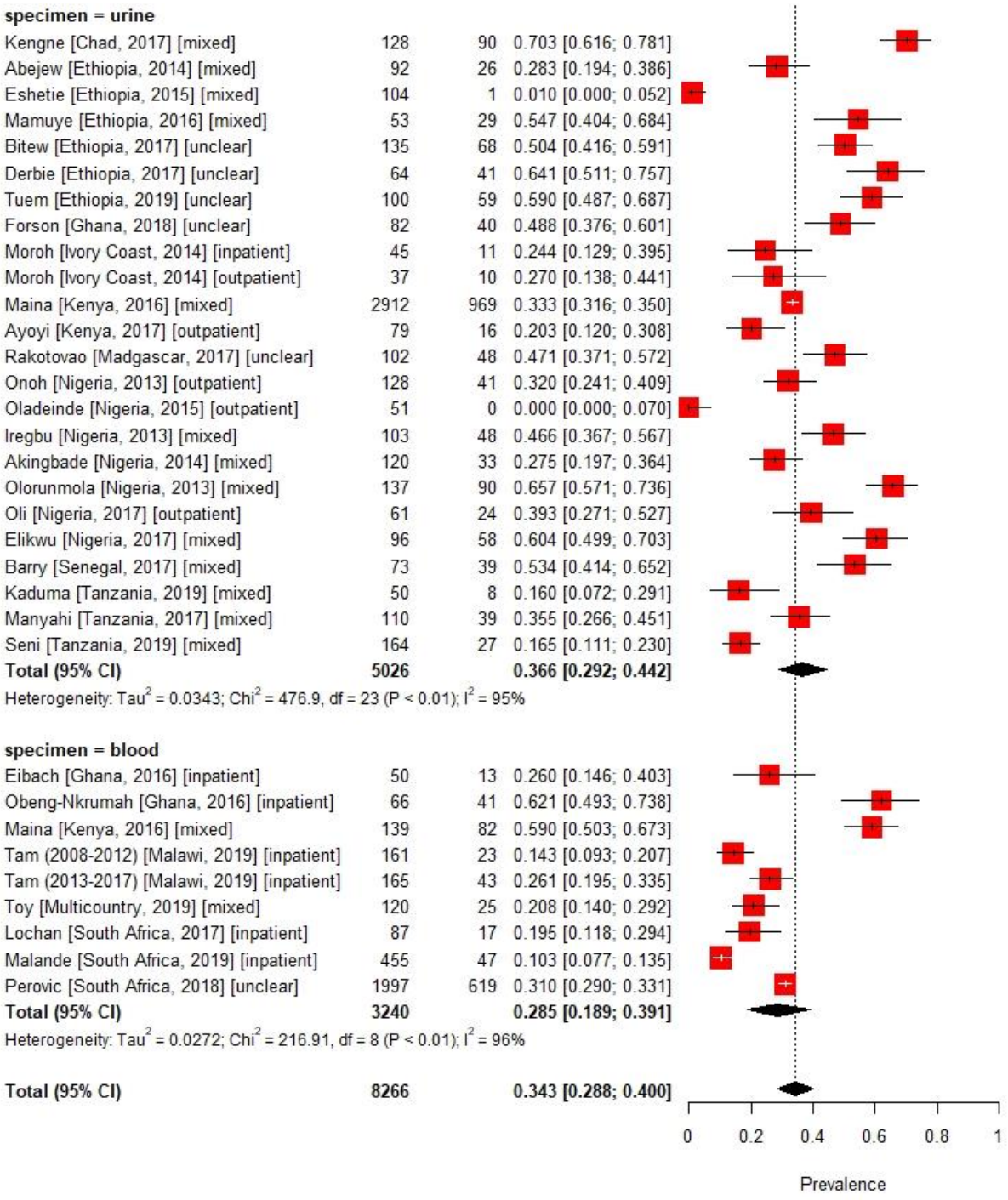
Panel A



Panel B


Study
Tested
Resistant
Prevalence [95% CI]
specimen = urine
Kengne [Chad, 2017] [mixed] 128 80 0.625 [0.535; 0.709]
Abejew [Ethiopia, 2014] [mixed] 196 92 0.469 [0.398; 0.542]
Eshetie [Ethiopia, 2015] [mixed] 104 12 0.115 [0.061; 0.193]
Mamuye [Ethiopia, 2016] [mixed] 53 24 0.453 [0.316; 0.596]
Bitew [Ethiopia, 2017] [unclear] 135 47 0.348 [0.268; 0.435]
Tuem [Ethiopia, 2019] [unclear] 23 10 0.435 [0.232; 0.655]
Moroh [Ivory Coast, 2014] [inpatient] 43 7 0.163 [0.068; 0.307]
Moroh [Ivory Coast, 2014] [outpatient] 91 8 0.088 [0.039; 0.166]
Maina [Kenya, 2016] [mixed] 2912 552 0.190 [0.175; 0.204]
Ayoyi [Kenya, 2017] [outpatient] 77 16 0.208 [0.124; 0.315]
Rakotovao [Madgascar, 2017] [unclear] 102 45 0.441 [0.343; 0.543]
Onoh [Nigeria, 2013] [outpatient] 128 48 0.375 [0.291; 0.465]
Iregbu [Nigeria, 2013] [mixed] 176 58 0.330 [0.261; 0.404]
Akingbade [Nigeria, 2014] [mixed] 120 28 0.233 [0.161; 0.319]
Oli [Nigeria, 2017] [outpatient] 61 56 0.918 [0.819; 0.973]
Elikwu [Nigeria, 2017] [mixed] 96 28 0.292 [0.203; 0.393]
Barry [Senegal, 2017] [mixed] 71 12 0.169 [0.090; 0.277]
Kaduma [Tanzania, 2019] [mixed] 50 10 0.200 [0.100; 0.337]
Manyahi [Tanzania, 2017] [mixed] 110 15 0.136 [0.078; 0.215]
Seni [Tanzania, 2019] [mixed] 164 22 0.134 [0.086; 0.196]
Total (95% CI) 4840 0.305 [0.230; 0.385]
Heterogeneity: Tau² = 0.0331; Chi² = 423.19, df = 19 (P < 0.01); I² = 96%
specimen = blood
Eibach [Ghana, 2016] [inpatient] 50 5 0.100 [0.033; 0.218]
Obeng-Nkrumah [Ghana, 2016] [inpatient] 104 73 0.702 [0.604; 0.788]
Maina [Kenya, 2016] [mixed] 139 68 0.489 [0.404; 0.575]
Tam (2008-2012) [Malawi, 2019] [inpatient] 154 24 0.156 [0.102; 0.223]
Tam (2013-2017) [Malawi, 2019] [inpatient] 165 47 0.285 [0.217; 0.360]
Toy [Multicountry, 2019] [mixed] 120 17 0.142 [0.085; 0.217]
Lochan [South Africa, 2017] [inpatient] 90 31 0.344 [0.247; 0.452]
Malande [South Africa, 2019] [inpatient] 455 136 0.299 [0.257; 0.343]
Perovic [South Africa, 2018] [unclear] 2777 524 0.189 [0.174; 0.204]
Total (95% CI) 4054 0.289 [0.199; 0.389]
Heterogeneity: Tau² = 0.0239; Chi² = 207.12, df = 8 (P < 0.01); I² = 96%
Total (95% CI) 8894 0.299 [0.248; 0.353]
0
0.2
0.4
0.6
0.8
1
Prevalence
Heterogeneity: Tau² = 0.0215; Chi² = 632.15, df = 28 (P < 0.01); I² = 96%
Test for subgroup differences: Chi² = 0.07, df = 1 (P = 0.80)

Panel C



Heterogeneity: $Tau^2 = 0.0268$; $Chi^2 = 751.82$, df = 32 ($P < 0.01$); $I^2 = 96\%$

Panel D

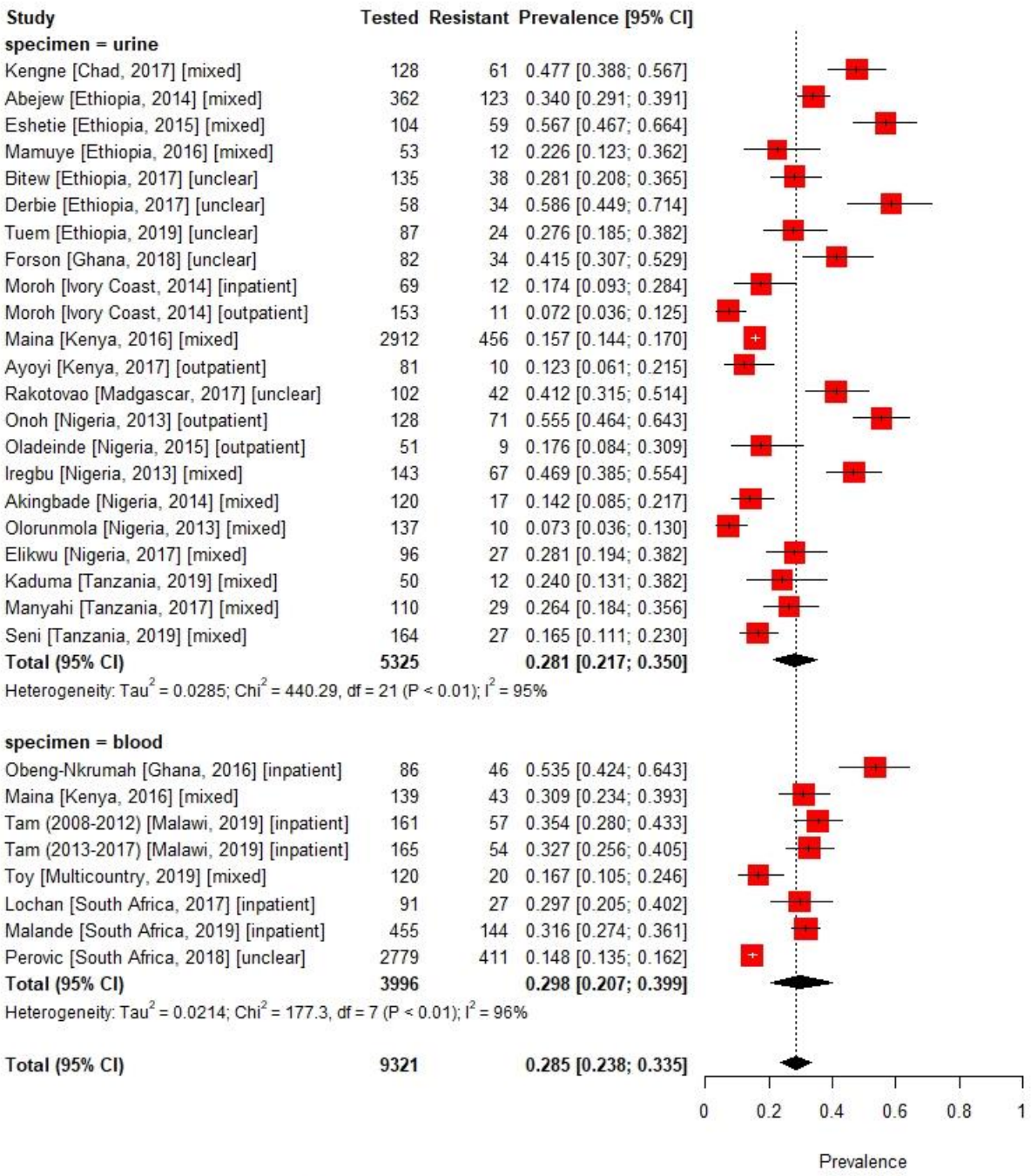
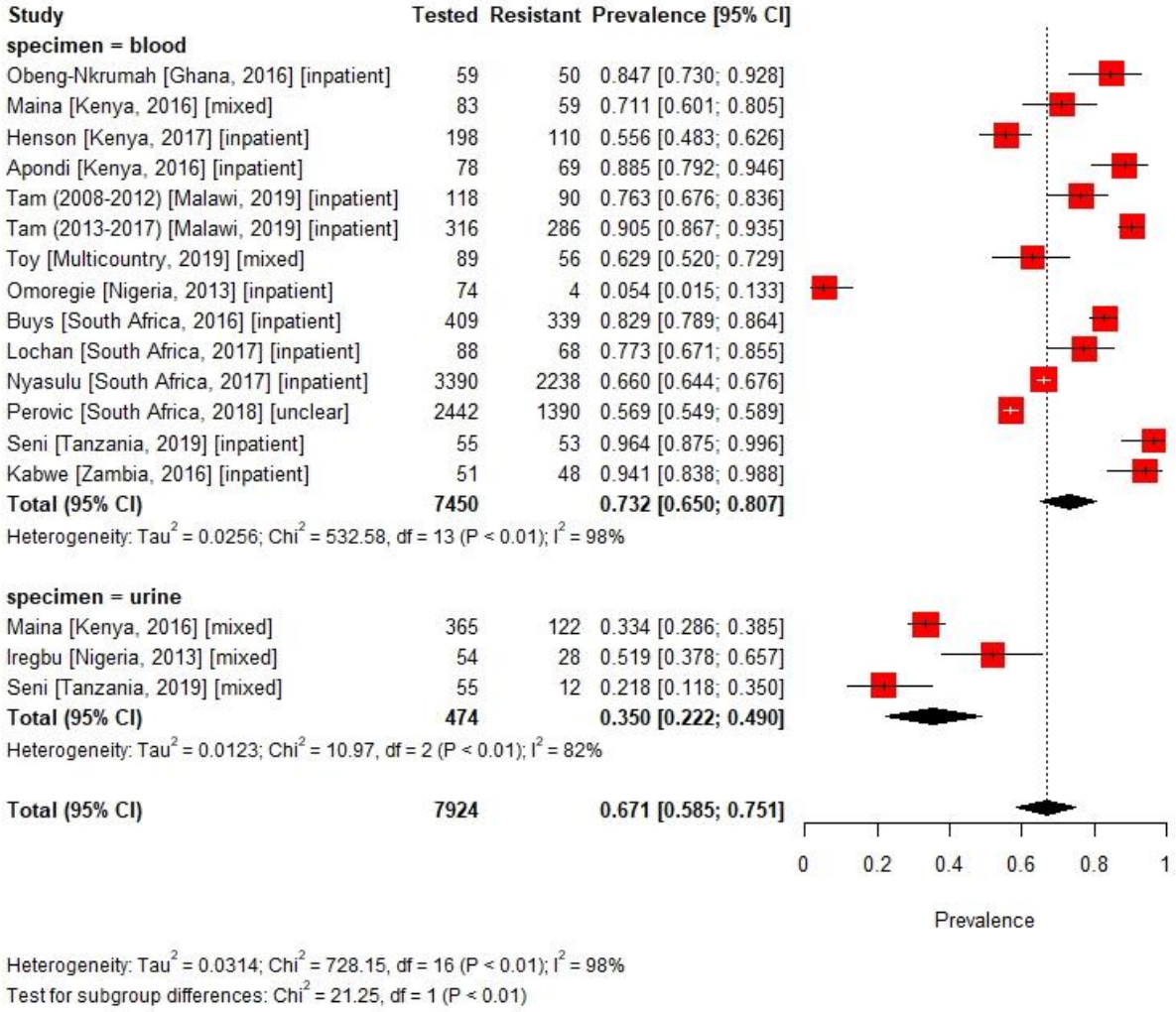
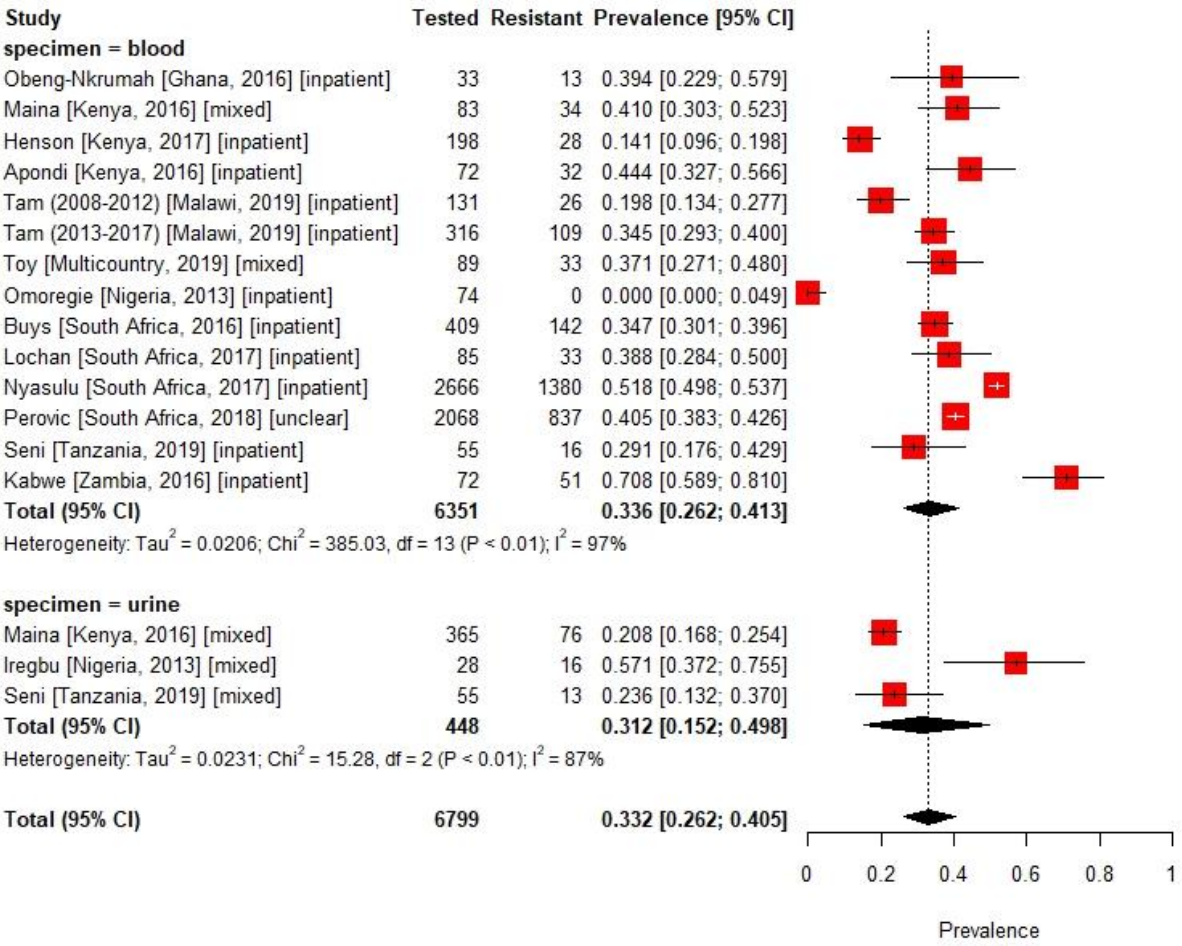

Study
Tested
Resistant
Prevalence [95% CI]
specimen = urine
Kengne [Chad, 2017] [mixed] 128 61 0.477 [0.388; 0.567]
Abejew [Ethiopia, 2014] [mixed] 362 123 0.340 [0.291; 0.391]
Eshetie [Ethiopia, 2015] [mixed] 104 59 0.567 [0.467; 0.664]
Mamuye [Ethiopia, 2016] [mixed] 53 12 0.226 [0.123; 0.362]
Bitew [Ethiopia, 2017] [unclear] 135 38 0.281 [0.208; 0.365]
Derbie [Ethiopia, 2017] [unclear] 58 34 0.586 [0.449; 0.714]
Tuem [Ethiopia, 2019] [unclear] 87 24 0.276 [0.185; 0.382]
Forson [Ghana, 2018] [unclear] 82 34 0.415 [0.307; 0.529]
Moroh [Ivory Coast, 2014] [inpatient] 69 12 0.174 [0.093; 0.284]
Moroh [Ivory Coast, 2014] [outpatient] 153 11 0.072 [0.036; 0.125]
Maina [Kenya, 2016] [mixed] 2912 456 0.157 [0.144; 0.170]
Ayoyi [Kenya, 2017] [outpatient] 81 10 0.123 [0.061; 0.215]
Rakotovao [Madgascar, 2017] [unclear] 102 42 0.412 [0.315; 0.514]
Onoh [Nigeria, 2013] [outpatient] 128 71 0.555 [0.464; 0.643]
Oladeinde [Nigeria, 2015] [outpatient] 51 9 0.176 [0.084; 0.309]
Iregbu [Nigeria, 2013] [mixed] 143 67 0.469 [0.385; 0.554]
Akingbade [Nigeria, 2014] [mixed] 120 17 0.142 [0.085; 0.217]
Olorunmola [Nigeria, 2013] [mixed] 137 10 0.073 [0.036; 0.130]
Elikwu [Nigeria, 2017] [mixed] 96 27 0.281 [0.194; 0.382]
Kaduma [Tanzania, 2019] [mixed] 50 12 0.240 [0.131; 0.382]
Manyahi [Tanzania, 2017] [mixed] 110 29 0.264 [0.184; 0.356]
Seni [Tanzania, 2019] [mixed] 164 27 0.165 [0.111; 0.230]
Total (95% CI) 5325 0.281 [0.217; 0.350]
Heterogeneity: Tau² = 0.0285; Chi² = 440.29, df = 21 (P < 0.01); I² = 95%
specimen = blood
Obeng-Nkrumah [Ghana, 2016] [inpatient] 86 46 0.535 [0.424; 0.643]
Maina [Kenya, 2016] [mixed] 139 43 0.309 [0.234; 0.393]
Tam (2008-2012) [Malawi, 2019] [inpatient] 161 57 0.354 [0.280; 0.433]
Tam (2013-2017) [Malawi, 2019] [inpatient] 165 54 0.327 [0.256; 0.405]
Toy [Multicountry, 2019] [mixed] 120 20 0.167 [0.105; 0.246]
Lochan [South Africa, 2017] [inpatient] 91 27 0.297 [0.205; 0.402]
Malande [South Africa, 2019] [inpatient] 455 144 0.316 [0.274; 0.361]
Perovic [South Africa, 2018] [unclear] 2779 411 0.148 [0.135; 0.162]
Total (95% CI) 3996 0.298 [0.207; 0.399]
Heterogeneity: Tau² = 0.0214; Chi² = 177.3, df = 7 (P < 0.01); I² = 96%
Total (95% CI) 9321 0.285 [0.238; 0.335]
0
0.2
0.4
0.6
0.8
1
Prevalence
Heterogeneity: Tau² = 0.0197; Chi² = 622.44, df = 29 (P < 0.01); I² = 95%
Test for subgroup differences: Chi² = 0.08, df = 1 (P = 0.78)

Figure 3. Prevalence of *Klebsiella pneumoniae.* resistance: third generation cephalosporins (Panel A), ciprofloxacin (Panel B), gentamicin (Panel C), and carbapenems (Panel D) of isolates from blood and urine samples

Panel A

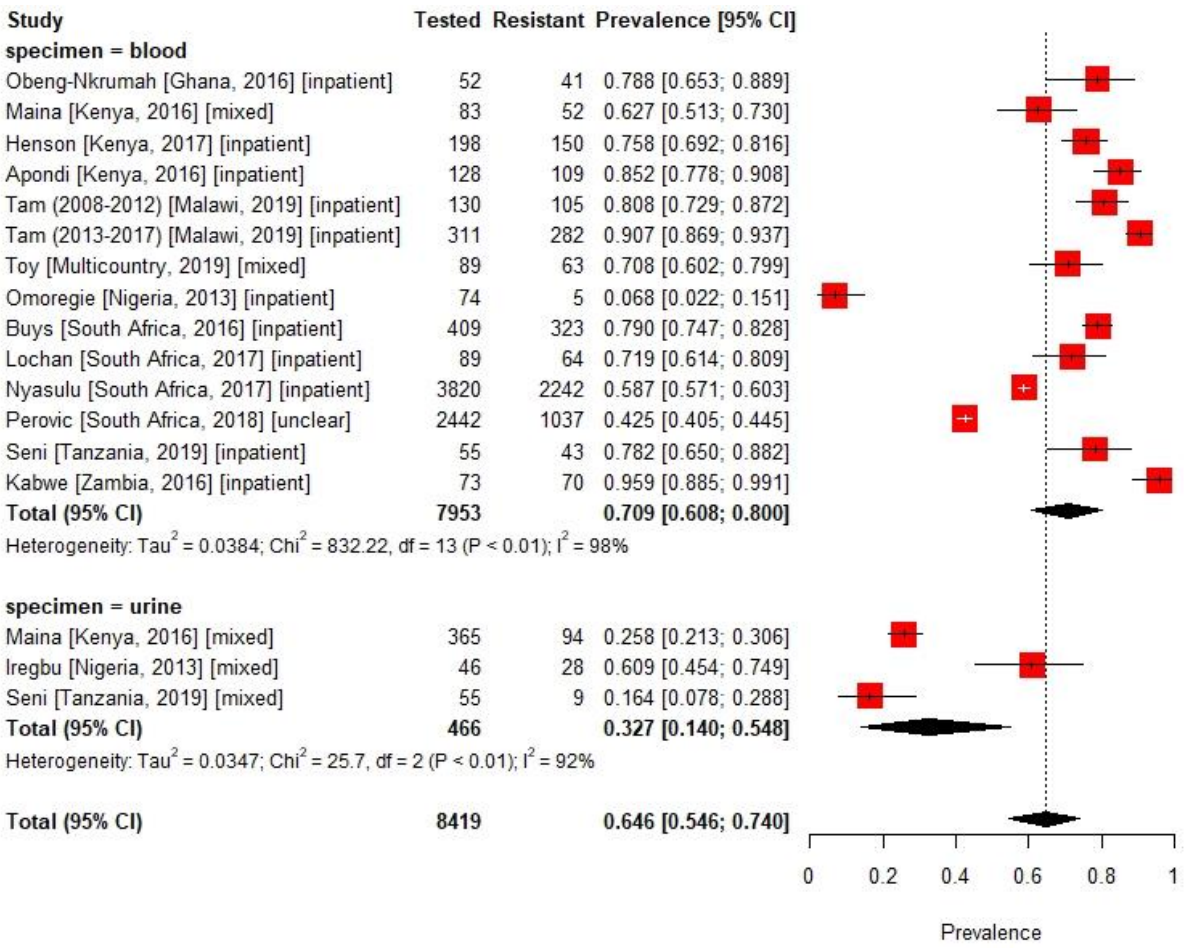


Panel B



Heterogeneity: $Tau^2 = 0.0226$; $Chi^2 = 472.63$, df = 16 ($P < 0.01$); $I^2 = 97\%$
Test for subgroup differences: $Chi^2 = 0.05$, df = 1 ($P = 0.82$)

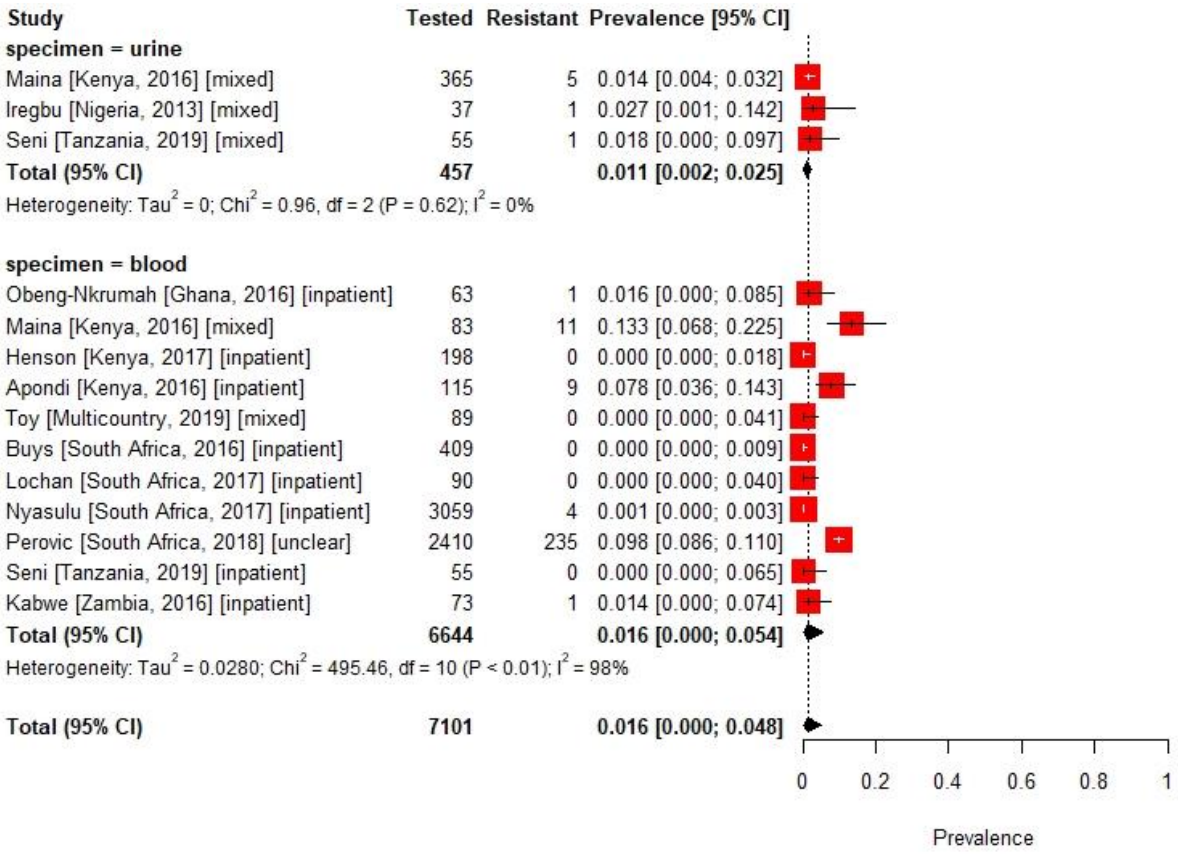
Panel C



Heterogeneity: $Tau^2 = 0.0428$; $Chi^2 = 1029.43$, df = 16 ($P < 0.01$); $I^2 = 98\%$

Test for subgroup differences: $Chi^2 = 9.58$, df = 1 ($P < 0.01$)

Panel D



Heterogeneity: $Tau^2 = 0.0246$; $Chi^2 = 496.89$, df = 13 (P < 0.01); $I^2 = 97\%$

Test for subgroup differences: $Chi^2 = 0.02$, df = 1 (P = 0.90)

Prevalence of antimicrobial resistance

A total of 22 studies reported AMR prevalence data for *E coli*, 7 for *K. pneumoniae* and 8 studies reported on both *E coli* and *K pneumoniae*.

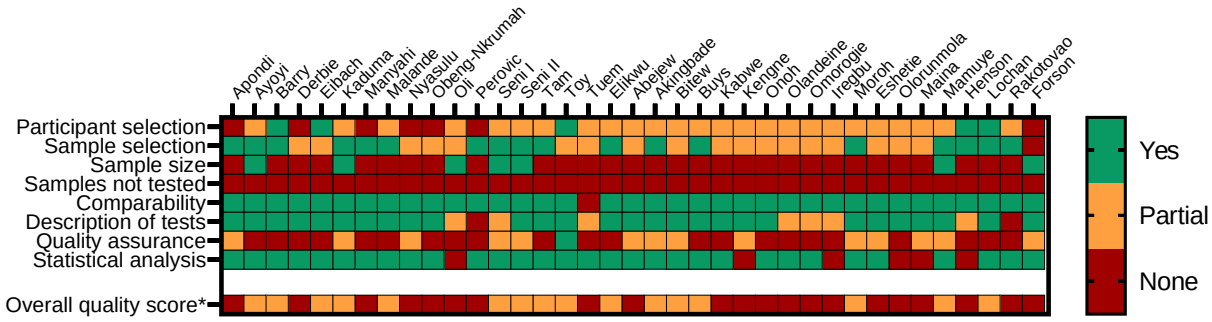
Twenty-three studies totalling 5583 isolates presented data for *E. coli* from urine and 8 studies reported on 4065 *E. coli* isolates from blood cultures. The pooled resistance prevalence across all settings for *E. coli* from blood samples was 87.1% (95%CI 80.7-92.3), 28.9% (95%CI 19.9-38.9), 28.5% (95%CI 18.9-39.1), 29.8% (95%CI 20.7-39.9), and 87.2% (95%CI 73.0-96.7) for ampicillin, third-generation cephalosporins, ciprofloxacin, gentamicin and co-trimoxazole, respectively. For urine samples, the pooled resistance prevalence was 90.5% (95%CI 86.5-93.9), 30.5% (95%CI 23.0-38.5), 36.6% (96%CI 29.2-44.2), 28.1% (95%CI 21.7-35.0), and 78.5% (95%CI 74.2-82.5) for ampicillin, third-generation cephalosporins, ciprofloxacin, gentamicin and co-trimoxazole. AMR prevalence was similar in both specimen types (Figure 2). Prevalence estimates were highly heterogeneous across studies.

Seven studies totalling 7972 isolates presented data for *K. pneumoniae* from blood cultures and 3 studies reported on 474 isolates from urine. Pooled resistance prevalence for *Klebsiella pneumoniae* (Figure 3) from blood cultures was 73.2% (95%CI 65.0-80.7), 33.6% (95%CI 26.2-41.3), 70.9% (60.8-80.0), and 87.0% (95%CI 79.4-93.1) for third generation cephalosporins, ciprofloxacin, gentamicin and co-trimoxazole respectively with high heterogeneity. For urine cultures, the pooled prevalence of resistance was 35.0% (95%CI 22.2-49.0), 31.2% (95%CI 15.2-49.8), 32.7% (95%CI 14.0-54.8), and 74.9% (95%CI 41.3-97.1) for third generation cephalosporins, ciprofloxacin, gentamicin and co-trimoxazole respectively. Isolates from blood had a considerably higher prevalence of resistance to all the antibiotics analysed (Figure 3). Carbapenem resistance in *Klebsiella spp.* isolates was low, with only one study reporting a prevalence of resistance exceeding 10%.[42]

Quality of studies included

According to the modified Newcastle-Ottawa Scale grading, 16 studies were considered to have moderate quality and 21 studies low quality (Table 2, Figure 4). Selection bias of patients and samples as well as potential ascertainment bias were the main reasons for low quality. Most studies included patients and samples from higher-level healthcare facilities or laboratories exclusively. Few studies were conducted in lower-level facilities or in a combination of lower and higher-level facilities. Furthermore, studies did not consistently provide details on missing data and samples excluded from the analysis. Also many studies did not provide sufficient information on the laboratory quality management to ensure high quality of laboratory results.

Figure 4. Quality ascertainment of the studies included in the analysis



**For the overall quality score, studies in red are low-quality and in orange medium-quality.*

Discussion

This systematic review confirms that high quality and representative data on Gram-negative AMR from sub-Saharan Africa are limited. The studies included in this review report concerningly high prevalence of resistance to first- and second-line antibiotics which threaten the ability to successfully treat severe Gram-negative infections in the future. Our search identified only 37 studies from 12 of the 47 (26%) sub-Saharan Africa countries. Furthermore, almost 80% of the isolates were from studies conducted in only two countries (Kenya and South Africa).

Data from these studies may have limited generalizability. More than half of the studies report data from a single-facility, mainly from tertiary-level and referral hospitals. Patients who present to higher-level facilities may be systematically different from the general population and outpatients. They are more likely to have complex illnesses putting them at increased risk of infections with resistant bacteria due to prior healthcare contact and antibiotic usage.[57] This may result in biased (overestimated) resistance prevalence. One of the explanations for the over-representation of higher-level facilities in studies from LMIC is the limited laboratory capacity in these countries.

Few studies identified by this review included patients presenting or admitted to district or regional hospitals highlighting the need for lower-tier laboratory strengthening and reporting of findings. In 2019 the WHO published the Model List of Essential *in vitro* Diagnostics. Culture-based diagnostics for bloodstream and UTIs and AST should be as a minimum standard available at district- and regional-laboratories.[58]

AMR prevalence is generally higher in hospital-acquired compared to community-acquired infections.[59] As such the information whether an infection is hospital or community-acquired is crucial when interpreting AMR data. However, few studies reported whether the samples were from community- or hospital-acquired infections or sufficiently described the patient population.

UTIs are a very common cause of presentation to primary care.[60] Only four of 23 studies presenting data on isolates from urine cultures, were conducted in outpatients settings, two from primary care clinics. All four studies included pre-selected populations (mostly pregnant women) from two countries. Although urinary cultures are not routine practice for the diagnosis and treatment of UTIs in primary care, they can be invaluable for AMR surveillance purposes and can provide information on community AMR prevalence.[7] Conversely, inclusion of urine isolates from hospitalized or referred patients only will result in high AMR prevalence estimates.[61,62] Urine samples are easy to collect and process for culture. While yield of cultures can vary according to setting, it is generally better than that of blood cultures. Therefore, urine cultures performed at sentinel sites should considered for estimating the burden of AMR in Gram-negative community-acquired infections.

The prevalence of resistance to most of the available and inexpensive antibiotic classes in sub-Saharan Africa was concerningly high thus drastically limiting treatment options for severe infections. The prevalence of ampicillin and co-trimoxazole resistance among *E. coli* was almost 90% which was similar to that reported in the previous systematic review.[4] Widespread use of amoxicillin as first line drug for treatment of a broad range of infections and co-trimoxazole prophylaxis in patients with HIV may explain these findings. Fluoroquinolones are the main oral treatment option for Gram-negative infections, while third-generation cephalosporins are the most commonly used antibiotic for treatment of severe Gram-negative infections in sub-Saharan Africa.[63] Over the last decade some settings have experienced a three-fold increase in resistance to third-generation cephalosporins among *Enterobacterales* with smaller increases recorded for fluroquinolones.[64] The findings of this review further highlight the high prevalence of resistance to these key drug classes. Almost a third of *E. coli* and two thirds of *K. pneumoniae* isolates were resistant to third-generation cephalosporins. Reassuringly carbapenem resistance is still rare in sub-Saharan Africa due to the limited availability and high cost of the drugs.

The quality of the studies was low or moderate. While the testing methods were well described in most publications, little additional information was provided about the laboratories processing the samples and the laboratory quality management. There was a general lack of

reporting of laboratory procedures employed to ensure the quality of laboratory data. Less than a third of studies reported on the performance of some component for internal quality control and only one study described external quality control for verification of results. Given the incomplete reporting on the quality of AST which may lead to misclassification and the importance of AMR globally, a checklist should be developed to ensure minimal information is provided for studies reporting on phenotypic AST.

This review is limited by the small number of studies and over-representation of samples from South Africa and Kenya. High heterogeneity between studies regarding study design, healthcare facilities, patient and isolate selection, testing methods and result interpretation limits comparability between studies. The strengths of this review include the comprehensive search strategy using multiple databases and inclusion of all studies irrespective of language.

In conclusion, this review highlights the substantial gap in high-quality, generalizable AMR prevalence data in priority pathogens, *E. coli* and *K. pneumoniae*, from sub-Saharan Africa alongside with concerningly high prevalence of resistance to key drug classes. In particular, there is a lack of data from lower-level facility-based studies which may preclude our understanding of AMR and thus impede the development of strategies to effectively combat resistance in these settings. These findings emphasize the need to strengthen laboratory capacity in LMICs and promote research into AMR.

1.1.2.2. Antimicrobial resistance and treatment recommendations in Zimbabwe

This section summarises the few studies available from Zimbabwe which were not eligible for inclusion in the systematic review presented in the previous section and puts the data in the context of the Zimbabwean national treatment recommendations. The studies were ineligible because they did not report AST according to bacterial species or sample type.

A few studies on AMR were conducted in Zimbabwe and suggest a high prevalence of resistance in Gram-negatives to key antibiotics. Third-generation cephalosporin resistance was 35%[65] in *Escherichia coli* from clinical isolates and 14-52% in colonising *Enterobacterales* in 2014-2015.[66,67] One of the studies, which was conducted between 2012 and 2017 in a private laboratory in Harare reported an increasing prevalence of resistance for several pathogen-drug combinations over the study period.[65] Although, the study included a large number of samples, resistance prevalence was not stratified by organism, specimen type or site of infection. Given that patients have to pay for diagnostic tests conducted in private laboratories results may not reflect the prevalence of AMR among the general population who cannot afford the costs of microbiological testing.

Insufficient AMR data both from routine surveillance as well as research means that antibiotic treatment guidelines are generic rather than setting-specific. For example, the Essential Medicines List and Standard Treatment Guidelines for Zimbabwe (EDLIZ) provides healthcare professionals with guidance for drug prescribing including antibiotics across all healthcare settings.[68] EDLIZ recommends amoxicillin or a fluoroquinolone for first-line treatment of UTI despite a >80% prevalence of resistance for amoxicillin among *E. coli* reported by studies conducted in the region.[4,64,68] For neonatal sepsis, the same guidelines recommend benzylpenicillin or ampicillin and gentamicin for early-onset neonatal sepsis.[69] According to a systematic review, *Klebsiella pneumoniae,* which is intrinsically resistant to aminopenicillins, is the most common cause of neonatal sepsis in Southern Africa and the prevalence of resistance to ceftriaxone and gentamicin is 94% and 83%, respectively.[70] In these scenarios, prescribers adhering to the guidelines will prescribe ineffective antibiotics which may result in unfavourable patient outcomes.

1.2. Laboratory testing for culture and antibiotic susceptibility testing

Insufficient availability of high-quality laboratory services for diagnosis of infections and antibiotic susceptibility testing is one of the main reasons for lacking AMR data in low-resource settings. WHO-AFRO has developed the Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA) checklist to guide laboratories towards accreditation with the aim to support laboratory strengthening and ultimately improve testing quality.[71] Overall, enrolment of laboratories from the African Region has been slower than anticipated and few received high ratings when audited.[72] The checklist used for the assessments comprises 12 items addressing specific domains of quality management. Table 3 summarises the challenges encountered by laboratories from low-resource settings according to the checklist domains. Similar to health system strengthening, laboratory strengthening requires long-term planning and sustained financial investment as well as political and institutional commitment. Sometimes simple interventions can address a very specific issue and result in sustained improvement while waiting for more holistic and comprehensive interventions to be implemented.

The lack of adequately maintained equipment, as well as high quality consumables and trained staff limit microbiology testing.[73] Laboratory consumables such as commercial culture media and supplies for antibiotic susceptibility testing require a well-maintained cold chain during shipment and storage, not easily achievable in situations with frequent power cuts. Lack of training and professional development opportunities negatively affect laboratory staff competence and motivation.[74] Low remuneration and poor working conditions result in high staff turnover and attrition increasing the work load among the remaining staff.[75,76]

For example, culture media prepared in-house requires frequent preparation which may be difficult in settings with shortages of trained staff or in lower-tier laboratories without autoclaves. Pre-prepared culture media with long shelf-lives is a possible solution particularly if costs were to decrease. Such media could be used to scale-up surveillance and enable access to microbiology tests in lower-level healthcare facilities.[77] Another solution would be the implementation of rapid simple molecular tests for diagnosis and detection of resistance. This has been very successful in the context of tuberculosis diagnostics which now primarily uses the GeneXpert platform.[78] Several other platforms using multiplexed PCRs are being evaluated for use in low-resource settings, but costs would need to be reduced to bring these tests to scale.

Time from specimen sampling to laboratory result is a key quality indicator. In LMICs, pre-analytic, analytic and post-analytic delays are common. Delayed results have limited impact

on clinical decision making. In a study on neonatal sepsis in Zimbabwe, delays in blood culture results meant that only 4% of results arrived in time to change clinical management.[79] Decentralised laboratory services, efficient sample (and result) transport systems and laboratory information management systems linked to electronic patient records would address some of the challenges leading to described delays.

Low laboratory quality erodes clinicians' trust in laboratory results sometimes making them reluctant to change clinical management based on laboratory results.[80] Timely, high-quality results together with improved communication between laboratory scientists and clinicians are paramount for improved patient care.

Point of care tests do not require a laboratory or any complex equipment, and are simple to conduct and interpret without extensive training. While these tests have revolutionised the diagnosis and management of a number of infectious diseases such as malaria, HIV and COVID-19, they are not yet available for the detection AMR. Of particular note is the C-reactive protein test performed at the point of care which has the potential to decrease antibiotic use in LMICs and thus influence the development of AMR.[81,82]

Table 3. Challenges in diagnostic testing across the twelve items of the laboratory accreditation checklist[71]

Item	Description
Organization	Centralized laboratories; few public sector laboratories services at lower level facilities Large number of tests conducted by private for-profit laboratories Reduced availability of diagnostics outside of public tertiary hospitals Out-of-pocket payments by patients for diagnostics
Personnel & training	Inadequate workforce (limited number, high turn-over) Insufficient staff (re)training Limited opportunities for continuous professional development
Equipment	Lack of funds for acquisition, maintenance, calibration and repair of equipment Limited technical support
Purchasing & inventory	Few commercial suppliers in-country: purchase through intermediaries; high costs, long lead-time to delivery, limited choice (no competition) and stocks; low quality of supplies Stock-outs Short shelf-life Cold chain requirements
Process control	Lack of sample rejection criteria for inappropriate samples Paper-based archiving systems making retrieval of samples challenging Insufficient quality control and assurance
Information management	Manual recording and reporting with high risk of data entry errors and delays in retrieval of results
Documents & records	Lack of an up-to-date laboratory quality manual which has been communicated to and implemented by staff Few version-controlled SOPs including SOPs for technical and managerial procedures Suboptimal systems for document control
Occurrence management	Insufficient occurrence investigation and feedback of results Lack of performance and documentation of root cause analyses
Assessment	Lack of regular internal audits Infrequent participation in EQA schemes: high enrolment costs; country specific schemes non-existent
Process improvement	Insufficient use of quality indicators (e.g. turnaround times to result not monitored – results available to clinicians too late to be used for patient management)
Customer service	Clinicians are not aware that they can provide feedback for improving laboratory services; laboratory staff are not requesting feedback from clinicians and do not consider themselves as service providers
Facilities & safety	Power supply disruptions Limited safety training Substandard infrastructure (laboratory premises)

EQA: external quality assurance; SOP: standard operating procedures.

1.3. HIV and AMR in sub-Saharan Africa

Individuals living in LMICs experience substantial morbidity and mortality due to infectious diseases.[83] Because of the high burden of infections, lack of diagnostic tests and difficulties in accessing healthcare, antibiotics are frequently prescribed[84] (unpublished data), often unnecessarily. Excessive antibiotic use is one of the main drivers of AMR and prior antibiotic treatment is a recognised risk factor for subsequent infections with resistant pathogens.

People living with HIV (PLWH) may be at increased risk of infections due to antibiotic resistant bacteria because of more frequent intercurrent infections, hospital admissions,[85,86] and antibiotic prescriptions[87,88] even prior to HIV diagnosis as compared to individuals without HIV infection. Of the 37.7 million people living with HIV worldwide, over two thirds are living in sub-Saharan Africa.[89] In 2020, an estimated 1.3 million Zimbabweans were living with HIV (12.9% prevalence among adults) with 31,000 new infections occurring every year.[90,91]

Because of the increased risk of infections with resistant pathogens, PLWH may need to be prioritised for diagnostic for AMR testing and AMR should be considered in the choice of antibiotics for empiric treatment. However, the evidence for an association between AMR and HIV has not been systematically reviewed to date. This section reviews the published studies on AMR and HIV and highlights the gap in evidence for the association between HIV and Gram-negative AMR.

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Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

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Where was the work published?	Clinical Microbiology and Infection		
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SECTION E

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Date	21-12-2022

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Date	21-12-2022

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Systematic review

The association between antimicrobial resistance and HIV infection: a systematic review and meta-analysis

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ABSTRACT

Objectives: People living with HIV (PLWH) are at increased risk of infections with resistant organisms due to more frequent healthcare utilization. Our objective was to investigate the association between HIV and antimicrobial resistance (AMR).

Methods: We searched MEDLINE, EMBASE, Web of Science, LILACS and African Journals Online. Studies were eligible if they reported on AMR for colonization or infection with bacterial pathogens (excluding mycobacteria and bacteria causing sexually transmitted infections) and were stratified by HIV status, species and antimicrobials tested. Pooled odds ratios were used to evaluate the association between HIV and resistance.

Results: In total, 92 studies published between 1995 and 2020 were identified. The studies included the following organisms: *Staphylococcus aureus* ($n = 47$), *Streptococcus pneumoniae* ($n = 28$), *Escherichia coli* ($n = 6$) and other Gram-negative bacteria. PLWH had a 2.12 (95%CI 1.36–3.30) higher odds for colonization and 1.90 (95%CI 1.45–2.48) higher odds for infection with methicillin-resistant *S. aureus*, a 2.28 (95%CI 1.75–2.97) higher odds of infection with *S. pneumoniae* with decreased penicillin susceptibility, and a 1.59 (95%CI 0.83-3.05) higher odds of resistance to third-generation cephalosporins in *E. coli* and *Klebsiella pneumoniae*.

Conclusion: This review shows an increased risk of AMR in PLWH across a range of bacterial pathogens and multiple drug classes. The lack of laboratory capacity for identifying AMR, and limited access to alternative treatment options in countries with the highest burden of HIV, highlight the need for more research on AMR in PLWH. Overall, the quality of studies was moderate or low, which may impact the findings of this review. **Ioana D. Olaru, Clin Microbiol Infect 2021;27:846**

Introduction

The discovery of antimicrobials has led to a 'golden age' in medicine which has enabled the effective treatment of a large number of once deadly infections. The emergence and worldwide dissemination of antimicrobial resistance (AMR) is threatening to overturn these advances, leading to increased morbidity, mortality and healthcare costs [1]. AMR is of particular concern in low- and middle-income settings where treatment options are limited due to cost and availability of drugs. Among the main drivers for AMR are inappropriate antimicrobial use, transmission of resistant pathogens in healthcare settings, and rapid dissemination of resistant pathogenic strains due to international travel and trade [2]. On an individual level, prior antimicrobial use, healthcare contact and underlying comorbid conditions are recognized risk factors for infection and colonization with resistant organisms [3].

There are almost 38 million people living with HIV (PLWH) worldwide, with the highest prevalence of infection in countries in sub-Saharan Africa [4]. PLWH have more frequent hospital admissions, clinic visits and antimicrobial treatment courses compared to

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individuals without HIV infection, putting them at increased risk of the acquisition of and infection with resistant bacteria. Hospital admissions for acute bacterial infections are more frequent in HIV-infected than in HIV-uninfected individuals [5,6]. Overall, bacterial infections (not including tuberculosis) are one of the main causes of hospitalization and death in PLWH worldwide, with almost a third of all hospital admissions and a quarter of all deaths due to bacterial infections [7]. More frequent hospital admissions may lead to an increased number of antimicrobial prescriptions and a higher risk of acquisition of resistant pathogens during hospitalization. Additionally, there is a higher prevalence of chronic conditions such as kidney and liver diseases, which also require more frequent clinic appointments and hospital admissions among PLWH [8,9]. In the United States, prescriptions for multiple antimicrobial drug classes are more common among elderly HIV-infected individuals than in their HIV non-infected counterparts [10]. Furthermore, antimicrobial prescriptions are more frequent among HIV-infected individuals even before HIV diagnosis, as shown by a study from Denmark [11]. With the roll-out of widespread antiretroviral therapy (ART) and improved early diagnosis and treatment of HIV, the impact of these factors on the prevalence of resistance may have changed over time.

Antimicrobials such as co-trimoxazole, macrolides and antimycobacterial agents are also recommended as prophylaxis for PLWH [12,13]. The indication may vary by CD4 cell count, age, geographical setting and prior medical history. Use of co-trimoxazole prophylaxis in high-income settings is decreasing due to early diagnosis of HIV and ART use. However, in low-income settings co-trimoxazole prophylaxis is recommended by the WHO for all children with HIV, and for adults with advanced HIV infection or who are living in areas where malaria and bacterial infections are prevalent, irrespective of ART [12]. Thus, co-trimoxazole prophylaxis, which has significantly reduced mortality [14], is currently widely used throughout sub-Saharan Africa.

Although HIV infection is not known to have a direct effect on the colonization and infection of an individual with resistant organisms, PLWH have a multitude of indirect risk factors. There are limited data on the association between HIV infection and bacterial AMR, and to our knowledge this has never been reviewed systematically.

The aim of this systematic review is to determine whether HIV infection is associated with colonization or infection with antimicrobial-resistant bacteria.

Methods

The review is focused on bacterial pathogens causing infection or colonizing individuals with HIV infection. Mycobacteria and organisms causing sexually transmitted infections were excluded because this association has recently been reviewed [15] and because of the differences in transmission pathways for sexually transmitted infections.

Eligible publications were identified using the search strategy described in the Supplementary Material Table S2. The search strategy was applied to MEDLINE, EMBASE, Web of Science and LILACS databases up to week 3 of 2020. The African Journals Online and PubMed databases were searched for additional publications. The search was updated to June 2020. References of included studies and other reviews were manually searched for relevant publications. Articles identified were imported into the bibliographic software manager Endnote X7 (Clarivate Analytics, Philadelphia, PA, USA). Titles and abstracts were screened for eligibility by IDO and KK in duplicate.

Studies were included if they reported resistance data for colonization or infection by HIV status and stratified the results by bacterial species and antimicrobials tested. Colonization and infection were considered as defined by the authors of the respective studies. Studies describing species from the same family—such as *Escherichia coli* and *Klebsiella pneumoniae* (Enterobacteriaceae)—which did not present data by bacterial species were grouped in the analysis. No restriction with regards to geographical region, language or year of publication was applied. Case series were excluded. The review followed the PRISMA guidelines. The study protocol was registered with PROSPERO (CRD42020178907). The search strategy and analyses are described in the Supplementary Material.

Analyses were conducted using STATA (version 15, Stata-Corp, TX, USA). A descriptive analysis was performed for all eligible studies. If at least five studies reporting on the same organism (or group) and resistance to the same antimicrobial class were identified, then a random effects meta-analysis using the DerSimonian and Laird method was performed in STATA (using 'metan'). The meta-analyses were also stratified according carriage/colonization or infection. The pooled odds ratio estimates were grouped by organism, infection versus colonization and drug tested. Sensitivity analyses were performed to determine whether findings were consistent across different groups (Supplementary Material). Meta-analyses for proportions were performed using the package 'metaprop'. Heterogeneity of studies was evaluated using Cochrane recommendations [16] using the I^2 statistic. Heterogeneity was considered substantial if I^2 >50%.

Completeness of reporting was assessed using the STROBE checklist [17], and for each item studies were graded into three categories: 'no information provided or unclear description', 'some information but insufficient', 'clear information provided'. The modified Newcastle–Ottawa scale was used to evaluate the quality of the studies [18].

Results

Characteristics of identified studies

Of 21 489 unique citations identified by the search, 299 were selected for full-text review, of which 71 were suitable for inclusion in the main analysis. An additional 21 publications were identified from reference review. A total of 92 citations were included in the qualitative analysis (Fig. 1). Most studies were from either the United States (n = 32, 35%) or South Africa (n = 21, 23%). Fig. 2 shows where the studies were conducted. About half of the studies were conducted in low- and middle-income countries (n = 50, 54%).

Most studies described resistance in a single pathogen (n = 83, 90%) while nine studies presented data for multiple organisms. AMR in *Streptococcus pneumoniae* was described in 28 studies (carriage n = 1, infection n = 27), *Staphylococcus aureus* in 47 (carriage n = 29; infection n = 18) and other organisms in 22 (carriage n = 6; infection n = 16). Studies in Enterobacteriaceae were on *E. coli* (n = 6), *Salmonella* spp. (n = 7), *K. pneumoniae* (n = 3), and *Shigella* spp. (n = 3). Supplementary Material Table S3 presents an overview of the studies included.

Patient characteristics

Studies included 40 625 PLWH and 17 187 704 individuals without HIV. Most studies were conducted in inpatients (n = 44, 48%), or a combination of inpatients and outpatients (n = 10, 11%); 18 studies (20%) reported on outpatients only, and six (7%) on other settings such as prisons (n = 3), youth centres (n = 1), shelters (n = 1) and individuals recruited from the community (n = 1) (Supplementary Material Table S3). One study reported on isolates

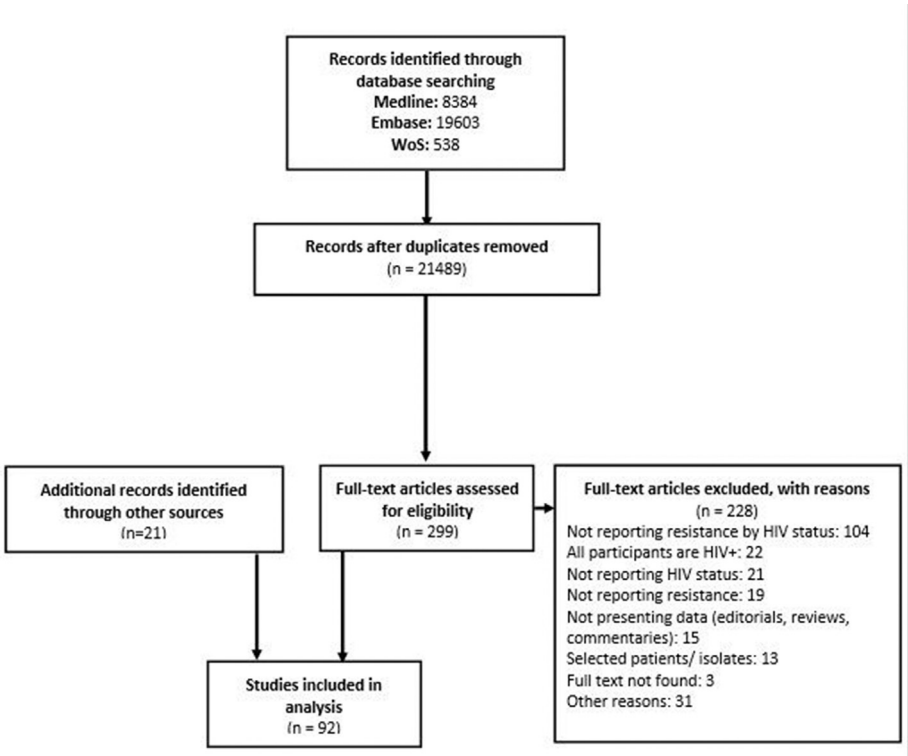

Fig. 1. PRISMA diagram for a systematic review of the risk of antimicrobial resistance in bacterial infections in adults with HIV infection. Other reasons for exclusion were: reported data from neonates or HIV-exposed and uninfected children ($n = 9$); presented data from studies with <20 participants ($n = 9$); studies were comparing different populations and/or settings ($n = 3$); no stratification by organism ($n = 3$); reported on inappropriate samples ($n = 2$); did not account for repeated sampling of the same individual ($n = 2$); publication based on data already included in the review ($n = 2$); excluded participants with HIV ($n = 1$).

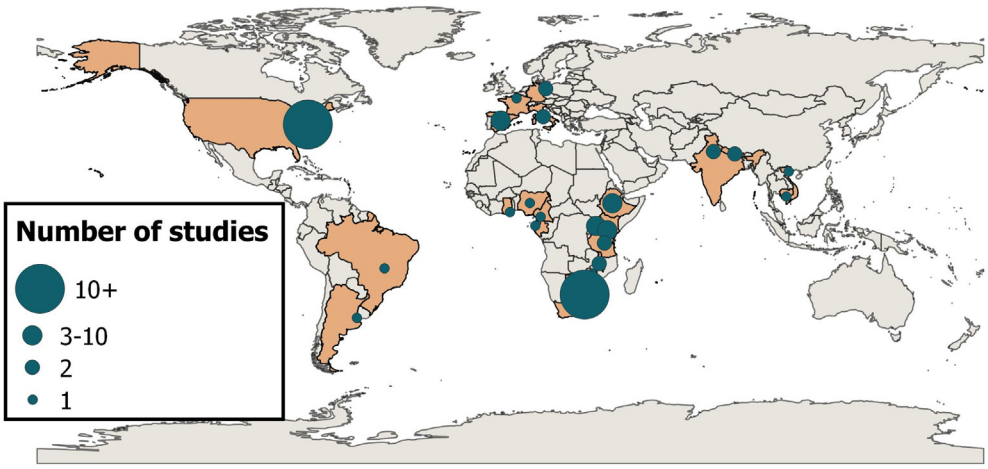

Fig. 2. Map of countries with studies included in a systematic review of the risk of antimicrobial resistance in bacterial infections in adults with HIV infection. Countries are shaded if at least one study on antibacterial resistance among HIV-positive and HIV-negative people was identified in this review. The size of the dot represents the number of studies from the respective country.

collected as part of laboratory surveillance and did not specify the source of the isolates; 13 studies did not provide details on the healthcare setting. Most of the studies were conducted in a single facility ($n = 54$, 59%).

Most studies reported data from adults ($n = 53$, 58%), while 15 (16%) included only children and 22 (24%) included both adults and children. For two studies, participant age was not reported.

HIV status was determined by performing an HIV test as part of the study procedures ($n = 26$ studies) or was obtained from patient records or as part of the medical history ($n = 52$ studies). In 14 studies, HIV status ascertainment was not clearly described.

Few studies reported on ART coverage ($n = 17$, 18%) and co-trimoxazole prophylaxis ($n = 22$, 24%) among HIV-infected patients. CD4 cell counts at the time of the study were reported for 39 studies (42%). Mean/median CD4 cell counts were <200 cells/μL in 12 studies (31%) and ≥200 in 25 studies (64%). For two studies the severity of the immunosuppression could not be determined. Forty-five (49%) of the studies collected data prospectively, while

43 (47%) extracted data from medical records or laboratory databases; one study used both prospective and retrospective data collection, and three (3%) did not specify how data collection was performed.

The pooled prevalence of resistance to selected antimicrobials by HIV status is shown in Table 1 and in Supplementary Material Figs S2 and S3.

Antimicrobial resistance in Staphylococcus aureus

Of the 47 studies describing AMR prevalence in *S. aureus*, 29 studies (62%) focused on colonization only (nasal: $n = 23$; multiple sites $n = 6$), and 18 (38%) on infections (bacteraemia: $n = 5$; skin and soft tissue infections: $n = 5$; respiratory infections: $n = 2$; multiple sites: $n = 6$). Among studies evaluating methicillin-resistant *S. aureus* (MRSA) colonization, six studies used methods enabling direct MRSA identification: chromogenic media ($n = 5$) and molecular methods ($n = 1$). Most studies reported predominantly on patients with community-acquired infections ($n = 10$, 56%), while four (22%) included patients with hospital-acquired infections and four (22%) did not describe the type of infection. The studies included a total of 27 364 PLWH and 17 159 190 individuals without HIV infection. Half of the studies (24/47) and 89% of PLWH were from United States. Most studies (41/47, 87%) were conducted after the introduction of widespread ART. The proportion of individuals on co-trimoxazole prophylaxis was not consistently reported ($n = 11$, 23%) and ranged from 0 to 89%.

PLWH had 2.12 (95%CI 1.36–3.30) higher odds for colonization and 1.90 (95%CI 1.45–2.48) higher odds for infection with MRSA compared with HIV-negative individuals. Eleven studies evaluated co-trimoxazole susceptibility of *S. aureus* isolates. The pooled OR for co-trimoxazole resistance was 1.40 (95%CI 0.49–3.99) for colonization and 2.89 (95%CI 0.48–17.37) for infection, comparing PLWH with HIV-negative individuals. Heterogeneity in ORs was high across studies (I^2 range 82–98%, Fig. 3c,d). Among individuals with *S. aureus* colonization, the odds of MRSA carriage were higher among PLWH than in those without HIV infection (Supplementary Material Fig. S4).

Antimicrobial resistance in Streptococcus pneumoniae

A total of 28 studies evaluated AMR in *S. pneumoniae*, including 9239 PLWH and 8368 individuals without HIV infection; South Africa contributed the majority of the HIV-positive patients (83%). Studies were mostly conducted before the widespread availability of ART ($n = 18$, 64%). Reporting of ART (1/28) and co-trimoxazole prophylaxis (8/28) usage was very rare. Where described, the proportion of PLWH reported to take co-trimoxazole prophylaxis ranged between 0 and 67%. More than two thirds of the studies described invasive pneumococcal disease ($n = 20$, 71%), while isolates were cultured from multiple sample types in seven studies (30%). One study reported on pneumococcal carriage.

Nine studies reported on co-trimoxazole resistance. *S. pneumoniae* isolates from PLWH had a 2.28 (95%CI 1.75–2.97) higher odds of having decreased penicillin susceptibility compared to isolates from individuals without HIV. The pooled OR for co-trimoxazole resistance was 2.29 (95%CI 1.31–4.00). Heterogeneity of studies was moderate to substantial (I^2 60% and 75%, respectively, Fig. 3a,b).

Antimicrobial resistance in Escherichia coli and Klebsiella pneumoniae

Four studies described resistance in *E. coli*, two in *K. pneumoniae*, and one in both *E. coli* and *K. pneumoniae* isolates from patients presenting with infections. These studies included 849 HIV-infected and 12 573 non-HIV-infected individuals. Isolates were from blood ($n = 3$), stool ($n = 2$), urine ($n = 1$) and respiratory samples ($n = 1$). The OR for the presence of resistance to third-generation cephalosporins and co-trimoxazole in *E. coli* and *K. pneumoniae* causing infections was 1.59 (95%CI 0.83–3.05) and 2.43 (95%CI 1.36–4.32) respectively (Fig. 3e,f). Heterogeneity was high across studies for both third-generation cephalosporin resistance (I^2 94%) and co-trimoxazole resistance (I^2 78%).

Quality of reporting in studies included

Completeness of reporting according to the STROBE checklist was moderate in the majority of studies (66/92, 72%) (Fig. 4). Sources of bias, sample size and generalizability of results were often not discussed. Also, most studies did not report on the number of participants or samples excluded from the analysis or the reasons for exclusion. When using the modified Newcastle–Ottawa Scale, most studies were of moderate ($n = 30$) or low ($n = 57$) quality. Only seven studies were of high quality.

Discussion

This review synthesizes for the first time data on AMR in PLWH compared to that in HIV-negative individuals. It shows that, for several important pathogens, PLWH are more likely to be infected or colonized with resistant bacterial strains than people without HIV infection. This holds true for two major Gram-positive and some Gram-negative organisms and for multiple antimicrobial classes. The higher prevalence of resistance among PLWH is of particular concern in low-income settings where alternative treatment options are limited by their availability and cost [19]. Co-trimoxazole may increase the risk of colonization with resistant Gram-negative and Gram-positive organisms [20–22]. Therefore, resistance is not surprising given the wide use of co-trimoxazole as

Table 1
Antimicrobial resistance to selected drugs

Organism	Antimicrobial	Number of studies reporting resistance by HIV status	Pooled prevalence of resistance (95%CI)	
			HIV+	HIV−
Staphylococcus aureus (infection)	MRSA	14	49% (32–65)	35% (25–44)
S. aureus (colonization)	MRSA	29	9% (7–11)	6% (4–8)
	Co-trimoxazole	5	65% (44–86)	55% (46–64)
Streptococcus pneumoniae (infection)	Penicillin	20	29% (23–36)	15% (11–19)
	Co-trimoxazole	7	38% (30–47)	20% (13–28)
	Tetracyclines	6	14% (8–20)	19% (12–26)
	Macrolides	6	8% (4–12)	8% (5–12)

Data were not pooled if there were three or fewer studies reporting prevalence by organism and drug class, or if the number of studies was small and estimates were highly heterogeneous. Studies where prevalence could not be calculated for both HIV-infected and non-infected individuals were also excluded. MRSA, methicillin-resistant *S. aureus*.

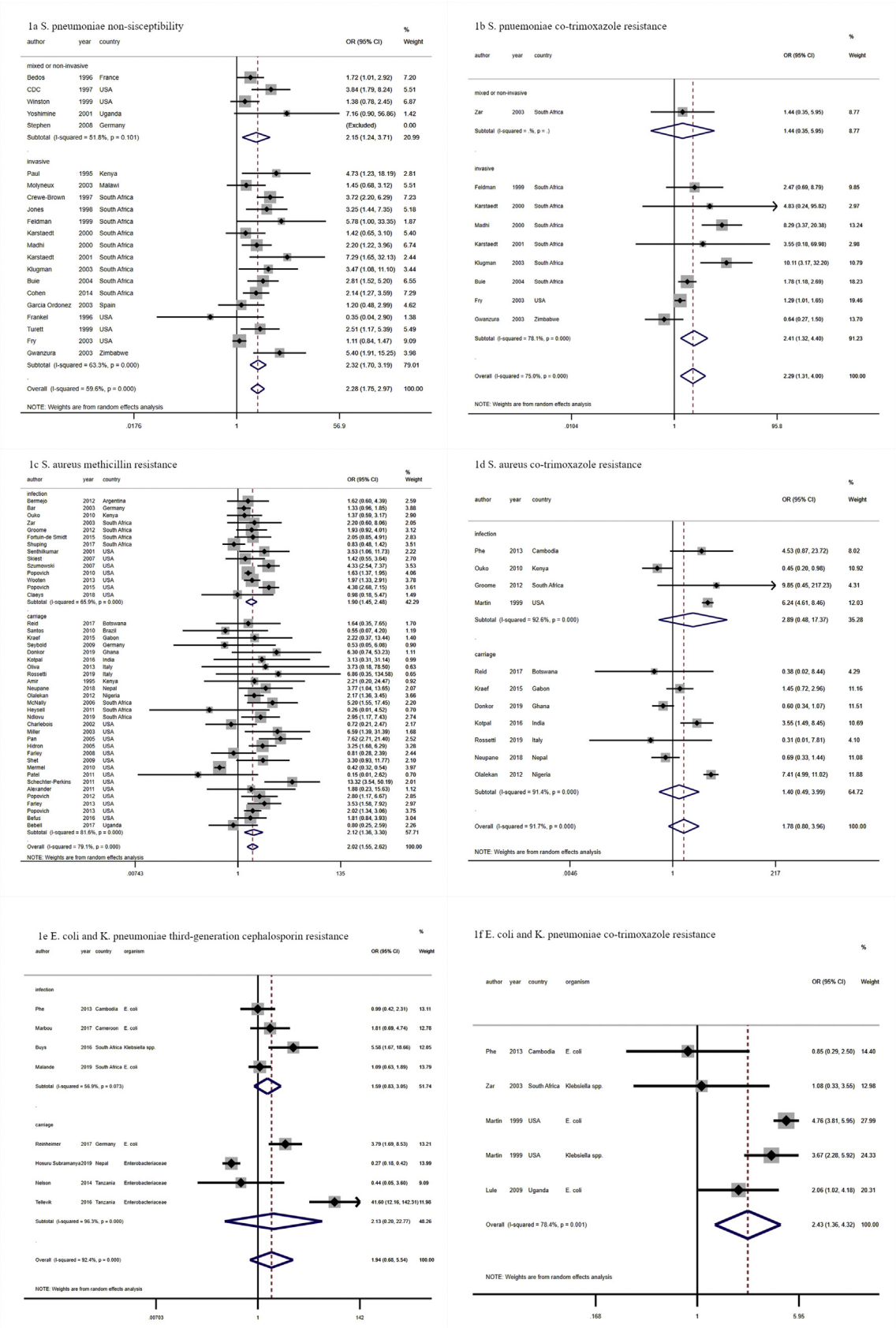

Fig. 3. Pooled odds ratios for antimicrobial resistance in bacteria isolated from HIV-infected individuals compared with those without HIV. *Streptococcus pneumoniae* penicillin non-susceptibility (a) and co-trimoxazole resistance (b); *Staphylococcus aureus* methicillin resistance (c) and co-trimoxazole resistance (d); *Escherichia coli* and *Klebsiella pneumoniae* third-generation cephalosporin resistance (e) and co-trimoxazole resistance (f).

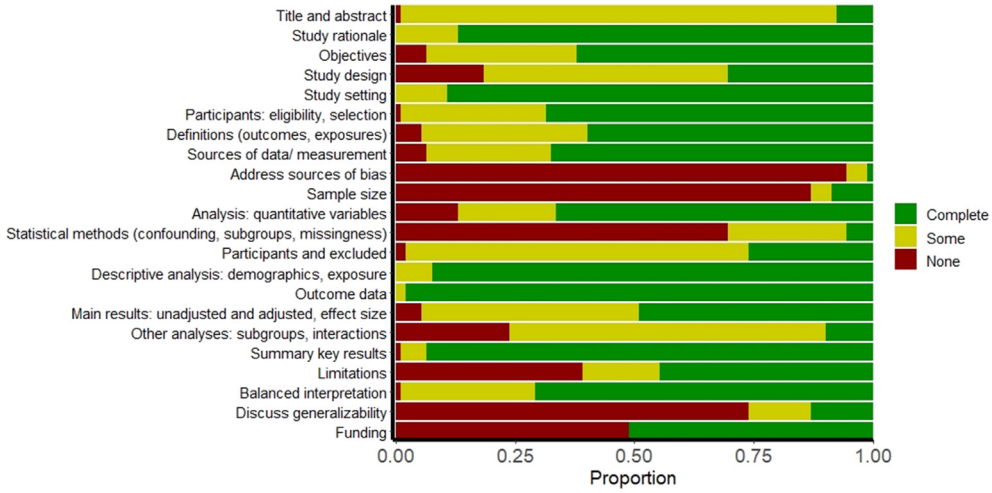

Fig. 4. Quality of reporting for studies according to the STROBE criteria. The horizontal bars represent the proportion of studies providing complete (green), some/insufficient (yellow) or no/unclear (red) information on the respective items.

prophylaxis in PLWH in general; however, only a fraction of the studies presented data on co-trimoxazole use among their participants. The degree of immunosuppression and ART influence the risk of infection and hospitalization [23], but less than 20% of the studies reported the proportion of PLWH on ART, and only 41% described CD4 cell counts among PLWH.

Many penicillins and cephalosporins are readily available, inexpensive drugs used to treat infections caused by a wide variety of organisms. For *S. aureus,* the methicillin resistance gene (*mecA*) is especially problematic as it confers resistance not just to flucloxacillin but to almost all β-lactam drugs, including third-generation cephalosporins. Treatment of systemic MRSA infections is hence particularly challenging in low- and middle-income settings, as vancomycin and linezolid are not widely available and are expensive and difficult to use safely in PLWH.

This systematic review found that HIV infection is associated with methicillin resistance in *S. aureus* in studies predominantly from the United States and conducted prior to 2010. The steady decrease in MRSA infections in the United States since 2005 is attributed to the rigorous introduction of enhanced infection control programmes [24]. This decline was also observed in PLWH, although MRSA incidence remained higher than in the general population [25]. People who inject drugs and men who have sex with men are at increased risk for MRSA [26,27]. Given that these populations are more likely to be HIV-infected than the general population in the United States [28,29], the higher prevalence of MRSA may be due to confounding by these factors. However, the association between HIV infection and MRSA was also found in studies from sub-Saharan Africa [30] where heterosexual transmission of HIV is predominant, and therefore the association cannot be fully explained by this confounder. Previous studies have found that low CD4 cell counts and unsuppressed HIV viral load, injected drug use and identifying as a man who has sex with men are all associated with MRSA in PLWH [31,32]. The high prevalence of MRSA colonization in PLWH may be attributed to hospital admissions caused by HIV-related immunosuppression [33]. On the other hand, the association between HIV infection and resistance may be confounded by the presence of high-risk sexual activities [34]. Most studies in this review reported on community-acquired MRSA infections reflecting the widespread dissemination of successful MRSA clones.

This review found that the higher odds for MRSA colonization and infection among people with HIV was consistent across different levels of immunosuppression, ART use and country income, although some comparisons may be limited by the very small number of studies in the respective subgroups.

Co-trimoxazole resistance in *S. aureus* occurs either by mutations in the dihydrofolate reductase gene or by acquisition of variant dihydrofolate reductase genes by horizontal gene transfer [35]. While in Europe the prevalence of co-trimoxazole resistance in *S. aureus* is relatively low [36], this is not the case for sub-Saharan Africa and Asia [37,38]. The higher prevalence of resistance in these settings may be explained by the widespread use of co-trimoxazole and other sulphonamide-based drugs for treatment and prevention of infections [37,39], and potentially by the expansion of particular clonal types within the HIV population [30]. This is also reflected by the findings of this review which suggest an association between HIV and co-trimoxazole resistance, although estimates varied widely across studies.

S. pneumoniae is a major cause of pneumonia and meningitis; individuals with immunodeficiencies are at increased risk for invasive disease [40]. β-Lactam drugs, namely benzylpenicillin or amoxicillin, are the mainstay treatment for pneumococcal disease. Recent antimicrobial use, young age, previous hospitalization, infection with certain capsular serotypes, and geographical location are all risk factors for resistant *S. pneumoniae* strains causing invasive disease [41]. The epidemiology of *S. pneumoniae* non-susceptible to penicillin varies geographically, and in the late 1990s the majority of invasive pneumococcal disease cases in the United States were attributed to pneumococcal conjugate vaccine (PCV7) serotypes [42].

Prevalence of co-trimoxazole resistance also varies geographically and is influenced by prior exposure either to co-trimoxazole [22] or to other sulphonamides such as sulfadoxine–pyrimethamine which are used in Africa for malaria treatment and preventive therapy in pregnancy [43].

Our review shows that HIV infection is associated with penicillin non-susceptibility and co-trimoxazole resistance in *S. pneumoniae*, with one in three isolates cultured from PLWH showing non-susceptibility to penicillin and co-trimoxazole, although this may be overestimated by preferential sampling of people with HIV infection. Given that all studies presenting co-trimoxazole resistance data were conducted in sub-Saharan Africa, it is highly likely that a considerable proportion of PLWH had received co-trimoxazole prophylaxis. Most studies on *S. pneumoniae* were published before the ART roll-out, and therefore these results may not reflect the current situation.

Our review identified only a small number of studies for the Enterobacteriaceae, but they suggest a higher odds of resistance to third-generation cephalosporins and co-trimoxazole in people with HIV infection, although the confidence interval crossed one for the former. Comparisons of AMR prevalence for other drug classes were precluded by the small number of studies identified. Given that third-generation cephalosporin resistance is associated with resistance to other drug classes, severe infections may require treatment with broad-spectrum antibiotics such as carbapenems. In contrast to high-income settings, where carbapenems usually are available but their use is restricted, these agents are usually unavailable in low-income settings or their use is cost-prohibitive.

Overall this systematic review shows an association between HIV infection and bacterial AMR across multiple pathogens and antimicrobial classes, although for co-trimoxazole, resistance may not be of clinical significance for some infections. Because of the diversity in study settings and populations, and the small number of studies identified, we were unable to assess the trend in AMR prevalence over time. Only 14 studies reported data collected after 2010 and five after 2015, thus precluding conclusions on the current epidemiology of AMR in these settings. Results were consistent across time periods, suggesting that the association between HIV infection and AMR is still relevant despite the relatively small number of more recent studies. This highlights the need for studies and surveillance to report on resistance prevalence according to HIV status, especially in studies from settings with a high HIV burden.

Despite the comprehensive search strategy, without restriction by language, year of publication and geography, only a small number of countries are represented in this review. For example, there were very few studies from South America and Asia. For individual pathogens, the majority of studies are from a single country; most of the studies of *S. aureus* were from the USA, and most of the studies of *S. pneumoniae* were from South Africa. Consequently, the results of this review may not be generalizable beyond these countries. Despite the high burden of HIV in sub-Saharan Africa, there are few data on HIV and AMR for most countries apart from South Africa. Additionally, there was substantial heterogeneity among studies which is not surprising given the differences in settings, patient populations, sample types and laboratory methods used for testing. Further heterogeneity may have been introduced by preferential sampling of people with HIV leading to more frequent diagnosis of infection as compared to individuals uninfected with HIV. The high heterogeneity between studies may impact on the generalizability of these findings. For most pathogens, a meta-analysis could not be performed because of the small number of studies. Because this review included observational studies, findings may have been affected by confounding.

This is, to our knowledge, the first systematic review evaluating the association between HIV infection and AMR in a wide range of bacterial pathogens. A comprehensive assessment of the quality of reporting was performed using the STROBE checklist.

The findings of this review underscore the need for further studies evaluating the association between HIV and AMR, especially from countries with a high HIV burden [7]. Most studies included were published before 2010 and were of moderate and low quality, which may affect the validity of our findings. This study further highlights the need for a better understanding of AMR prevalence in low-income settings and on the extent to which people with HIV may be at increased risk for infections with resistant bacterial pathogens, emphasizing the need for improved antimicrobial resistance testing and reporting. Increasing diagnostic capacity and AMR surveillance, as well as promoting further research into AMR and HIV, could ultimately lead to improved management of HIV-infected patients.

Transparency declaration

The authors declare that they have no conflicts of interest. IDO received funding though the Wellcome Trust Clinical PhD Programme awarded to the London School of Hygiene & Tropical Medicine (grant number 203905/Z/16/Z).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.03.026.

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Supplementary materials for the above publication *"The association between antimicrobial resistance and HIV infection: a systematic review and meta-analysis"* are included in the Appendix 1.

Contents of Appendix 1

1.4. Understanding antibiotic prescribing and use

Antibiotics play a key role in LMICs and are regarded as life-saving medicines as well as solutions for restoring productivity and addressing healthcare access inequalities.[10,92] In high-income settings 90% of antibiotics are prescribed in primary care[93] and 10% of outpatient encounters result in antibiotic prescriptions.[94,95] This contrasts with LMICs where antibiotics are prescribed more frequently with approximately 60% of outpatients receiving an antibiotic prescription.[96,97] Healthcare workers from LMICs are aware that antibiotics are overprescribed and that over-prescription is closely associated with AMR. However, the awareness does not necessarily lead to changes in their prescribing practice.[98,99] Furthermore, training on antibiotic prescribing is felt to be insufficient, particularly in the outpatient setting.[99]

Outside of the clinic setting, people may purchase antibiotics without a prescription directly from pharmacies or from other informal sources. Altogether antibiotics acquired without a prescription may represent a considerable share of total antibiotic use in low-resource settings. Their use is driven by the lack of access to healthcare and high costs thereof that have to be met by the patients and by the deficits in regulatory systems that control antibiotic procurement and dispensing[100,101]. Access to antibiotics outside the formal health system reduce costs associated with healthcare and avoid long clinic waiting times.[98] An estimated two thirds of antibiotics are purchased at pharmacies without prescription. Informal markets, drug shops and left-over medicines from relatives and neighbours are other sources of antibiotics.[92,102] In Zimbabwe, antibiotic prescriptions were historically highly regulated with only 8% of dispensing occurring without a prescription.[103] However, the rapidly declining economy and the rise in costs for accessing basic healthcare led to an increase in the sale of antibiotics over the counter and unregistered drug vendors.[102]

Beyond its impact on promoting AMR, easy access to antibiotics without prescriptions results in self-medication with antibiotics prior to clinic presentation. This in turn reduces the yield of cultures.[104,105] Antibiotic use, as reported by patients, may not be reliable because of social desirability. This poses difficulties in ascertaining prior antibiotic exposure and complicates interpretation of microbiology tests.

2. RATIONALE FOR THIS PHD

This thesis addresses some of the gaps in our understanding of factors that contribute to excessive antibiotic use and AMR in Zimbabwe which were outlined in Figure 1. Firstly, it contributes to the limited literature and provides data on the burden of Gram-negative AMR in Zimbabwe. Prevalence of AMR is described by investigating urine samples using conventional phenotypic testing. Furthermore, the underlying molecular mechanisms of resistance are explored through whole genome sequencing and put into the context of other studies from sub-Saharan Africa.

Secondly, it investigated whether HIV, which is highly prevalent in southern Africa, is a risk factor for infections with resistant Gram-negative bacteria and should be considered when developing treatment recommendations for high-HIV prevalence settings.

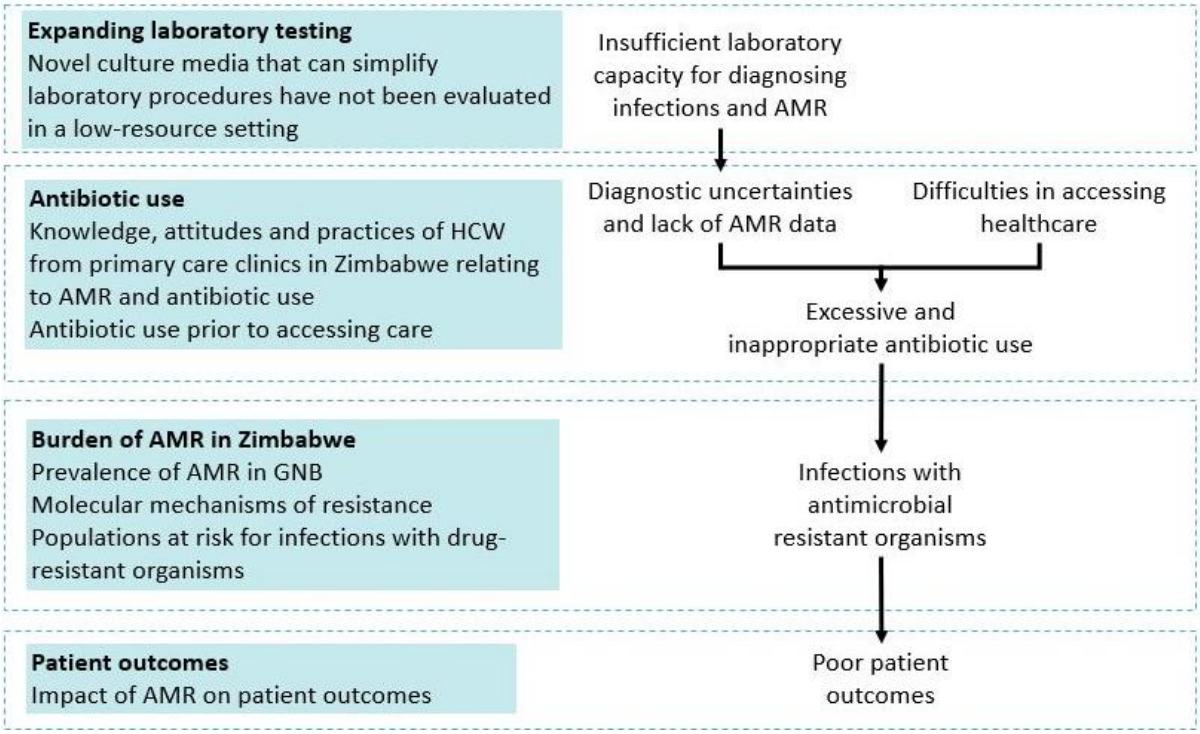
Thirdly, it evaluates whether infections with antibiotic-resistant organisms impact on clinical outcomes.

Fourthly, it contributes to our understanding of antibiotic use in the community and includes the perspective of primary care prescribers. Antibiotic use was investigated among outpatients prior to clinic presentation comparing self-report with an antibiotic assay. Also, healthcare workers' knowledge and practices with regards to AMR and antibiotic prescribing as well as their training needs were examined.

Lastly, it evaluates strategies aimed at simplifying, expediting, and decentralizing laboratory testing which may be used for AMR surveillance. This was done by evaluating two novel culture media suitable to overcome some of the disadvantages of conventional media.

Figure 2 displays the different topics addressed by this PhD and their relationship with excessive and inappropriate antibiotic use and AMR.

Figure 2. Conceptual framework representing the knowledge gaps that are addressed by this PhD and their relationship with AMR and antibiotic use



AMR: antimicrobial resistance; GNB: Gram-negative bacilli; HCW: healthcare workers.

3. AIMS AND OBJECTIVES FOR THIS PHD

The aim of this PhD was to determine the prevalence of AMR in key Gram-negative pathogens, to evaluate simplified phenotypic laboratory methods, and to understand antibiotic use in the low-resource setting of Zimbabwe.

Objectives:

1. To determine the prevalence of AMR in priority Gram-negative pathogens;
2. To describe the molecular mechanisms of resistance and virulence factors in *E. coli*;
3. To determine if there is an association between HIV status and Gram-negative AMR;
4. To investigate the association between effective antibiotic treatment and patient outcome;
5. To explore antibiotic prescribing and determine prior antibiotic use in the outpatient setting;
6. To evaluate the performance of novel culture media for diagnosis of Gram-negative infections and rapid detection of resistance that could be used in LMICs.

4. THESIS OUTLINE

This thesis comprises six manuscripts that are published or under review, a manuscript in preparation for publication on the molecular epidemiology of *E. coli*, and the published study protocol. Chapter 2 is the published protocol for the ARGUS (Antimicrobial Resistance in Gram-negatives from Urinary Specimens) study which includes most of the studies which are part of this PhD. Chapter 3 is a published study reporting on the prevalence of AMR in patients presenting with UTIs to primary care clinics in Harare and enrolled into the ARGUS study. This study also evaluates the association between AMR and HIV infection. Chapter 4 is currently being prepared for publication and describes the molecular epidemiology and mechanisms of resistance in *E. coli*. Chapter 5 is a manuscript under review and reports on clinical and bacteriological outcomes of patients enrolled in the ARGUS study. Chapter 6 is a manuscript under review exploring the knowledge, attitudes and practices as well as training needs of healthcare workers from primary care clinics in Zimbabwe. Chapter 7 is a published study investigating antibiotic use prior to accessing healthcare. Chapter 8 and 9 are published studies evaluating novel culture media that could be used to reduce turnaround times and expand AMR surveillance in low-resource settings. The two studies are conducted among patients with UTIs from the ARGUS study and neonates with suspected sepsis.

Table 4. Overview of objectives and chapters for this PhD

Research gap	PhD objective	Method	Chapter #
Burden of Gram-negative resistance	Prevalence of Gram-negative resistance to key antibiotics Association between HIV infection and AMR	1. Cross-sectional analysis of patients presenting with UTI symptoms to primary care clinics	**Chapter 3:** Prevalence of ESBL-producing *Escherichia coli* in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe (publication)
	Molecular mechanisms of resistance	2. Analysis of *E. coli* sequencing data	**Chapter 4:** Genomic epidemiology of *Escherichia coli* causing urinary tract infections in Harare, Zimbabwe (manuscript in preparation)
	Outcomes of patients with UTIs	3. Cohort study of patients presenting with UTI symptoms to primary care clinics	**Chapter 5:** Clinical and bacteriological outcomes in patients with urinary tract infections presenting to primary care in Harare, Zimbabwe: a cohort study (publication)
Antibiotic prescribing and overuse	Antibiotic prescribing and use in the outpatient setting	1. Survey of healthcare workers on antibiotic prescribing practices and AMR 2. Prior antibiotic use in patients presenting to primary clinic	**Chapter 6:** Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe (publication, under review) **Chapter 7:** Sexually transmitted infections and prior antibiotic use as important causes for negative urine cultures among adults presenting with urinary tract infection symptoms to primary care clinics in Zimbabwe: a cross-sectional study (publication)
How can microbiology diagnostics be improved and expanded in LMICs	Performance of novel culture media	1. Evaluate two novel culture media and compare them to conventional microbiology techniques	**Chapter 8:** Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe (publication) **Chapter 9:** Evaluation of a novel culture system for rapid pathogen identification and detection of cephalosporin resistance in neonatal Gram-negative sepsis at a tertiary referral unit in Harare, Zimbabwe (publication)

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CHAPTER 2

Methods of this PhD

This chapter presents the methods employed for attaining the objectives of this PhD. The published ARGUS (Antimicrobial Resistance in Gram-negative bacteria from Urinary Specimens) study protocol includes the following components: (1) a cross-section study evaluating the prevalence of resistance to key antibiotics and the risk of AMR among PLWH; (2) the molecular epidemiology of *E. coli* causing UTIs in the study population; (3) a cohort study evaluating bacteriological and clinical outcomes of patients with UTIs; (4) a cross-sectional analysis evaluating antibiotic exposure prior to seeking healthcare; and (5) a cross-sectional evaluation of novel culture media that can be used for bacterial identification and detection of resistance in patients with UTIs. The ARGUS study in its entirety received ethical approvals from the Medical Research Council of Zimbabwe (MRCZ/A/2406), and the London School of Hygiene and Tropical Medicine (Ref. 16424) and permission from the Harare City Health Department which oversees activities in PHCs from Harare.

In addition, this PhD includes a survey of healthcare workers from primary care exploring knowledge, attitudes and practices relating to AMR and antibiotic prescribing. This analysis was not described in the published protocol but received ethical approval as part of the ARGUS study. A separate study evaluating novel culture media for improving the diagnosis and management of neonatal sepsis is also part of this PhD. This study was included because it addresses the problem of Gram-negative AMR and describes a strategy that may potentially improve diagnostics. The methods for these analyses are presented in Chapters 7 and 9 as part of the respective publications.

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RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student ID Number	1805237/RITD	**Title**	
First Name(s)	Ioana Diana		
Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Wellcome Open Research		
When was the work published?	June 2020		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	This is the study protocol for the main study included in this PhD. I am the principal investigator for the ARGUS study. I am the first author of the manuscript. I designed the study with support from my supervisors and acquired the funding for conducting the study. I wrote the study protocol and obtained the regulatory approvals for conducting the study. I wrote the first draft of the manuscript, revised it following feedback from supervisors and co-authors, prepared and submitted the final draft.

SECTION E

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Supervisor Signature	Katharina Kranzer
Date	21-12-2022

STUDY PROTOCOL

Antimicrobial Resistance in Gram-negative bacteria from Urinary Specimens: a study of prevalence, risk factors and molecular mechanisms of resistance (ARGUS) in Zimbabwe – a study protocol [version 1; peer review: 2 approved]

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Abstract

Antimicrobial resistance (AMR) is compromising our ability to successfully treat infections. There are few data on gram-negative AMR prevalence in sub-Saharan Africa especially from the outpatient setting. This study aims to investigate the prevalence of and underlying molecular mechanisms for AMR in gram-negative bacilli causing urinary tract infections (UTIs) in Zimbabwe. Risk factors for AMR and how AMR impacts on clinical outcomes will also be investigated.

Adults presenting with UTI symptoms at primary health clinics in Harare will be included. A questionnaire will be administered, and urine samples will be collected for culture. Participants with positive urine cultures will be followed up at 7-14 days post-enrolment. All participants will also be followed by telephone at 28 days to determine clinical outcomes.

Bacterial identification and antibiotic susceptibility testing will be performed on positive cultures.

The results from this study will be used to inform policy and development of treatment recommendations. Whole genome sequencing results will provide a better understanding of the prevalent resistance genes in Zimbabwe, of the spread of successful clones, and potentially will contribute to developing strategies to tackle AMR.

Keywords

AMR, antibiotic resistance, Escherichia coli

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 12 Jun 2020	✓ view	✓ view

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Antimicrobials have revolutionized modern medicine leading to important reductions in mortality, morbidity and disability. Their discovery and use in medical practice was, however, accompanied by the rapid development of resistance[1]. Antimicrobial resistance (AMR) can reverse the benefits brought by these drugs, leading to increased patient deaths and healthcare costs[2,3]. Considering the current trends of increasing AMR, it is estimated that by 2050, 10 million deaths per year globally will be caused by antimicrobial resistant infections, exceeding the number of deaths due to cancer[4].

The increase in AMR is mainly driven by inappropriate antimicrobial use in humans and animals and insufficient infection control systems. Exposure to antimicrobials selects for spontaneous mutations or the acquisition and propagation of bacterial clones harbouring resistance genes[5]. Resistance genes are then mobilized and can disseminate to other commensal and pathogenic organisms[6]. This in turn may lead to increased carriage of resistant organisms in the population and an increase in use of second-line antimicrobial drugs[7]. At an individual level, other risk factors for infections due to resistant organisms are underlying co-morbid conditions and healthcare contact[8].

AMR is a global problem affecting all countries irrespective of income and geographical location[9]. However, countries differ widely with regards to their detection and reporting capabilities. Surveillance plays a key role in understanding the epidemiology of AMR and informs interventions and control measures. Global surveillance networks, such as the Global AMR Surveillance System (GLASS), were established to ensure standardised data collection and analysis and facilitate data sharing regionally and globally. However, thus far few African countries contribute data to these networks[10], and the WHO Africa region has limited AMR prevalence data[9,11,12]. GLASS focuses on a number of priority pathogens including *Escherichia coli* and *Klebsiella pneumoniae* isolated from priority specimens such as blood and urine[13]. Figure 1 and Table 1 illustrate the lack of data from sub-Saharan Africa on AMR in key pathogens, as well as the high prevalence of resistance where such data are available.

Due to limited availability of diagnostics, insufficient laboratory capacity and suboptimal funding of health care systems, in sub-Saharan Africa infections are often treated using a "syndromic" approach[14]. Samples for microbiological investigations are rarely collected outside of national tuberculosis, malaria and

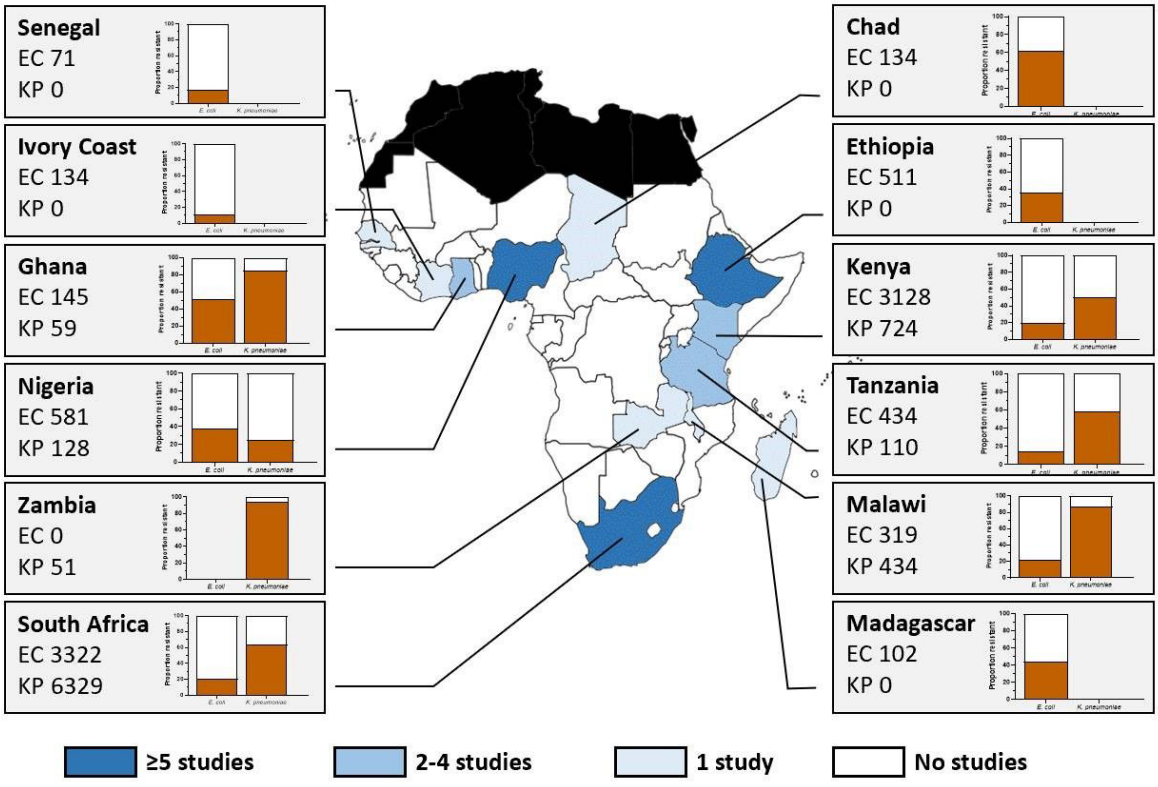

Figure 1. Studies from sub-Saharan Africa and prevalence of third-generation cephalosporin resistance in *E. coli* and *K. pneumoniae*. Only studies describing antimicrobial resistance prevalence in isolates from blood or urine cultures are included. The numbers in the small boxes represent the number of isolates with a reported third-generation cephalosporin test result. The orange bars in the graphs represent the proportion of isolates with third-generation cephalosporin resistance for E. coli (left) and K. pneumoniae (right). The white bars represent the proportion of susceptible isolates. The shaded boxes at the bottom of the picture represent the number of studies according to country. EC: Escherichia coli; KP: Klebsiella pneumoniae. One study presented data from multiple countries and was not included on the map. This figure was compiled using the studies listed in Table 1.

Table 1. Prevalence of resistance in *E. coli* and *Klebsiella spp.* in Sub-Saharan Africa in studies published since 2013. AST antimicrobial susceptibility testing; BSAC British Society for Antimicrobial Chemotherapy; CA community acquired; CLSI Clinical and Laboratory Standards Institute; EUCAST European Committee on Antimicrobial Susceptibility Testing; HA hospital acquired HCA healthcare associated; ISO International Organization for Standardization; LIMS laboratory information management system; QC quality control; UTI urinary tract infection.

Author (year)	Country	Study period	Setting	Patient population	Laboratory type	AST method	Sample type	Number of isolates tested	Quality assurance
Apondi *et al.* [2016][15]	Kenya	2002–2013, retrospective	One teaching/ referral public hospital	Inpatients, all blood culture isolates with *K. pneumoniae* most from neonatal unit	Hospital laboratory	CLSI, automated blood culture, disc diffusion	blood	281 *K. pneumoniae*	ISO accredited laboratory, internal QMS
Ayoyi *et al.* [2017][16]	Kenya	NA, prospective	Antenatal clinics from informal settlements in Nairobi	Booking appointment for antenatal clinics, asymptomatic	Not specified	CLSI, disc diffusion	urine	85 *E. coli*	Not specified
Barry *et al.* [2017][17]	Senegal	08/2012-03/2013, prospective	Urban hospitals and primary clinics	Outpatients and inpatients admitted <72h; CA-infections	Not clearly specified	Automated (Vitek) for identification and AST	urine	74 *E. coli*	Not specified
Derbie *et al.* [2017][18]	Ethiopia	01/2012-12/2014, retrospective	Referral hospital, health centers, private clinics	Not clearly specified, no antibiotics <2 weeks	Research laboratory, referral center	CLSI, Biochemical tests for identification, disc diffusion	urine	72 *E. coli*	Not specified
Eibach *et al.* [2016][19]	Ghana	09/2007-07/2009; 01/2010-12/2012, prospective	Regional hospital (rural)	Inpatients of all ages presenting with fever, neonates with suspected sepsis; CA-infections and HAI in neonates	Not specified	EUCAST; Automated blood culture, biochemical identification confirmed by MALDI-TOF abroad; AST by Vitek2	Blood	50 *E. coli*	Not specified
Kaduma *et al.* [2019][20]	Tanzania	03/2017-05/2017, prospective	Regional referral hospital	Pregnant women with and without pre-eclampsia attending antenatal clinics or admitted in a matched case-control design; no symptoms of UTI	Not specified	CLSI, AST by disc diffusion	urine	50 *E. coli*	Use of reference strains for culture and AST
Malande *et al.* [2019][21]	South Africa	01/2005-12/2014, retrospective	Major urban tertiary-level referral hospital for children	Children admitted to hospital; 47% CA-infections; 28% HAI; 24% HCA	External hospital laboratory	CLSI, automated blood culture, identification and AST using Vitek2	blood	583 *E. coli*	Not specified
Manyahi *et al.* [2017][22]	Tanzania	06/2004-01/2005, retrospective	Tertiary care hospital	Inpatients and outpatients presenting to the hospital; 49% HAI; 51% CA	Hospital laboratory	CLSI; identification using biochemical tests and Vitek2	urine	110 *E. coli*	Not specified

Wellcome Open Research 2020, 5:140 Last updated: 23 MAR 2022

Author (year)	Country	Study period	Setting	Patient population	Laboratory type	AST method	Sample type	Number of isolates tested	Quality assurance
Nyasulu *et al.* [2017][23]	South Africa	07/2005-12/2009, retrospective	7 tertiary public hospitals with laboratories linked to the National Health Laboratory Service	Samples from hospitals	Hospital laboratories reporting to a central laboratory	CLSI, automated blood cultures, identification with Vitek2, or Microscan or conventional biochemical methods, AST manual (disc diffusion or Etest) or automatic (Vitek2 or microscan)	blood	4466 *K. pneumoniae*	The individual hospital laboratories had good QA practices, and computarised LIMS
Obeng-Nkrumah *et al.* [2016][24]	Ghana	01/2010-12/2013, retrospective	Tertiary-care teaching hospital	Inpatients who had a blood culture collected and were aged >28 days; either presenting to the hospital directly or referred for investigations	Hospital laboratory	CLSI, automated blood cultures, identification using biochemical tests, AST by disc diffusion	blood	118 *E. coli* 63 *K. pneumoniae*	Not specified
Oli *et al.* [2017][25]	Nigeria	05-07/2016, prospective	Regional teaching hospital	Women with symptoms of UTI presenting to various outpatient clinics	Laboratory within university	CLSI, biochemical tests for identification, AST by disc diffusion	urine	61 *E. coli*	Not specified
Perovic *et al.* [2018][26]	South Africa	01-12/2016, retrospective	Not specified	Not specified	Four private laboratories	CLSI	blood	2781 *E. coli* 2466 *K. pneumoniae*	Not clearly specified, accredited laboratories
Seni *et al.* [2019][27]	Tanzania	07/2016-10/2017, prospective	4 referral hospitals urban and rural	Children presenting to the hospitals with suspected blood stream infections	Hospital laboratory and university laboratory	CLSI; manual blood cultures, AST by disc diffusion	blood	55 *K. pneumoniae*	Use of reference strains
Seni *et al.* [2019][28]	Tanzania	03/2016-05/2017, prospective	7 healthcare facilities: tertiary hospital, regional referral hospital, district hospital, health centers	Pregnant women, inpatients and outpatients	Not specified	CLSI, biochemical identification, AST by disc diffusion	urine	164 *E. coli* 55 *K. pneumoniae*	Use of reference strains for culture, biochemical tests and AST

Author (year)	Country	Study period	Setting	Patient population	Laboratory type	AST method	Sample type	Number of isolates tested	Quality assurance
Toy *et al.* [2019][29]	Burkina Faso, Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, Sudan, Tanzania	01/2010-09/2013, prospective	12 healthcare facilities from the 9 participating countries	Patients of all ages presenting with fever as in- or outpatients; CA-infections	Laboratories at each site	CLSI; Mostly automated blood cultures, one setting with manual; biochemical identification;AST by disc diffusion	blood	120 *E. coli* 89 *Klebsiella spp.*	IQA established for the study, use of reference strains; central coordinator performing QA and working towards establishing a QMS; isolates sent to reference laboratories for confirmation
Tuem *et al.* [2019][30]	Ethiopia	01/2012-12/2017, retrospective	Tertiary hospital	Patients attending the microbiology laboratory	Laboratory of the hospital	NCCLS; AST by disc diffusion	urine	148 *E. coli* 50 *K. pneumoniae*	Not specified
Elikwu *et al.* [2017][31]	Nigeria	12/2015-04/2016, prospective	University teaching hospital	In- and outpatients with suspected UTIs, all ages	Laboratory of the hospital	CLSI, AST by disc diffusion	urine	96 E. coli	Not specified
Onoh *et al.* [2013][32]	Nigeria	04/2010-03/2011, prospective	2 tertiary referral hospitals, district-level – antenatal clinic	Pregnant women with UTI symptoms presenting to the antenatal clinics, outpatients	Not specified	Not specified, manual (disc diffusion)	urine	128 *E. coli*	Not specified
Oladeinde *et al.* (2015)[33]	Nigeria	06/2011-11/2011, prospective	Antenatal clinics at traditional birth center	Pregnant women, asymptomatic presenting for antenatal care, outpatients	Not specified	BSAC, not specified	urine	51 *E. coli*	Not specified
Omoregie *et al.* (2013)[34]	Nigeria	02/2010-01/2011, prospective	University, referral hospital	Neonatal sepsis	Not specified	BSAC, manual (disc diffusion)	blood	74 *K. pneumoniae*	Not specified
Iregbu *et al.* (2013)[35]	Nigeria	01/2010-12/2012, prospective	Tertiary hospital	Not clearly specified, suspected UTI, mostly outpatients; likely most were CA-infections	Microbiology laboratory of the national hospital	Not specified, manual (disc diffusion)	urine	323 *E. coli* 202 *K. pneumoniae*	Not specified
Abejew *et al.* (2014)[36]	Ethiopia	09/2002-09/2011, retrospective	Public and private hospitals, primary care centers	Not clearly specified, inpatients and outpatients	Regional health research laboratory	National standards, manual (disc diffusion)	urine	410 *E. coli*	Use of reference strains for AST

Author (year)	Country	Study period	Setting	Patient population	Laboratory type	AST method	Sample type	Number of isolates tested	Quality assurance
Akingbade *et al.* (2014)[37]	Nigeria	Not specified, unclear	Inpatient and outpatient clinics from public health facilities	Inpatients and outpatients	Not specified	National standards, manual (disc diffusion)	urine	120 *E. coli*	Use of reference strains for AST
Moroh, *et al.* (2014)[37]	Ivory Coast	2000–2011, retrospective	Not clearly specified	Inpatients and outpatients	Central laboratory of a teaching hospital	Not specified, manual (disc diffusion)	urine	879 *E. coli* (345 from inpatients, 534 from outpatients)	Use of reference strains
Eshetie *et al.* (2015)[38]	Ethiopia	02/2014-05/2014, prospective	1 teaching referral hospital	Inpatients and outpatients with symptomatic UTI	Accredited referral laboratory	CLSI, manual (disc diffusion)	urine	112 *E. coli*	Clearly describes QC, use of reference strain for media
Olorunmola *et al.* (2013)[39]	Nigeria	05/2003-12/2005, unclear	2 tertiary care hospitals	Inpatients and outpatients with suspected UTI	Not specified	Not specified, manual (disc diffusion, agar dilution)	urine	137 *E. coli*	Not specified
Buys *et al* (2016)[40]	South Africa	01/2006-12/2011, retrospective	1 public teaching and referral hospital for children	Inpatients, children, CA 5%, HA 86% and HCA 9% infections	Laboratory in the national health laboratory service	CLSI, automated culture, identification and AST (Vitek), (disc diffusion and Etests)	blood	409 *K. pneumoniae*	Has a LIMS
Kabwe *et al.* (2016)[41]	Zambia	10/2013-05/2014, prospective	NICU of a teaching hospital	Inpatients, neonates with suspected sepsis	Not specified	CLSI, automated culture, manual ID and AST (disc diffusion)	blood	77 *K. pneumoniae*	Not specified
Maina *et al.* (2016)[42]	Kenya	09/2010-2014, retrospective	1 teaching hospital, internationally funded	Inpatients and outpatients attending a private hospital (middle- and high-income); mixed between CO and hospital-onset	Hospital laboratory	CLSI, mainly automated culture and identification and AST (Vitek), if required manual (disc diffusion, E-tests)	Blood and urine	2912 *E. coli* (urine) 139 *E. coli* (blood) 365 *K. pneumoniae* (urine) 83 *K. pneumoniae* (blood)	ISO accredited, data extracted from LIMS
Mamuye *et al.* (2016)[43]	Ethiopia	08/2013-01/2014, prospective	1 teaching tertiary hospital	Inpatients and outpatients	Not specified	CLSI, manual (disc diffusion)	urine	53 *E. coli*	Reference strains for AST
Bitew *et al.* (2017)[44]	Ethiopia	05/2015-05/2016, prospective	private laboratory	Suspected UTI	Private laboratory	Not specified, automated (Vitek)	urine	135 *E. coli*	Reference strains for AST

Author (year)	Country	Study period	Setting	Patient population	Laboratory type	AST method	Sample type	Number of isolates tested	Quality assurance
Henson *et al* (2017)[45]	Kenya	Since 1994, retrospective	District hospital with international funding	Inpatients adults and children with CA (49%) and HA infections (51%)	In-hospital laboratory, international funding	CLSI, automated culture; semi-automated (broth microdilution with automated reading)	blood	198 *K. pneumoniae*	Not specified, samples collected within ongoing surveillance for invasive infections
Kengne et al (2017)[46]	Chad	07-11/2014, prospective	1 general hospital	Inpatients and outpatients	Not specified	Not specified, automated (Vitek)	urine	128 *E. coli*	Reference strain
Lochan et al (2017)[47]	South Africa	01/2011-12/2012, retrospective	1 tertiary care paediatric hospital	Inpatients with CA (36%), HA (54%) and HCA infections (10%)	National health laboratory	CLSI, automated culture; automated identification and AST (Vitek)	blood	92 *E. coli* 92 *K. pneumoniae*	Not specified
Rakotovao-Ravahatra et al (2017)[48]	Madagascar	01/2014-10/2016, retrospective	1 teaching hospital	Not specified	Laboratory of the teaching hospital	French standards, manual (disc diffusion)	urine	102 *E. coli*	Not specified
Forson *et al.* (2018)[49]	Ghana	02-08/2016, prospective	5 hospitals	Asymptomatic pregnant women	Not specified	CLSI, manual (disc diffusion)	urine	82 *E. coli*	Use of reference strains
Iroh Tam *et al.* (2019)[50]	Malawi	1998–2017, retrospective	1 large central hospital with international funding	Inpatients, reported data on children under 5 years with suspected sepsis	Hospital laboratory with international funding	BSAC, manual and automated blood cultures, biochemical tests for identification, AST by disc diffusion	blood	1998–2017: 857 *E. coli* 578 *K. pneumoniae* *2008–2012:* 163 *E. coli* 132 *K. pneumoniae* *2013–2017:* 165 *E. coli* 316 *K. pneumoniae*	Not specified, part of an institutional blood culture surveillance

HIV programmes and a few academic centers. Consequently, there is an overuse of antimicrobials resulting from a "just-in-case" approach to treating infections and a lack of resistance data on which to base prescribing[51]. This is the case of ceftriaxone, which is commonly prescribed to patients admitted to hospitals with suspected infections[52,53]. The AWaRe classification is a framework developed by the WHO for categorizing essential antimicrobials as 'watch', 'access' or 'reserve' and for guiding their prescription and usage. According to this classification ceftriaxone, the most widely available third-generation cephalosporin, is in the "watch" group and should be used judiciously for restricted indications[54].

Third-generation cephalosporins are essential drugs for the treatment of severe bacterial infections. Resistance usually develops through the acquisition of extended-spectrum beta-lactamases (ESBL), which hydrolyze the beta-lactam ring rendering third-generation cephalosporins ineffective[55]. ESBL genes are transferrable between different species of *Enterobacteriaceae* and also are often associated with other mechanisms that cause fluoroquinolone, aminoglycoside and sulphonamide resistance, thus leading to resistance to the main classes of antimicrobials used to treat Gram-negative infections[56]. In sub-Saharan Africa, access to amikacin or to carbapenems for treatment of third-generation cephalosporin resistant infections is extremely limited and cost-prohibitive[57].

Over two thirds of the 37.9 million people living with HIV (PLWH) are in sub-Saharan Africa[58]. Southern Africa is particularly severely affected with most countries having an adult HIV prevalence exceeding 10%[58]. PLWH attend health care facilities frequently for scheduled and unscheduled visits, receive more antimicrobial prescriptions and experience more hospital admissions than people without HIV, and therefore may be at increased risk for infections with antimicrobial-resistant organisms[59,60].

Co-trimoxazole prophylaxis has been shown to reduce mortality and hospital admissions in PLWH[61], and is currently recommended for all children and adults with advanced HIV or who are at risk for malaria and severe bacterial infections[62]. However, co-trimoxazole has been reported to increase carriage of resistant organisms in PLWH[63,64]. The increase in colonization with resistant organisms is not limited to an increase in co-trimoxazole resistance but also extends to other drug classes including cephalosporins and fluoroquinolones[65,66]. For Gram-negative bacilli (GNB), this may be due to the co-localization of resistance genes on the same mobile genetic elements which are transferrable between bacterial species[67].

Urinary tract infections (UTIs) are the most common infections caused by GNB, with an estimated incidence of 10 per 100 person years among women[68]. Resistance patterns of GNB causing UTIs reflect the community burden of resistance with the added advantage that samples are easy to obtain, and processing easy to standardise[69]. *E. coli* is the most common cause of UTIs especially in the community setting and *K. pneumoniae* the second most common[70].

Protocol

Study hypotheses

This study hypothesizes that among patients presenting with symptoms of UTI, PLWH have a higher risk of infections with resistant organisms than individuals without HIV. Additionally, because of the AMR prevalence in this setting, the current first-line treatment recommendations of amoxicillin or norfloxacin for UTI treatment will be suboptimal in terms of bacterial antimicrobial susceptibility and resolution of infection.

Study aims and objectives

The aims of this study are i) to determine if infections in PLWH are more commonly due to antimicrobial resistant organisms, compared with infections in individuals without HIV infection; ii) to explore the prevalence of and underlying molecular mechanisms for AMR in GNB causing UTIs, iii) to investigate risk factors for AMR, and iv) to examine how AMR impacts on clinical outcome.

Primary objective:

1 To determine if there is an association between HIV status and infections with ESBL-producing *E. coli* in adults (aged 18 years or older) who present with symptoms of UTI to primary healthcare services in Harare, Zimbabwe.

Secondary objectives:

2 To estimate the prevalence of third-generation cephalosporin resistance due to ESBL production in *E. coli* isolated from individuals who present with symptoms of UTI to primary healthcare services in Harare;

3 To determine the prevalence of resistance to amoxicillin and quinolones (first-line drugs for UTIs according to Zimbabwean National guidelines[71]) in bacteria causing UTIs;

4 To identify the risk factors associated with UTIs with bacteria resistant to amoxicillin and quinolones, and with ESBL-producing and multidrug-resistant bacterial strains;

5 To determine the impact of resistance to first-line antimicrobials (amoxicillin and ciprofloxacin) on clinical outcomes (defined as complete resolution of symptoms at follow-up);

6 To evaluate the causes of negative urine cultures in this setting;

7 To determine the molecular mechanisms leading to AMR, virulence factors and population diversity of *E. coli*.

Methods

Study setting

The study is conducted in primary healthcare clinics (PHCs) in Harare, Zimbabwe. PHCs provide acute primary care, including treatment for common infections. In addition, all PHCs have 1) maternity services to record and follow pregnancies in their catchment area and perform uncomplicated deliveries, 2) family planning and well-child services for growth monitoring and vaccinations, and 3) HIV services for regular follow-ups and provision of antiretroviral therapy.

The study sites are selected based on the number of clinic presentations, their catchment population and their location within Harare. The catchment population of the clinics, of over 800,000 people[72], belong mostly to the low-income strata and live in densely populated communities. The clinics selected serve the populations of the following suburbs: Budiriro, Glen View, Glen Norah, Mufakose, Highfields, Kuwadzana, Warren Park, Dzivarasekwa, Kambuzuma and Mbare.

The PHCs are primarily nurse-led and prescriptions are issued in accordance with national guidelines[54]. UTIs are usually diagnosed clinically and first-line treatment is with a fluoroquinolone or amoxicillin. Patients purchase antimicrobials according to prescription either at the PHC pharmacy or at other pharmacies in the community. Study-specific procedures are performed by the study staff who are trained on the protocol and relevant study procedures.

Study design

This is a prospective cohort study enrolling adults (aged ≥18 years) who present with symptoms of UTI at PHCs in southwest Harare, Zimbabwe. Recruitment into the study will be over an 18-month period. All participants are followed up by telephone at 28 days post-enrolment. In addition, participants with a positive urine culture at enrolment are followed up between 7 and 14 days post-enrolment to provide a urine sample to assess for clearance of infection (Figure 2).

Participant recruitment

Study staff screen and enrol participants according to the eligibility criteria. Recruitment is conducted five days per week during regular PHC opening hours. A total of 1500 participants with suspected UTIs will be enrolled. The reason for exclusion of screened participants is recorded.

Eligibility

Patients are enrolled into the study if they fulfil all the inclusion criteria and do not have any of the exclusion criteria.

Inclusion criteria:

- age ≥18 years
- presenting with symptoms of UTI (≥2 of the following: dysuria, urgency, frequency, suprapubic pain and/or flank pain). The presence of at least two symptoms is required in order to exclude those who are more likely to have other conditions (e.g. sexually transmitted infections)
- onset of symptoms within two weeks prior to presentation
- presence of symptoms within the last 24 hours
- provision of written informed consent

Exclusion criteria:

- discharge from hospital within the previous 72 hours
- having a urinary catheter in-situ

Individuals with catheters are excluded because they are likely to represent a different population with more healthcare exposure, and are more likely to have previously been prescribed antimicrobials and to have infections with resistant organisms. These infections are more likely to be healthcare associated infections rather than community acquired infections, which is the focus of this study. Recruiting individuals with urinary catheters would therefore likely lead to an over-estimation of community-level resistance.

Procedures at enrolment

Clinical and demographic data collection. Data on age, sex, socio-economic status (measured using standardised asset ownership tool, education and employment of the head of the household[73]), clinical history, prior health care seeking (traditional healer, private practitioner, pharmacy), and risk factors for AMR (prior antimicrobial use or hospitalization during the previous six months, comorbidities including HIV status, antiretroviral treatment, co-trimoxazole prophylaxis, chronic kidney disease and diabetes, current or recent pregnancy, recurrent UTIs) are

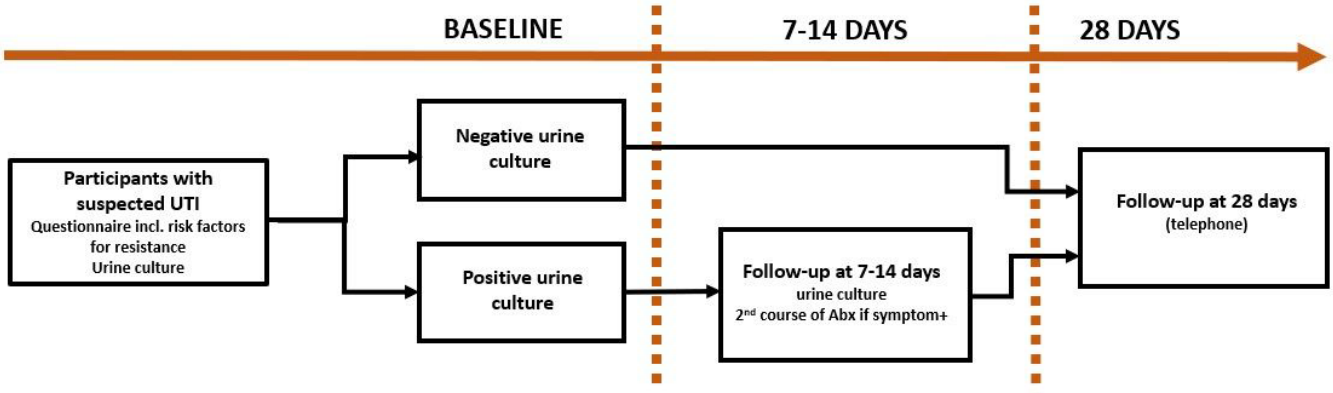

Figure 2. Outline of procedures at enrolment and follow-up. *UTI: urinary tract infection, Abx: antibiotics.*

collected using an interviewer-administered questionnaire, confirmed by patient-held records. Drug treatment (if any) and duration of treatment prescribed by the health care worker is recorded. Results of HIV tests, which are routinely carried out at the PHCs, are documented.

Sample collection and laboratory processing

A midstream urine sample for microscopy, culture and antimicrobial susceptibility testing (AST) is collected in a sterile container. The samples are transported to the laboratory as soon as possible and if a prolonged time to delivery is anticipated (>4 hours), the samples are cooled to prevent overgrowth of contaminants.

Urine samples undergo dipstick and microscopy for leucocytes, and culture. A standardised sample volume (1 µl) is inoculated on chromogenic agar (Brilliance UTI agar, Oxoid, UK). Presumptive bacterial identification is performed according to the manufacturers' instructions. A urine culture is considered positive if $\geq 10^3$ colony forming units (CFU)/mL are present with either pure culture or predominance of one organism[74]. If cultures grow a non-uropathogen or if ≥2 organisms are isolated in the absence of a clear predominance of one organism, the culture is considered contaminated. When GNBs cannot be identified by colony appearance on chromogenic agar, biochemical testing with APIs (Analytical Profile Index, bioMérieux, France) is used. AST is performed using the Kirby-Bauer disc diffusion method and interpreted using EUCAST standards[75]. Screening for ESBL production is performed according to EUCAST recommendations[76]. Briefly, if resistance to cefpodoxime alone or ceftriaxone and ceftazidime is detected, double-disc synergy testing between a cephalosporin and clavulanic acid is performed. Similarly, for AmpC detection in isolates with cefoxitin and ceftazidime resistance, synergy testing between cefoxitin and cloxacillin is carried out. In addition, for isolates resistant to third-generation cephalosporins, the minimum inhibitory concentration for ceftriaxone is determined using E-tests (bioMérieux, France).

All bacterial isolates are stored on storage beads at -80°C. Stored *E. coli* isolates will be used to re-establish cultures on agar plates from which DNA will be extracted using the DNA QIAmp Mini Kit (Qiagen, Hilden, Germany). *E. coli* isolates will undergo whole genome sequencing to ascertain molecular determinants of AMR, virulence factors and population diversity. For whole genome sequencing, DNA libraries will be prepared using the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, USA) as per the manufacturer's instructions. The libraries will be sequenced using the Illumina HiSeq platform (Illumina, San Diego, USA). Trimmed reads will be assembled into contigs using SPAdes and using a publicly available *E. coli* reference genome. Antimicrobial genotype and virulence gene prediction will be performed using ABRicate. Phylogeny will be determined using FastTree and viewed in FigTree.

Evaluation of negative urine cultures

Pilot data and data from other studies from sub-Saharan Africa[77,78] have shown that a large proportion of urine cultures from patients with symptoms suggestive of UTI are negative (60–75%) as compared to 25% in Europe[79]. This may be due to various causes such as antimicrobial use prior to sample collection, low bacterial load, delayed sample inoculation leading to overgrowth of contaminants or depletion of pathogen, or symptoms due to sexually transmitted infections rather than UTIs. These alternative causes will also be investigated in a subset of participants from this study (Figure 3).

To determine recent antimicrobial use, information on antimicrobials prior to clinic presentation and on co-trimoxazole use for HIV-positive individuals will be collected. In addition, urine samples will be evaluated for antimicrobial residues using a disc-diffusion adapted from Driscoll *et al.*[80]. Low bacterial loads will

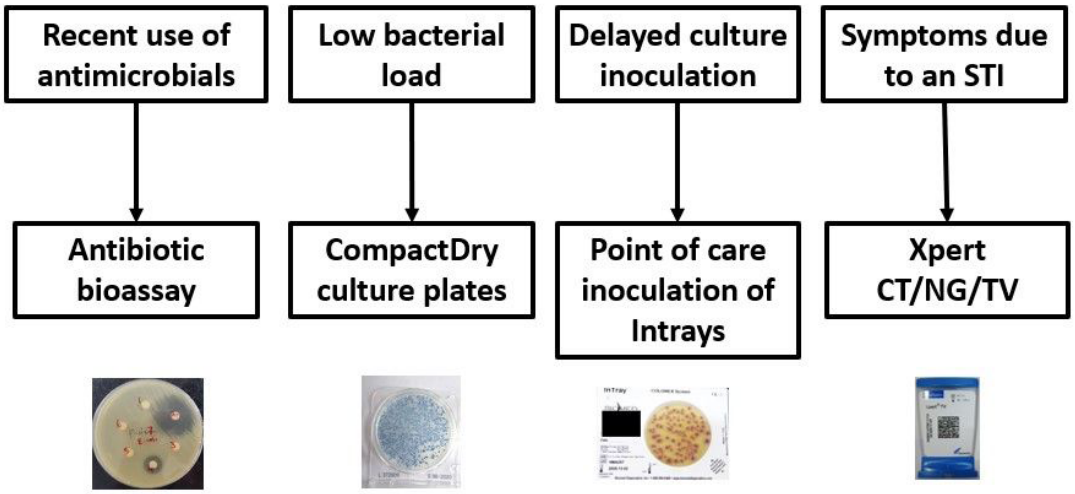

Figure 3. Evaluation of causes for negative urine cultures. *STI: sexually transmitted infections; CT: Chlamydia trachomatis; NG: Neisseria gonorrhoeae; TV: Trichomonas vaginalis.*

be investigated using a highly sensitive culture system that is employed for testing coliform contamination of water and food (CompactDry EC, Nissui, Japan). Point of care inoculation of urine samples using InTrays (BioMed Diagnostics) will be used to determine if sample transportation delays may contribute to contamination and pathogen loss. The prevalence of sexually transmitted infections in Zimbabwe can be as high as 15–20% (unpublished data from Ferrand R.A. *et al.*). A subset of urine samples will be tested for gonorrhoea and chlamydia using Xpert CT/NG (Cepheid, Sunnyvale, CA, USA) and for trichomonas vaginalis using Xpert TV (for women only).

Provision of routine care for study participants

Clinical care for study participants remains the responsibility of routine health care providers. Urine dipstick, microscopy and culture results are provided to the clinic health care workers, with advice from the study physician on management for complicated cases (such as prior treatment failure, isolation of multidrug resistant bacteria, pregnancy, or severe kidney or liver disease requiring dose-adjustment).

Procedures at follow-up

Participants who have a positive urine culture at enrolment have a follow-up visit between 7 and 14 days after enrolment. Participants are asked to provide information on their symptoms, antimicrobial use and healthcare seeking since their enrolment. If a participant has not taken a prescribed antimicrobial, the reasons are also recorded. A second urine culture is collected to assess for clearance. If UTI symptoms have not resolved, a second course of antimicrobials based on results of the AST is considered, as clinically appropriate.

All participants, irrespective of enrolment culture results, are followed up by telephone 28 days after enrolment to assess clinical outcomes (i.e. symptom resolution, hospital admission, UTI symptom recurrence).

Participants due to come to the clinic for a follow-up visit will be notified in advance of their appointment. If participants are unable to come to the clinic, a home visit will be performed. For the telephone follow-up visit at 28 days, participants will be called on at least three separate occasions on two different days. If they cannot be reached by telephone, a home visit will be attempted. Loss to follow-up will only impact on the outcome analysis and was accounted for in the sample size calculation.

Outcome measures

A UTI is classified as confirmed if the urine culture is positive with a recognised urinary pathogen or possible if the culture is negative or shows contamination. Bacteriological cure is defined as a negative urine culture following an initial positive urine culture. Clinical cure represents resolution of symptoms at the 7- to 14-day follow-up. Relapse is defined as the absence of a positive culture and symptoms after seven days but reappearance or re-presentation with symptoms within 28 days of the initial presentation. AMR to specific drugs and ESBL are defined using the EUCAST standards and guidelines for detection of resistance mechanisms[75,81]. Multidrug resistance is defined as resistance to one agent from at least three different antimicrobial classes[82].

Data management

All processes related to data collection, management and storage are governed by standard operating procedures (SOPs) and follow the principles of Good Clinical Practice.

All participants are identified throughout the study by a unique identifying number that is assigned at recruitment using uniquely numbered and barcoded consent forms. Apart from age and sex no personal data are collected on the clinical report forms.

All data are collected and entered on handheld tablets into pre-designed forms using the Open Data Kit (ODK, www.opendatakit.org) software. Electronic data entry quality is ensured by real-time error capture, internal validation, consistency checks and stringent formatting constraints. For the instances when the data cannot be entered directly into the electronic form (e.g. laboratory results that are only available after 24–49h), data are recorded onto paper forms. Upon completion of the laboratory tests, the data from the paper forms are entered electronically. Paper forms are available in case of failure of electronic data entry in the field.

Data analysis

Categorical variables will be analysed using counts and percentages and continuous variables using means/medians and standard deviations/interquartile ranges. The proportions of study participants with a positive, contaminated and negative urine culture will be determined. Prevalence and 95% confidence intervals will be presented for each causative organism and for resistance to antimicrobials. Univariate associations between risk factors and the presence of first-line and first- and second-line resistance and clinical and bacteriological outcome will be assessed using the χ^2 test for categorical variables. STATA (version 14, Stata-Corp, TX, USA) will be used for data analysis.

For the primary objective (association between HIV infection and ESBL presence in *E. coli*), a logistic regression model will be built, which will include age and sex as the pre-specified confounders and which will be controlled for the other variables which show an association in the univariate analysis (e.g. recent hospitalization, recent antimicrobial use, pregnancy). The molecular mechanisms of resistance and virulence factors will be reported in a descriptive analysis.

Sample size estimates

The sample size calculations used the following assumptions from published studies and a pilot study: 30% of urine cultures are positive[40,41], 90% of the positive cultures yield *E. coli*, ESBL prevalence in *E. coli* is 15% in HIV-negative and 30% in HIV-positive individuals, 25% of participants are HIV-positive and 90% of study participants know their HIV status. In order to determine if there is a difference in proportions of ESBL-producing *E. coli* between HIV-positive and HIV-negative individuals, 1404 participants presenting with symptoms of UTI

would need to be recruited into the study, of which 405 would be included in the primary outcome analysis.

For the clinical outcome analysis, UTI with a bacterial strain showing AMR (defined as resistance to ciprofloxacin or amoxicillin according to Zimbabwean guidelines)[71] will be considered as the exposure, and complete resolution of symptoms (clinical cure) at the day 7 follow-up visit the outcome. Preliminary data from this study have shown that the prevalence of AMR in UTI isolates is 83%. The assumptions used will be: 500 cultures are positive, loss to follow up is 10%, 80% of isolates are resistant to amoxicillin or ciprofloxacin, 30% of participants with symptoms have a positive culture, and 20% of patients did not take antimicrobials. Estimating a positive impact of treatment on clinical cure in participants without AMR of 80–90% and 40–50% in participants with AMR, the study will have >80% power to detect a difference between the groups.

Study status

The study began recruiting participants in June 2019 and recruitment is ongoing.

Discussion

Although there are indications that PLWH are at increased risk for infections with resistant organisms as compared to the general population, most studies have focused on gram-positive pathogens such as *Streptococcus pneumoniae*[83] and *Staphylococcus aureus*[84]. The ARGUS study will investigate if there is an association between HIV infection and AMR in GNB causing UTIs in Harare. The study will also investigate other risk factors for AMR in this setting. Results may contribute to the development of specific treatment recommendations based on the risk of AMR. Furthermore, the study will provide important data on the prevalence of AMR in community-acquired infections caused by GNB in this setting which will inform antibiotic prescribing guidelines, as well as the development of strategies to prevent further dissemination of resistance. The findings of this study are especially important since data on priority organisms for AMR surveillance from a large number of clinics across Harare will be collected. The information on outcomes of infections will guide the design of future management algorithms including identification of patients at risk for persistent infections and for complications.

The study is limited by its recruitment from a single city in Zimbabwe and therefore results might not be generalizable to the whole country which has a predominantly rural population. However, participants are recruited from ten PHCs across Harare and are therefore representative of the urban population. Individuals accessing healthcare at the clinics are required to pay a consultation fee. Due to the economic challenges in Zimbabwe, there has been an increase in consultation fees alongside rapid inflation. Therefore, individuals with mild symptoms may not access the clinic and will therefore not be included.

Ethics and dissemination

The study was approved by the ethics committees of the Medical Research Council of Zimbabwe (MRCZ/A/2406), the London School of Hygiene and Tropical Medicine (Ref. 16424), and the Biomedical Research and Training Institute. The study was granted permission from the City of Harare Department of Health. All study participants have provided/must provide written informed consent prior to enrolment into the study.

The study results will be disseminated to healthcare workers at the clinics through leaflets and dissemination meetings with the aim to enhance understanding, discuss the findings and ultimately improve future patient management. Significant microbiological results from individual patients will be reported to the attending healthcare worker as soon as they are available, in order to optimise treatment for individual patients. A report of the study results will be provided to the PHC healthcare workers, the Ministry of Health and Child Care, Harare City Health and other relevant stakeholders and policy makers. Data from this study may be used to inform treatment guidelines in order to improve patient management. The results of this study will be presented at national and international conferences to a wider audience and will be published in peer-reviewed journals.

Data availability

No data are associated with this article.

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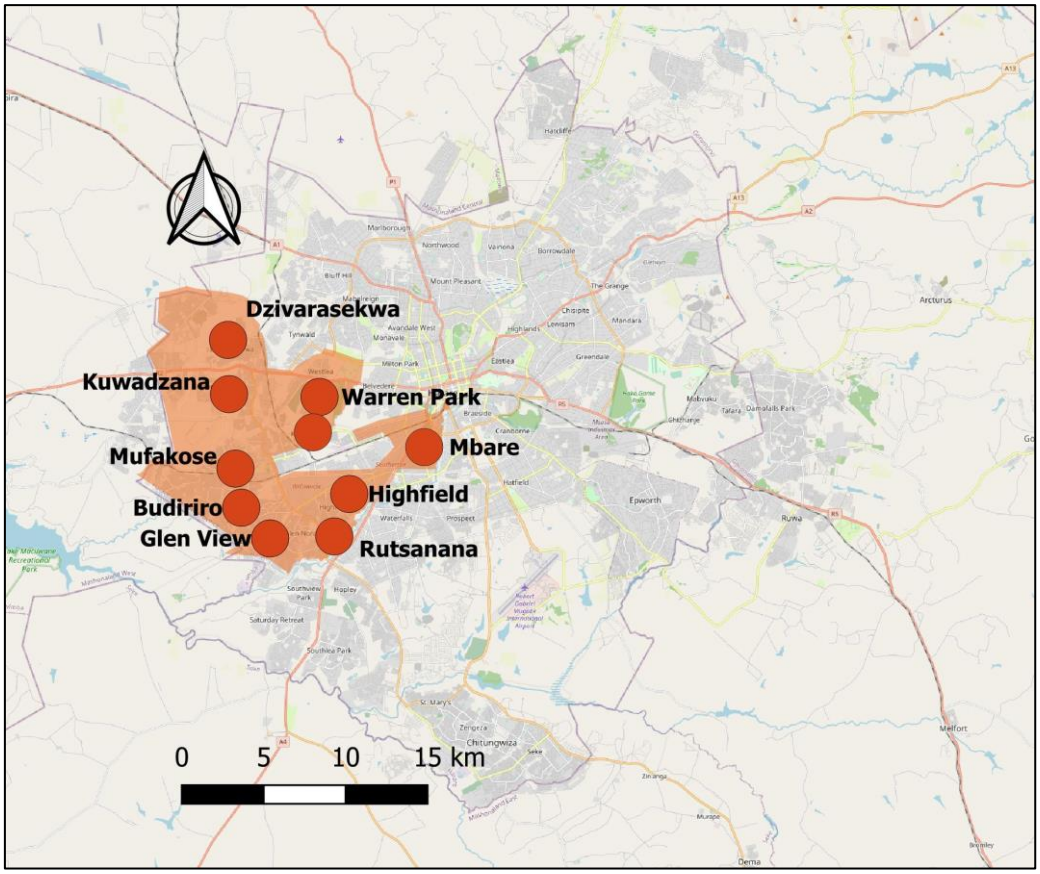
EXTENDED METHODS

Study setting



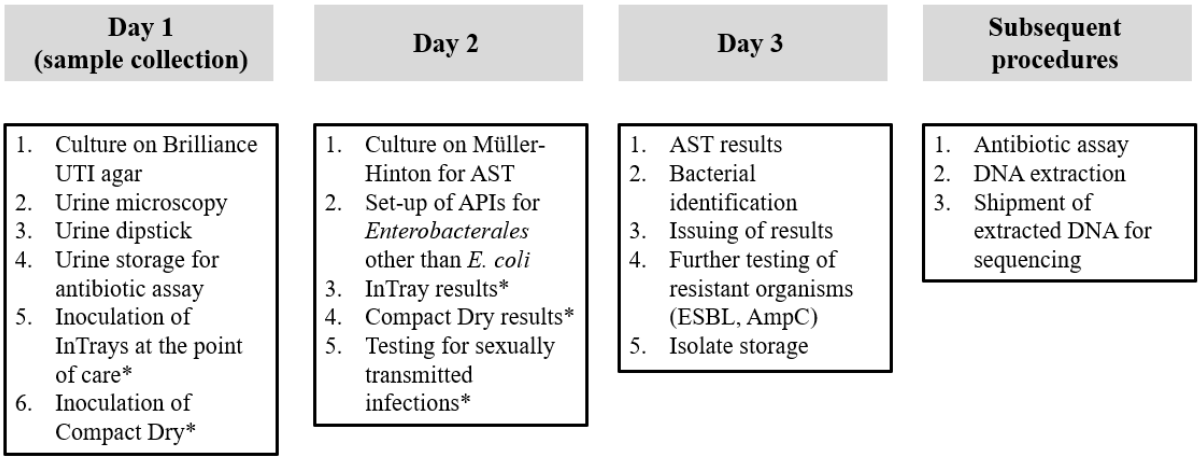
Map of Harare showing the locations of the ten clinics where recruitment was conducted (orange circles) and their respective catchment areas (shaded areas). According to the 2022 Zimbabwe census data, the total catchment population was 911,841 people (population of Harare: 1,491,740). The median catchment population per clinic was 76,824.

Source: Zimbabwe National Statistics Agency. 2022 population and housing census. Preliminary report on population figures. 2022, Zimbabwe. Available from https://zimbabwe.unfpa.org/en/publications/2022-population-and-housing-censuspreliminary-results.

Extended microbiology procedures

Microbiology methods for the ARGUS study

Outline of procedures



** performed in a subset of patients*

Urine sample collection and transport

A urine sample was collected from all participants during the baseline visit. Participants were instructed by trained research assistants on how to collect a midstream urine sample in a sterile urine container. The samples were then placed in a cooler box prior to transportation to the laboratory on the same day, usually within a few hours of collection.

Urine microscopy and dipstick testing

Urine microscopy and dipstick testing were performed upon receipt of the sample at the laboratory, on the same day of sample collection. Urine microscopy was performed after gently mixing the sample and using a counting chamber to determine the number of white and red blood cells per µL. For dipstick testing, the strip was briefly immersed in the sample and read for the presence of nitrites and leucocytes at 1 and 2 minutes, respectively according to the colour chart provided by the manufacturer. Leukocyturia was considered to be present if the sample contained $\geq 10^6$ white blood cells/L or if the dipstick was positive for the presence of leucocytes.

Sample storage

For the antibiotic assay, urine samples were aliquoted in 2-ml cryovials on the day of collection and stored at -80°C. The remainder of the sample was refrigerated in the original container until bacterial identification and antimicrobial susceptibility testing were completed.

Urine culture, bacterial identification and antimicrobial susceptibility testing

Samples were inoculated onto Brilliance UTI agar (Oxoid, UK) using 1µL calibrated loops and incubated aerobically at 37°C for 18-24 hours. Cultures were read after incubation by trained microbiologists who recorded semi-quantitative growth of a uropathogen and presumptive identification. Cultures were classified as follows: i) no growth; ii) 10^3-10^4 CFU/mL; iii) 10^4-10^5 CFU/mL; iv) >10^5 CFU/mL; or v) contaminated. Cultures were considered contaminated if they had growth of non-pathogenic bacteria or mixed growth without clear predominance of an uropathogen. Presumptive bacterial identification was done according to colony colour on chromogenic media. All *Enterobacterales* other than *E. coli* underwent further biochemical testing using APIs (Analytical Profile Index, bioMérieux, France) for identification. *S. aureus* was identified using catalase followed by slide and tube coagulase. For *Enterobacterales*, API 20E were used according to the manufacturer's instructions. A bacterial suspension was prepared using 5 mL saline and inoculated onto the test strips. The test strips were then incubated at 37°C for 18-24 hours prior to reading. Bacterial identification was done using the online database (https://apiweb.biomerieux.com).

Antimicrobial susceptibility testing was done by disc-diffusion on Müller-Hinton agar. A bacterial suspension of 0.5 McFarland was prepared and inoculated within 15 minutes of preparation onto the agar plates. Antimicrobials tested according to bacterial species are shown in Table 1. Plates were incubated aerobically at 37°C for 18 hours. Reading and interpretation was done according to the EUCAST standards.[1] Isolates were preserved for subsequent DNA extraction and sequencing at -80°C using Viabank cryogenic storage beads.

Table 1. Antimicrobial susceptibility testing according to bacterial species

Bacterial species	**Antimicrobials tested, disc concentrations in µg are shown in parentheses**
Staphylococcus saprophyticus	Cefoxitin (30), nitrofurantoin (100), trimethoprim (5), ciprofloxacin (5)
Enterococcus spp.	Ampicillin (2), vancomycin (5), ciprofloxacin (5), nitrofurantoin (100)
Enterobacterales	Ampicillin (10), cefpodoxime (10), ciprofloxacin (5), gentamicin (10), co-trimoxazole (25), imipenem (10), amoxicillin/ clavulanic acid (20-10), ceftazidime (10), ceftriaxone (30), cefoxitin (30), chloramphenicol (30), nitrofurantoin (100), fosfomycin (200), amikacin (30)
Pseudomonas spp.	Ceftazidime (10), ciprofloxacin (5), gentamicin (10), imipenem (10)

Testing for ESBL and AmpC production

Phenotypic testing was done in accordance to EUCAST recommendations.[2] For bacterial species without inducible AmpC production (e.g. *E. coli, K. pneumoniae*) whenever resistance to either cefpodoxime, ceftriaxone or ceftazidime was detected, double-disc synergy testing was performed to confirm the presence of ESBL. The test was done by placing cefotaxime (30 µg) and amoxicillin/ clavulanic discs 20 mm apart on a Müller-Hinton plate. The test was considered positive if the inhibition zones around the cephalosporin discs following incubation were augmented or a key-hole effect was seen. Bacterial species without inducible chromosomal AmpC exhibiting resistance to ceftazidime and cefoxitin underwent cloxacillin synergy testing.

Laboratory data management and issuing of results

Microbiological data were collected using paper-based case report forms. Once bacterial culture and susceptibility testing were completed, results were entered into a specifically designed electronic form using Epi Info (Version 7, Center for Disease Control and Prevention, USA) which was then used to issue the laboratory results. Results were printed in duplicate, verified by a senior microbiologist and a copy was provided to the clinic for routine care.

Quality assurance for laboratory procedures

The quality of laboratory testing was ensured by using standardised operating procedures for all processing steps. Culture media were prepared in accordance with the manufacturers' instructions and checked for sterility and the ability to support growth prior to use. Supporting growth and bacterial identification using chromogenic media was verified using ATCC strains (*E. coli* ATCC 25922, *E. faecalis* ATCC 29212). The Brilliance UTI agar was used within two weeks of preparation. The quality control for antimicrobial susceptibility testing was done using ATCC strains and the EUCAST recommendations.[3]

InTray processing and interpretation

InTrays (Biomed Diagnostic, USA) are ready-to-use small-sized chromogenic media that can be used for detection and presumptive identification of different bacterial species. InTray COLOREX ESBL contain antimicrobial substrates for the selective detection of ESBL-producing organisms. The InTrays require refrigeration and have a long shelf-life compared to

other pre-prepared chromogenic media of 6 (InTray COLOREX ESBL) to 12 months (InTray COLOREX Screen).

On each working day, InTrays were prepared and issued to trained research assistants working in the field. Following sample collection, sterile cotton swabs were used to distribute the urine sample on the surface of the agar on each of InTray COLOREX Screen and ESBL. The procedure was performed at the point of care immediately after sample collection. Upon receipt at the laboratory, the InTrays were incubated at 37°C for 18-24 hours. The plates were read the following day by an experienced microbiologist. Colony counts were recorded for each colony type separately. Uropathogens were pink (*E. coli*), blue (KESC: *Klebsiella, Enterobacter, Serratia, Citrobacter*), brown halo (*Proteus spp.*), cream/ translucent (*Pseudomonas spp.*), turquoise (*Enterococcus spp.*), golden (*S. aureus*), or pink (*S. saprophyticus*). Bacteria were classified as non-pathogenic if colonies were white, grey, pale blue or purple. For *Enterobacterales* growth was classified into: i) no growth (or 1-4 colonies); ii) 5-49 colonies; iii) 50-100 colonies; iv) confluent growth (uncountable). The results of testing using InTrays were not issued to clinicians.

Compact Dry processing and interpretation

Compact Dry EC (Nissui Pharmaceutical Co. Ltd., Japan) are small, dehydrated agar plates that can be used for differentiation of *E. coli* (blue colonies) from other coliforms (purple colonies). Compact Dry EC are routinely used for assessing of water quality. The plates can be stored at room temperature, have a very long shelf-life of 18 months, and have a grid to facilitate colony counts. For optimal colony counting, the manufacturer recommends diluting samples to a target of 100 colonies/ mL.

Compact Dry plates were inoculated with serially diluted urine samples on the day of sample collection. Aliquots of 990 µL phosphate-buffered saline were pipetted into five 2 mL sterile tubes. Upon receipt in the laboratory, urine samples were mixed gently, and 1.1 mL was transferred to a 2 mL tube. Using the phosphate-buffered saline aliquots, samples were diluted successively up to $1{:}10^6$. A volume of 1 mL from the $1{:}10^3$ and $1{:}10^6$ dilutions were then inoculated onto the Compact Dry plates and incubated at 37°C for 18-24 hours. The optimal dilutions were identified during a previous pilot study performed in the same laboratory when multiple dilutions were inoculated and compared. Bacterial counts were performed according to colony colour and recorded in a specially designed form. Growth was categorised as follows: i) no growth; ii) growth of 1-49 colonies; iii) growth of 50-250 colonies; iv) semi-confluent growth (individual colonies still visible but colonies could not be counted); v) confluent growth

(uncountable, no individual colonies visible). The results of the Compact Dry testing were not provided to the clinicians and were not used to inform clinical care.

Testing for sexually transmitted infections

Testing for sexually transmitted infections (STIs) was done for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* using the GeneXpert platform (Cepheid, USA) according to the manufacturer's instructions. All urine samples submitted to the laboratory between March and July 2020 were eligible for STI testing. Following receipt at the laboratory, 7 mL urine were transferred to the kit-provided urine transport tube and mixed. Then 1 mL sample was transferred to the Xpert CT/NG and Xpert TV (women only) cartridge and tested on the GeneXpert instrument. The urine transport tubes were stored at 4°C degrees for future re-testing. Re-testing was conducted in the case of invalid, error or no result. The test results were recorded using specific result sheets and patients were actively recalled if they had a positive result and were followed-up at the clinic for treatment. Treatment was provided free of charge by the study team. Multiple attempts were made to contact the participants.

Antibiotic assay

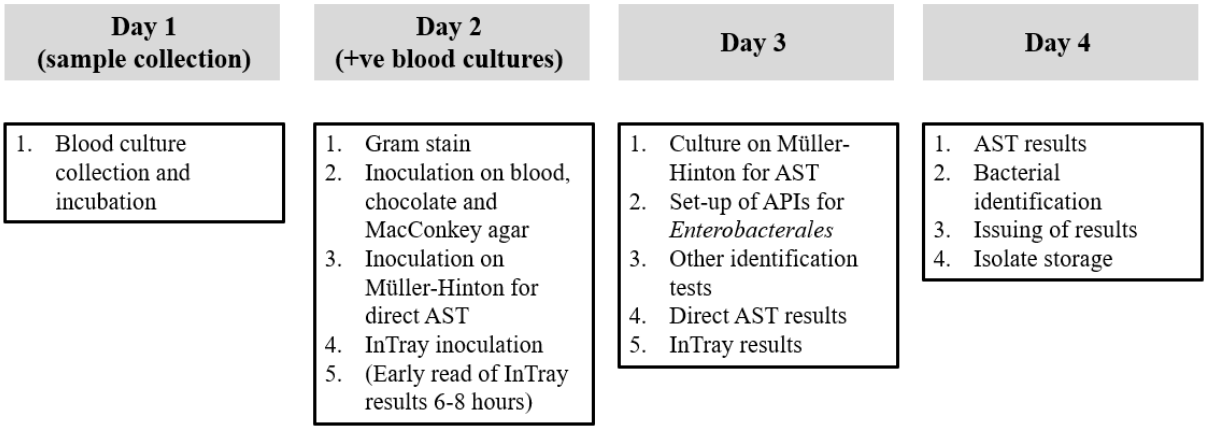
Frozen urine aliquots were allowed to thaw completely prior to testing. A bacterial suspension was prepared using a single colony of *E. coli* ATCC 25922, inoculated onto Müller-Hinton agar and allowed to dry. Blank 6 mm filter paper discs were placed on the media. Urine samples were mixed and 20 µL urine were inoculated onto the discs followed by incubation at 37°C for 24 hours. The diameter of inhibition was recorded. The sample was considered to have antibacterial activity against the indicator organism if any visible inhibition of growth was present.

DNA extraction and shipping

Cryopreserved *E. coli* isolates were retrieved and re-cultured on blood agar. Following overnight incubation and after review for potential contamination or mixed growth, one colony was re-cultured into tryptic soy broth and incubated overnight at 37°C. 1 mL of the liquid media was used for DNA extraction using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions for the isolation of genomic DNA from Gram-negative bacteria. Following extraction, the DNA was quantified using the Qubit (Invitrogen) fluorometer. At the end of the study, the extracted DNA was shipped for whole genome sequencing which was done commercially by MicrobesNG (Birmingham, UK)

Microbiology methods for the neonatal study

Outline of laboratory procedures for the neonatal study



Blood culture collection and transport

Samples were collected from neonates with suspected sepsis whose mothers provided informed consent for study participation. Following skin disinfection with 70% alcohol wipes, 2 mL of blood were collected for culture (BacT/ALERT PF Plus, bioMérieux, France). The blood culture bottles were transported to the laboratory by a dedicated study driver within 4 hours of collection. All blood culture bottles were weighed prior to issuing to the hospital and upon receipt at the laboratory to determine the volume of blood inoculated into the bottle. Blood culture bottles were incubated using the BacT/ALERT (bioMérieux, France) automated system and continuously monitored for up to five days. Positive blood cultures were processed immediately during laboratory working hours (Mondays to Fridays from 8 am to 4:30 pm, Saturdays and Sundays 10 am to 12 pm). Positive samples underwent Gram staining and inoculation onto blood, chocolate and MacConkey agar (HiMedia, India). The sample was also inoculated onto Müller-Hinton agar for direct antimicrobial susceptibility testing. Blood, MacConkey and Müller-Hinton agar were incubated aerobically at 37°C for 18-24 hours. Chocolate agar was incubated under 5-10% CO_2. Gram positive organisms were identified using their morphology on microscopy and culture followed by catalase and coagulase tests. If the slide coagulase was negative, a tube coagulase was performed. Presumptive enterococci were inoculated onto bile-aesculin agar. *Enterobacterales* were identified using APIs as described above. Antimicrobial susceptibility testing was done by disc diffusion using a 0.5

McFarland inoculum on Müller-Hinton agar. The following antimicrobial discs were used: ceftriaxone, imipenem, gentamicin, amikacin, ciprofloxacin, and chloramphenicol for *Enterobacterales*; ampicillin and vancomycin for enterococci, and cefoxitin for coagulase negative staphylococci. Vancomycin E-tests (bioMérieux, France) were used for coagulase-negative staphylococci which were tested only if the organisms were considered significant. Antimicrobial susceptibility testing results were interpreted according to the EUCAST recommendations.[4] The quality of laboratory testing was ensured by using standardised operating procedures and by performing quality controls for laboratory tests. Every new batch of prepared media was first checked for sterility of supporting growth using ATCC strains prior to use. Quality control for antimicrobial susceptibility testing was also performed according to EUCAST recommendations.[3]

InTray processing and interpretation

Following the Gram stain result, positive blood cultures with Gram negative bacilli and Gram positive cocci in chains were inoculated onto InTrays COLOREX Screen and ESBL. A drop from the positive blood culture broth was inoculated onto the plate and streaked using a calibrated loop. The plates were then incubated at 37°C. For ceftriaxone-resistant organisms, growth was usually apparent on the ESBL plate after a short incubation (6-8 hours). The final InTray result was read after 18-24 hours incubation.

Laboratory data management and issuing of results

Data were entered using pre-designed paper-based case-report forms. Upon completion of testing, the results were entered into an electronic form in Epi Info which was also used to issue the results for the clinicians. All results were verified by a senior microbiologist. Clinicians were informed of results as follows: i) immediately following Gram-staining using a messaging platform, clinicians were also required to acknowledge receipt of the message to ensure that significant results were actioned; ii) upon availability of the direct antimicrobial susceptibility testing results or visible growth on the ESBL InTrays using the same messaging platform with acknowledgement of receipt; iii) whenever new information became available that would potentially inform a modified clinical management ; iv) upon completion of testing when a paper-based result was issued to the clinicians.

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CHAPTER 3

Prevalence of ESBL-producing *Escherichia coli* in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe

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This chapter comprises the paper describing the results from the first component of the ARGUS study i.e. the description of the prevalence of AMR and the association between HIV and infections with extended-spectrum beta-lactamase producing *E. coli*. Resistance to third-generation cephalosporins is of particular concern because it limits treatment options for severe Gram-negative infections in low- and middle-income settings where alternatives may not be available.

The study shows that there was a high prevalence of AMR to first-line drugs for UTIs and the prevalence of resistance was higher among participants with HIV across most antimicrobial drug classes. Patients with HIV were more than twice as likely to have an infection with ESBL-*E. coli* than individuals without HIV.

This suggests that HIV status should be considered when prescribing empiric antimicrobial treatment to ensure effective treatment for both in- and outpatients. The high prevalence of resistance to first-line antimicrobials highlights the urgent need for up-to-date data on AMR prevalence to inform antimicrobial treatment guidelines.

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SECTION A – Student Details

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Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
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If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	JAC Antimicrobial Resistance		
When was the work published?	June 2021		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	NA		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the principal investigator for the study. I have designed and planned the study, prepared the study protocol, obtained the regulatory approvals, prepared the study forms and the standard operating procedures. Patient enrolment and data collection was done by research assistants which I trained and supervised. I prepared the laboratory standard operating procedures, trained the laboratory technicians and oversaw the laboratory work. I ensured that laboratory testing is conducted in accordance to quality standards. I performed the data management, cleaned, and analysed the data, and interpreted the results. I wrote the first draft of the manuscript, revised it following feedback from supervisors and co-authors, prepared and submitted the final draft. I revised the manuscript following peer-review.

SECTION E

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JAC-Antimicrobial Resistance

Prevalence of ESBL-producing *Escherichia coli* in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe

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Background: People living with HIV may be at increased risk for infections with resistant organisms. Infections with ESBL-producing organisms are of particular concern because they limit treatment options for severe Gram-negative infections in low-resource settings.

Objectives: To investigate the association between HIV status and urinary tract infections (UTIs) with ESBL-producing *Escherichia coli*.

Patients and methods: Cross-sectional study enrolling adults presenting with UTI symptoms to primary care clinics in Harare, Zimbabwe. Demographic and clinical data were collected during interviews and a urine sample was collected for culture from each participant. Antimicrobial susceptibility testing was performed according to EUCAST recommendations.

Results: Of the 1164 who were enrolled into the study, 783 (64%) were female and 387 (33%) were HIV infected. The median age was 35.8 years. Urine cultures were positive in 338 (29.0%) participants, and the majority of bacterial isolates were *E. coli* ($n = 254$, 75.2%). The presence of ESBL was confirmed in 49/254 (19.3%) *E. coli*. Participants with HIV had a 2.13 (95% CI 1.05–4.32) higher odds of infection with ESBL-producing *E. coli* than individuals without HIV. Also, the prevalence of resistance to most antimicrobials was higher among participants with HIV.

Conclusions: This study found an association between HIV and ESBL-producing *E. coli* in patients presenting with symptoms suggestive of UTI to primary care in Harare. HIV status should be considered when prescribing empirical antimicrobial treatment.

Introduction

The increase in antimicrobial resistance (AMR) challenges our ability to effectively treat infections leading to excess morbidity and mortality.[1] Although AMR has been highlighted as a priority on the global health agenda,[2] surveillance data on the burden of AMR are lacking from many low- and middle-income countries (LMICs).[3]

In understanding the burden of AMR, specific attention needs to be paid to populations who may be at increased risk for infections with resistant organisms such as people living with HIV. Globally, there are currently 38 million people living with HIV; two-thirds in sub-Saharan Africa.[4] Although there is evidence of an association between HIV and infection and colonization with resistant organisms, research has mainly focused on Gram-positive pathogens while data for Gram-negative infections are sparse.[5–7]

ESBL-producing Gram-negative organisms are of particular concern in LMICs. ESBLs confer resistance to third-generation

cephalosporins, which are the drugs of choice for the treatment of severe Gram-negative infections.[8] A better understanding of the epidemiology of AMR allows optimization of treatment recommendations with the ultimate aim of improving patient management.

Urine cultures performed in outpatients with symptoms of urinary tract infection (UTI) allow estimation of the burden of community-level Gram-negative resistance. The most frequently isolated organism from urine is *Escherichia coli*,[9] which is also the main cause of Gram-negative sepsis[10] and a WHO priority pathogen for AMR surveillance.[3]

This study aimed to investigate the association between HIV status and UTIs with ESBL-producing *E. coli* and to investigate the prevalence of AMR in bacteria causing UTIs among adults presenting to primary healthcare clinics (PHCs) in Harare, Zimbabwe.

Patients and methods

Ethics

Ethical approval for the ARGUS study was obtained from the Medical Research Council Zimbabwe (MRCZ/A/2406), the Institutional Review Board of the Biomedical Research and Training Institute in Zimbabwe and the London School of Hygiene and Tropical Medicine Ethics committee (Ref. 16424). The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards. All study participants provided written informed consent.

Study setting and participants

Data were collected as part of the Antimicrobial Resistance in Gram-negative bacteria from Urinary Specimens (ARGUS) study.[11] This was a cross-sectional analysis of consecutively enrolled participants recruited from 10 PHCs in southwest Harare between 1 July 2019 and 24 July 2020. Adult HIV prevalence in Zimbabwe is estimated at 13%.[4] According to national guidelines UTIs should be treated with either a fluoroquinolone or amoxicillin.[12]

Eligibility criteria included age 18 years or older, having at least two symptoms suggestive of UTI, onset of symptoms within the previous 2 weeks, presence of symptoms within the last 24 h, and provision of written informed consent. Those who were discharged from hospital in the previous 72 h, who had a urinary catheter *in situ* or who were enrolled into the study on a previous occasion were excluded.

Interviewer-administered questionnaires determined potential risk factors for AMR and clinical history. Responses were entered in electronic form using the Open Data Kit (ODK, www.opendatakit.org). HIV status was ascertained by self-report and confirmed by patient-held records.

Laboratory methods

A mid-stream urine sample was collected from every participant and transported to the laboratory within 4 h of collection. A volume of 1 μL of the sample was inoculated on chromogenic agar (Brilliance UTI agar, Oxoid, UK) and incubated at 37 °C for 24 h. Bacterial identification was performed using chromogenic media and APIs (Analytical Profile Index, bioMérieux, France) for Enterobacterales other than *E. coli*. Urine cultures were considered positive if there was growth of $\geq 10^3$ cfu/mL of a uropathogen either in pure culture or when a uropathogen was predominant. Antimicrobial susceptibility testing (AST) was done by disc diffusion using the Kirby-Bauer method and interpreted using EUCAST standards.[13] Testing for ESBL and AmpC production was performed according to EUCAST recommendations.[14] ATCC reference isolates were used for quality control of bacterial identification and AST (for further details on laboratory testing see Supplementary data, available at *JAC-AMR* Online).

The χ^2 test was used to evaluate categorical variables. Differences between continuous variables were assessed using the Mann–Whitney *U*-test. The level of significance was considered to be $P \leq 0.05$. For the association between HIV infection and infection with ESBL-producing *E. coli*, a multivariate analysis using logistic regression was performed (Figure S1). The model excluded individuals with unknown HIV status. Statistical analyses were performed using STATA v.15 (StataCorp, TX, USA).

Data availability

All data are presented in the main manuscript and additional supporting files. Data can also be provided on reasonable request to the corresponding author.

Results

Of the 1374 individuals with UTI symptoms presenting at the clinics, 1164 were eligible and were enrolled into the study. Reasons for ineligibility are detailed in Figure S2.

The median age was 35.8 years (IQR 26.3–47.7) and 743 (63.8%) were female of whom 102 (13.7%) were pregnant (Table 1).

The study included 387 (33.2%) participants with HIV infection, 680 who were HIV negative and 97 (8.3%) who did not know their HIV status. ART coverage among participants with HIV was 97.2% and 214 (55.6%) were receiving co-trimoxazole prophylaxis.

Uropathogens and AMR

Cultures were positive in 338 (29.0%) participants, 761 (65.4%) were negative and 65 (5.6%) contaminated. The majority of bacterial isolates were *E. coli* ($n = 254$, 75.2%) followed by other coliforms ($n = 33$, 9.8%), *Enterococcus* spp. ($n = 40$, 11.8%), *Staphylococcus saprophyticus* ($n = 3$, 0.9%) and *Staphylococcus aureus* ($n = 2$, 0.6%).

The prevalence of resistance to first-line antimicrobials was 245 (79.0%) for amoxicillin and 66 (19.8%) for ciprofloxacin. Co-trimoxazole resistance was present in 247 (84.0%) of isolates. Table S3 shows the prevalence of resistance according to bacterial species. In Enterobacterales resistance to amoxicillin/clavulanic acid was 40.1% (117/292), to nitrofurantoin 9.6% (28/292) and to fosfomycin 2.2% (5/228). Ceftriaxone resistance was present in 52/292 (17.8%) of isolates. The presence of ESBL was confirmed in 49/254 (19.3%) *E. coli*. Exposure to fluoroquinolones in the previous year but not to other antimicrobials was also associated with infections with ESBL-producing *E. coli* (20.4% versus 4.4% for no prior exposure, $P < 0.001$, Table S5). ESBL-producing *E. coli* were resistant to ciprofloxacin, gentamicin, nitrofurantoin and fosfomycin in 69.4% (34/49), 30.6% (15/49), 18.4% (9/49) and 9.8% (4/41) of isolates, respectively.

HIV and AMR in E. coli

Amoxicillin resistance was present in 67 (81.7%) *E. coli* isolates from participants with HIV and in 117 (80.7%, $P = 0.851$) from individuals without HIV infection while ciprofloxacin resistance was detected in 24 (29.3%) and 27 (18.6%, $P = 0.065$), respectively (Figure 1). The prevalence of co-trimoxazole resistance was 91.5% among individuals with HIV and 86.9% in those without

Table 1. Characteristics of individuals presenting with symptoms of UTI to public health clinics in Harare, Zimbabwe, stratified by urine culture result

	Urine culture result			
Characteristic	total, $N = 1164$	positive, $N = 338$	negative, $N = 761$	contamination, $N = 65$
Age, years, median (IQR)	35 (26–48)	34 (25–50)	36 (27–47)	34 (25–47)
Female sex, *n* (%)	743 (63.8)	263 (77.8)	431 (56.6)	49 (75.4)
Pregnancy, *n* (%)[a]				
not pregnant	599 (81.9)	222 (85.4)	341 (80.8)	36 (73.5)
first trimester	19 (1.6)	9 (2.7)	9 (1.2)	1 (1.5)
second trimester	32 (2.7)	8 (2.4)	20 (2.6)	4 (6.2)
third trimester	49 (4.2)	7 (2.1)	35 (4.6)	7 (10.8)
unknown	30 (4.1)	14 (5.4)	15 (3.6)	1 (2.0)
Place of recruitment, *n* (%)				
acute clinic	840 (72.2)	258 (76.3)	539 (70.8)	43 (66.2)
maternity	85 (7.3)	17 (5.0)	56 (7.4)	12 (18.5)
HIV clinic	239 (20.5)	63 (18.6)	166 (21.8)	10 (15.4)
Clinical symptoms				
duration of symptoms, days, median (IQR)	7 (4–12)	7 (3–10)	7 (5–12)	7 (5–12)
reported fever, *n* (%)[b]	122 (10.6)	36 (10.8)	79 (10.5)	7 (10.9)
dysuria, *n* (%)	942 (81.8)	295 (88.1)	595 (79.1)	52 (81.3)
frequency, *n* (%)	824 (71.6)	261 (77.9)	515 (68.5)	48 (75.0)
suprapubic pain, *n* (%)	730 (63.4)	193 (57.6)	490 (65.2)	47 (73.4)
haematuria, *n* (%)	185 (18.8)	77 (23.0)	96 (12.8)	12 (18.8)
limitation of daily activities, *n* (%)	168 (14.6)	60 (17.9)	99 (13.2)	9 (14.1)
Comorbidities, *n* (%)				
diabetes	23 (2.0)	8 (2.4)	13 (1.8)	2 (3.1)
chronic kidney disease	10 (0.9)	3 (0.9)	7 (0.9)	0 (0)
hypertension	126 (11.0)	42 (12.6)	78 (10.5)	6 (9.2)
HIV infection, *n* (%)[c]	387 (36.3)	110 (36.5)	259 (36.8)	18 (29.0)
co-trimoxazole prophylaxis, *n* (%)[d]	214 (55.6)	58 (52.7)	145 (56.4)	11 (61.1)
on ART, *n* (%)[d]	351 (97.2)	99 (97.1)	237 (97.9)	15 (88.2)
Outcome of their clinic visit, *n* (%)				
discharged home	1069 (97.9)	318 (98.8)	691 (97.6)	60 (96.8)
referred to outpatient specialist	12 (1.1)	4 (1.2)	8 (1.1)	0 (0)
referred to hospital	11 (1.0)	0 (0)	9 (1.3)	2 (3.2)

Missing data: clinical symptoms ($n = 13$: duration of symptoms, fever, dysuria, frequency, suprapubic pain, haematuria, limitation of daily activities); comorbidities ($n = 23$: diabetes, chronic kidney disease, hypertension); ART ($n = 26$); outcome of the clinic visit ($n = 72$).
[a]Denominator: women.
[b]For 939 (80.7%) participants a temperature measurement was available and 44 (4.7%) had an axillary temperature of $\geq$37.5 °C.
[c]97 patients did not know their HIV status.
[d]Denominator: participants with HIV infection.

HIV infection. In participants with HIV who were taking co-trimoxazole prophylaxis the prevalence of co-trimoxazole resistance was 97.8%. Infections with ESBL-producing organisms were more common among participants with HIV than in participants without HIV infection (26.8% versus 13.1%, $P = 0.010$). Participants with HIV had a 2.43 (95% CI 1.22–4.83) higher odds of infection with ESBL-producing *E. coli* than individuals without HIV, and the association persisted after adjusting for age and sex (adjusted OR (aOR) 2.13; 95% CI 1.05–4.32, Tables S5 and S6, Figure S1).

Discussion

This study shows a high prevalence of resistance to first-line antibiotics for UTI treatment and of ESBL-producing *E. coli* among individuals presenting with symptoms suggestive of UTIs to PHCs in Harare. Also, the study found an association between HIV infection and the presence of ESBL-producing *E. coli* in this setting. In general, the prevalence of resistance was higher in participants with HIV infection compared with those without for almost all antimicrobials tested.

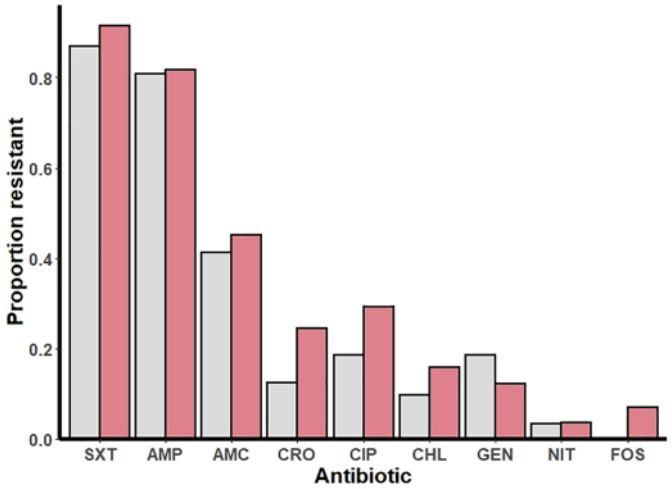

Figure 1. AMR among *E. coli* isolates in individuals with HIV (red) and individuals without HIV infection (light grey). AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; FOS, fosfomycin; GEN, gentamicin; NIT, nitrofurantoin; SXT, co-trimoxazole. None of the isolates had imipenem resistance.

Almost one in five of all study participants, and one in four of those with HIV infection, had a UTI due to ESBL-producing *E. coli*. The prevalence of ESBL among participants with HIV was 2-fold higher compared with those without. While resistance to third-generation cephalosporins is less important in primary care settings the associated resistance to oral antimicrobials limits treatment options in outpatients. Furthermore, a high ESBL prevalence in the community setting will translate to high levels of infections due to ESBL-producing organisms among severely ill patients admitted to hospital. This is particularly problematic since third-generation cephalosporins are reserved for the treatment of severe Gram-negative infections in hospitalized patients and alternative treatment options such as carbapenems are not easily available or affordable in most LMICs. This is compounded by limited access to laboratory diagnostics that would enable diagnosis of infections with resistant organisms to guide targeted antibiotic treatment.

Bacterial infections remain a major cause of hospitalization and death among people living with HIV.[15] The higher prevalence of ESBL-producing *E. coli* infections among participants with HIV may be explained by the more frequent healthcare seeking in this group leading to colonization and infection with resistant organisms. Comorbidities, hospital admissions, clinic visits and antimicrobial prescriptions, which are well-recognized risk factors for infections with resistant pathogens, are more common in those infected with HIV compared with those without.[16–19] HIV status therefore should be taken into consideration when prescribing antimicrobials.

In this study, there was a high prevalence of resistance to first-line treatment. This is not surprising given the frequent use of amoxicillin for a broad range of indications. Furthermore, the high prevalence of co-trimoxazole resistance can be explained by its use as prophylaxis in HIV-infected individuals.[20] On the other hand, the prevalence of resistance to nitrofurantoin was <10%, making it an effective and affordable treatment option for uncomplicated infections in this setting at a cost per treatment of approximately $1. Fosfomycin would serve as a good alternative for treatment given the low prevalence of resistance, low cost (around $5), and single-dose treatment regimens.

This study focused on patients presenting to primary care facilities, providing a good estimate for community-level resistance prevalence in Harare. The study recruited from 10 PHCs in the largest city in Zimbabwe across different socioeconomic strata, making the results generalizable to the urban population of Harare. Antimicrobials taken prior to clinic presentation may have led to an overestimation of resistance. Furthermore, the study was underpowered to detect an association between HIV and AMR other than ESBL.

This study found an association between HIV and ESBL-producing *E. coli* in patients presenting with symptoms suggestive of UTI to primary care in Harare. HIV status should be considered when prescribing empirical antimicrobial treatment in this setting to ensure effective treatment for both in- and outpatients. The high prevalence of resistance to first-line antimicrobials highlights the urgent need for up-to-date data on AMR prevalence to inform antimicrobial treatment guidelines. Consolidating laboratory capacity, strengthening AMR surveillance and promoting research in AMR in LMICs is paramount to ensure treatment guidelines are tailored to the local context and epidemiology.

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Transparency declarations

None to declare.

Disclaimer

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The views expressed do not necessarily reflect the UK government's official policies.

Supplementary data

Methods, Tables S1 to S6 and Figures S1 to S2 are available as Supplementary data at *JAC-AMR* Online.

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Supplementary materials for the above publication *"Prevalence of extended-spectrum beta-lactamase producing Escherichia coli in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe"* are included in the Appendix 2.

Contents of Appendix 2

CHAPTER 4

Genomic epidemiology of *Escherichia coli* causing urinary tract infections in Harare, Zimbabwe

The previous chapter showed that the prevalence of resistance to first-line drugs which are recommended for the treatment of UTIs is high and that there is a relatively high prevalence of resistance to third-generation cephalosporins. The current chapter explores the underlying molecular mechanisms for resistance and describes the *E. coli* lineages causing UTIs in this setting. The findings of this study are also put into the wider context of the molecular epidemiology of extraintestinal pathogenic *E. coli* (ExPEC) in sub-Saharan Africa. The findings from this chapter are currently being prepared for publication.

Introduction

Escherichia coli is the leading pathogen causing Gram-negative infections and a major cause of death associated with antimicrobial resistance (AMR).[1] Phylogenetically *E. coli* is very diverse with strains associated with particular ecological niches and commensal and infection-associated phenotypes.[2] Although, mainly a commensal colonizing the human gut, some strains can acquire virulence traits allowing them to cause intestinal and extraintestinal infections.[2] Pathogenic organisms can be broadly categorised into intestinal pathogenic *E. coli*, comprising strains associated with diarrheal disease, and ExPEC.[3] ExPEC causes around 80% of community-acquired urinary tract infections (UTIs) with certain strains also associated with sepsis and neonatal meningitis.[2] Uropathogenic *E. coli* (UPEC) are the most commonly encountered ExPEC.[4] Traditionally, isolates were characterised by multi-locus sequence typing (MLST) using seven housekeeping genes which were amplified by polymerase chain reaction followed by DNA sequencing. Currently, whole genome sequencing can be used to determine sequence types and allows for a better discrimination between isolates and for determining phylogenetic relationships.[5] Pandemic ExPEC lineages with global distribution and associated with resistance to multiple antibiotic classes, such as sequence type (ST) 131 are widely recognized. There are geographical differences in the prevalence of different STs and resistance mechanisms.[6] Additionally, most studies reporting on the genomic epidemiology of ExPEC have been conducted in high-income settings with few studies reporting on strains from sub-Saharan Africa.[6]

The aim of this study was to describe the genomic epidemiology and mechanisms of resistance of *E. coli* isolated from patients presenting with UTIs to primary care clinics in Zimbabwe.

Methods

Patients presenting with symptoms of UTI to ten primary care clinics in Harare between June 2019 and July 2020 were recruited into the Antimicrobial Resistance in Gram-negative bacteria from Urinary Specimens (ARGUS) study. Study procedures were previously described.[7] Briefly, adult patients presenting with at least two symptoms of UTI were eligible and invited to participate. After providing written informed consent, data on demographics, risk factors, clinical history and a urine sample for culture were collected. Urine culture, bacterial identification, and antimicrobial susceptibility testing was performed at the Biomedical Research and Training Institute laboratory using standard microbiology procedures. All significant bacterial isolates were cryopreserved.

DNA from overnight cultures of the *E. coli* isolates was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. DNA libraries were prepared using the Nextera XT Library Prep kit according to the manufacturer's instructions (Illumina, San Diego, USA). Pooled libraries were quantified using the Kapa Biosystems Library Quantification kit for Illumina according to the manufacturer's instructions. Libraries were sequenced on the Illumina HiSeq platform using 250bp paired reads protocol. Reads were trimmed using Trimmomatic v.0.30.[8] De novo assembly was performed using SPAdes v.3.7 with default settings.[9] Species identification was performed using Kraken. In silico MLST was performed using MLST version 2.19.0 from the PubMLST database according to the Achtman scheme.[10,11] Novel sequences were identified using Enterobase (www.enterobase.warwick.ac.uk). Resistance genes, virulence factors and serotypes were identified using the ABRicate pipeline, version 0.9.8 [12] and the ResFinder,[13] Virulence Factor,[14] and EcOH[15] databases. A resistance gene was considered to be present if it had at least 80% match in identity and coverage with a gene in the database. *E. coli* phylogroups were determined *in silico* using ClermonTyping.[16] The *fimH* alleles were determined using FimTyper.[17] A core genome alignment was generated using snippy[18] with the *E. coli* K-12, MG1655 as a reference (GenBank accession number NC000913.3). Phylogenetic trees were generated using Fasttree and visualised and annotated using R version 4.1.1 (package ggtree).

Results

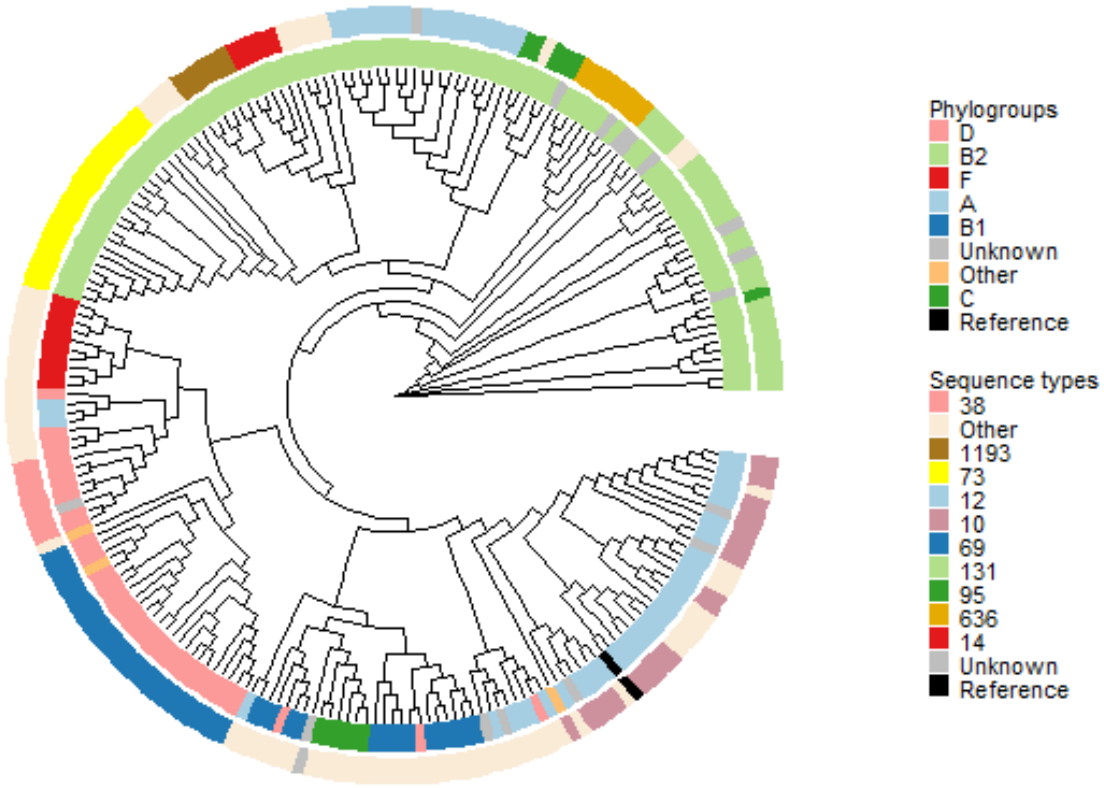
A total of 227 out of 254 isolates identified as *E. coli* using conventional microbiological techniques could be retrieved and had growth on reculture. Of those, four isolates were identified as other species than *E. coli* using whole genome sequencing and were excluded from the analysis.

Serotypes and sequence types

Most isolates belonged to the B2 phylogenetic group 97/223 (43%) followed by A and D with 38 (17%) isolates each, B1 with 16 (7%); F with 10 (5%), and C with 6 (3%). Five isolates belonged to other phylogroups and for 13 (6%), the phylogroup could not be determined. There was a high diversity of STs among the isolates which belonged to 63 STs. The most common STs were ST69 (n=25), ST131 (n=25), ST10 (n=23), ST73 (n=20), and ST12 (n=18). Two new STs not previously described were identified. Of the 97 isolated from phylogroup B2, 60 (62%) belonged to three STs ST131, ST73 and ST12. 24/38 isolates from phylogroup D belonged to

ST69. Only 3 isolates belonged to ST410. Figure 1 shows the phylogenetic tree alongside *E. coli* phylogroup and ST distribution.

Figure 1. A phylogenetic tree depicting the genetic relationship among the 223 *E. coli* isolates analysed in this study. The tree was reconstructed using core genome SNP alignment and inferred maximum likelihood using snippy and fasttree. The tree is shown as a cladogram and was annotated in RStudio (package ggtree). The inner circle represents the phylogroups and outer circle the sequence types. Colour codes for the phylogroups and sequence types (STs) are depicted in the legend.



Antimicrobial resistance genes

The following beta-lactamase genes encoding for extended-spectrum beta-lactamases (ESBLs) were identified: *bla*$_{\text{CTX-M-15}}$ in 31/223 (14%), *bla*$_{\text{CTX-M-14}}$ in 3 (1%), *bla*$_{\text{CTX-M-27}}$ in 9 (4%), *bla*$_{\text{CTX-M-55}}$ in 1 (<1%). Two isolates co-harboured two ESBL genes each, *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{CTX-M-55}}$ in one isolate and *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTXM-27}}$ in the other isolate. Among CTX-M-15 isolates, 13 were in ST131, 7 in ST636, 9 in other STs, and for 2 the ST could not be determined. bla$_{\text{CTX-M-27}}$ were present in 3 ST131, in 2 ST38 and one of each for ST10, ST95,

ST1193, and ST2172. Beta-lactamase genes determining third-generation cephalosporin resistance were mainly *bla*$_{\text{CTX-M}}$ with only two isolates encoding for AmpC beta-lactamases. *bla*$_{\text{OXA1}}$ was present in 25 (11%) isolates of which 12 were ST131, *bla*$_{\text{TEM1}}$ in 163 (73%). Resistance genes identified included genes encoding for resistance to trimethoprim-sulfamethoxazole *sul1*, *sul2* and *dfrA* in 120 (54%), 179 (80%), and 201 (90%), respectively; *aac(6')-Ib-cr* for fluoroquinolone resistance in 25 (11%), *aph(6)-Id*, and *aadA5* for aminoglycoside resistance in 172 (77%) and 57 (26%) and *mphA* encoding for macrolide resistance in 56 (25%) (Figure 2 and 3). The *qnrS* gene encoding plasmid-mediated fluoroquinolone resistance and *qepA* gene encoding a fluroquinolone efflux pump were identified in 6 and 2 isolates respectively (Figure 3). Point mutations associated with fluoroquinolone resistance were present as follows: *gyrA* D87N in 45 (20%), *gyrA* S83L in 75 (34%), and *parC* S80I in 49 (22%). Mutations in the *gyrA* gene were present in 19/25 isolates belonging to ST131, in 6/6 of ST1193 and 8/8 of ST636. Genes conferring resistance to at least three antibiotic classes were present in 194 (87%) of isolates (Figure 4).

Figure 2. Prevalence of resistance genes among *E. coli* isolates included in this study

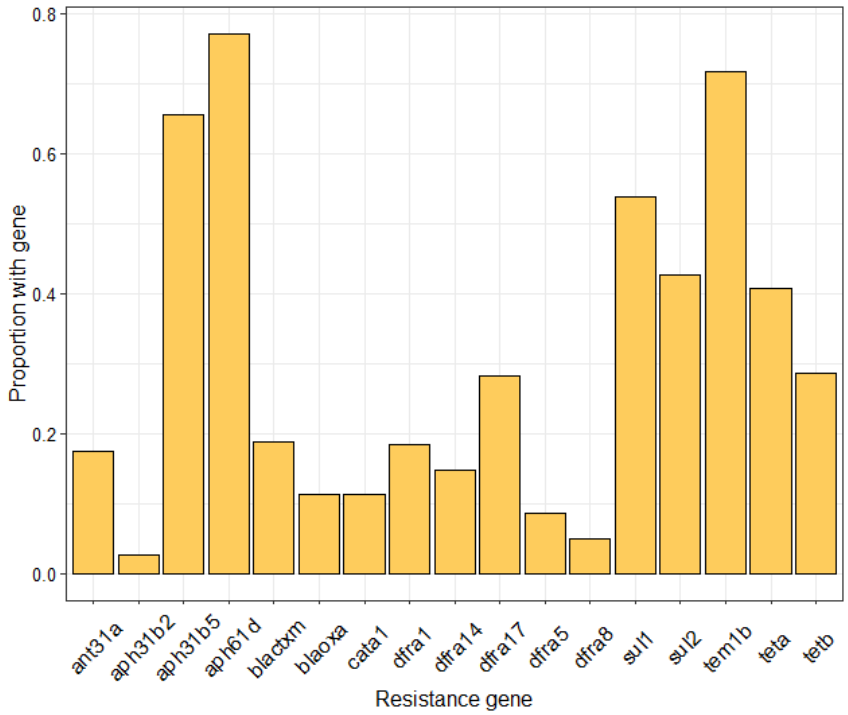
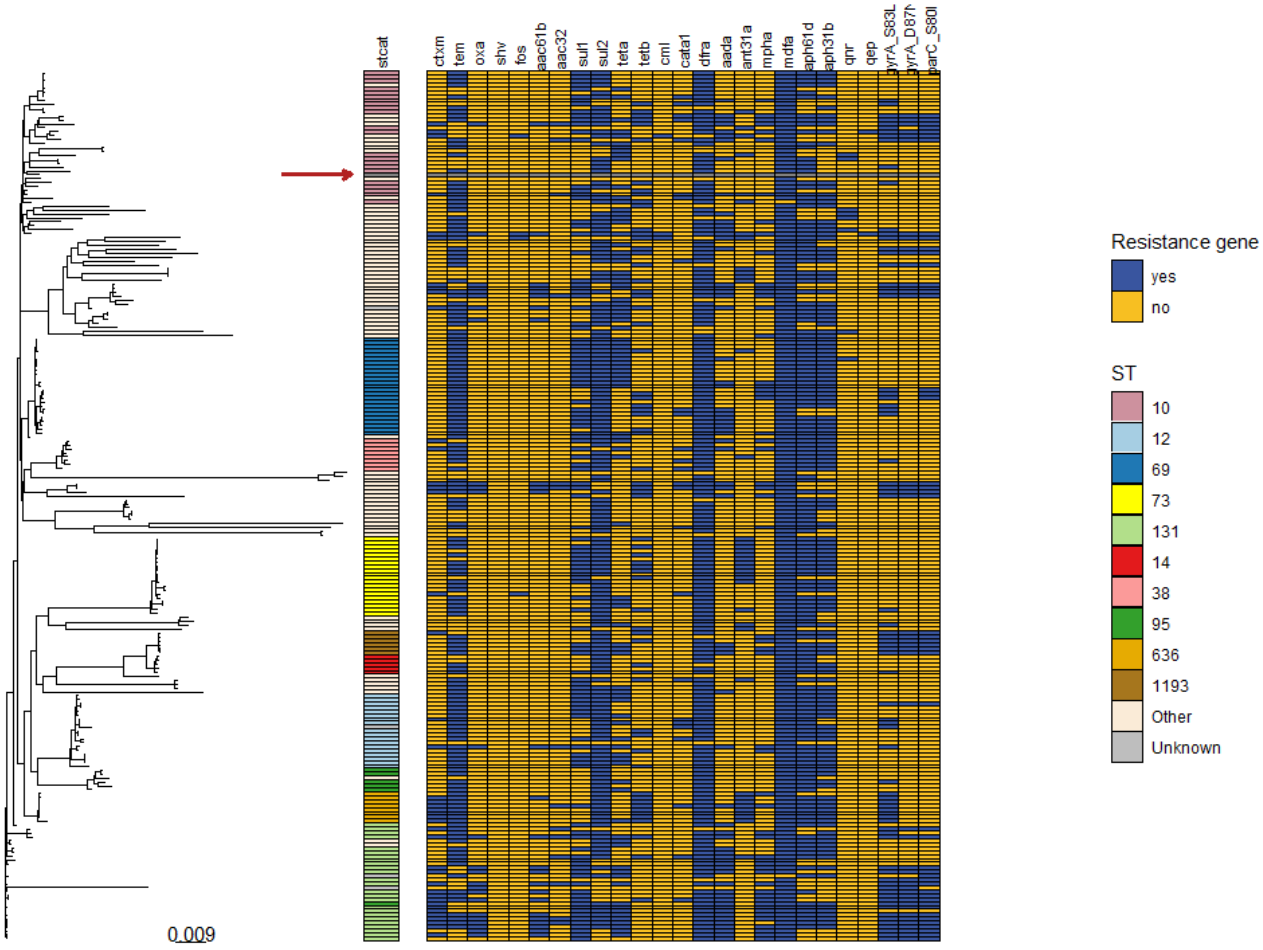
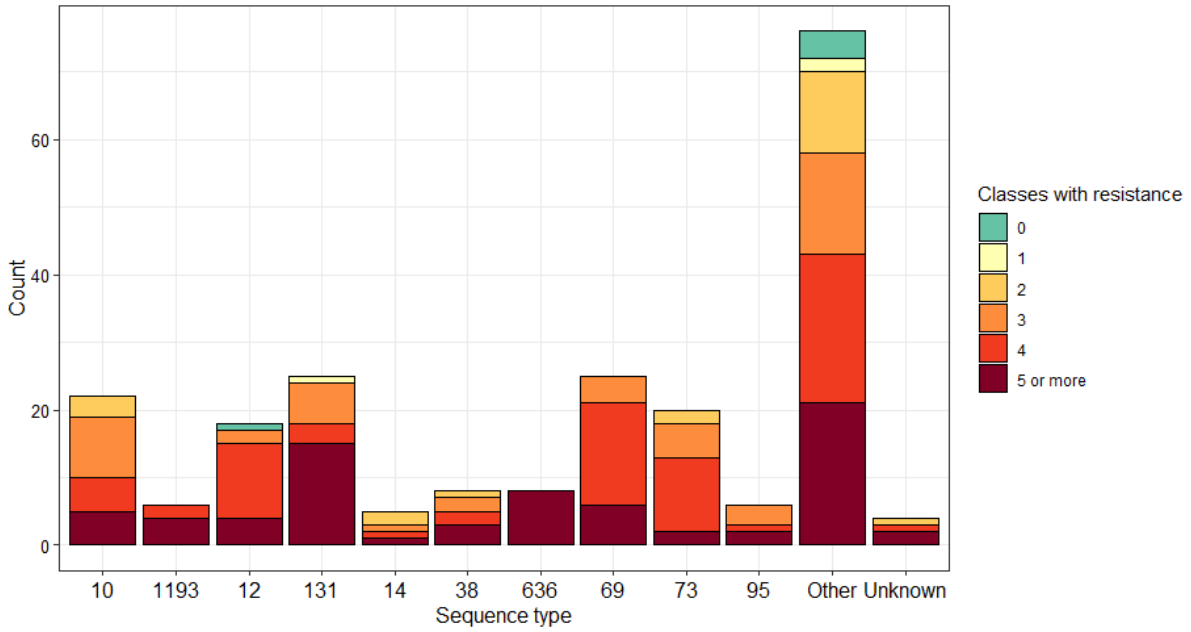


Figure 3 Phylogenetic tree showing the distribution of antimicrobial resistance genes and point mutations across *E. coli* sequence types.



The reference is represented as a dark grey line (red arrow). ST: sequence type. The phylogenetic tree was constructed using core genome alignment and inferred maximum likelihood in snippy and fasttree and was annotated in R (package ggtree).

Figure 4. Antimicrobial resistance according to sequence type



Penicillins and third-generation cephalosporins are counted separately. Antibiotic classes considered are: penicillins, cephalosporins, aminoglycosides, fluoroquinolones, phenicoles, folate pathway inhibitors, phosphonic acids and tetracyclines.

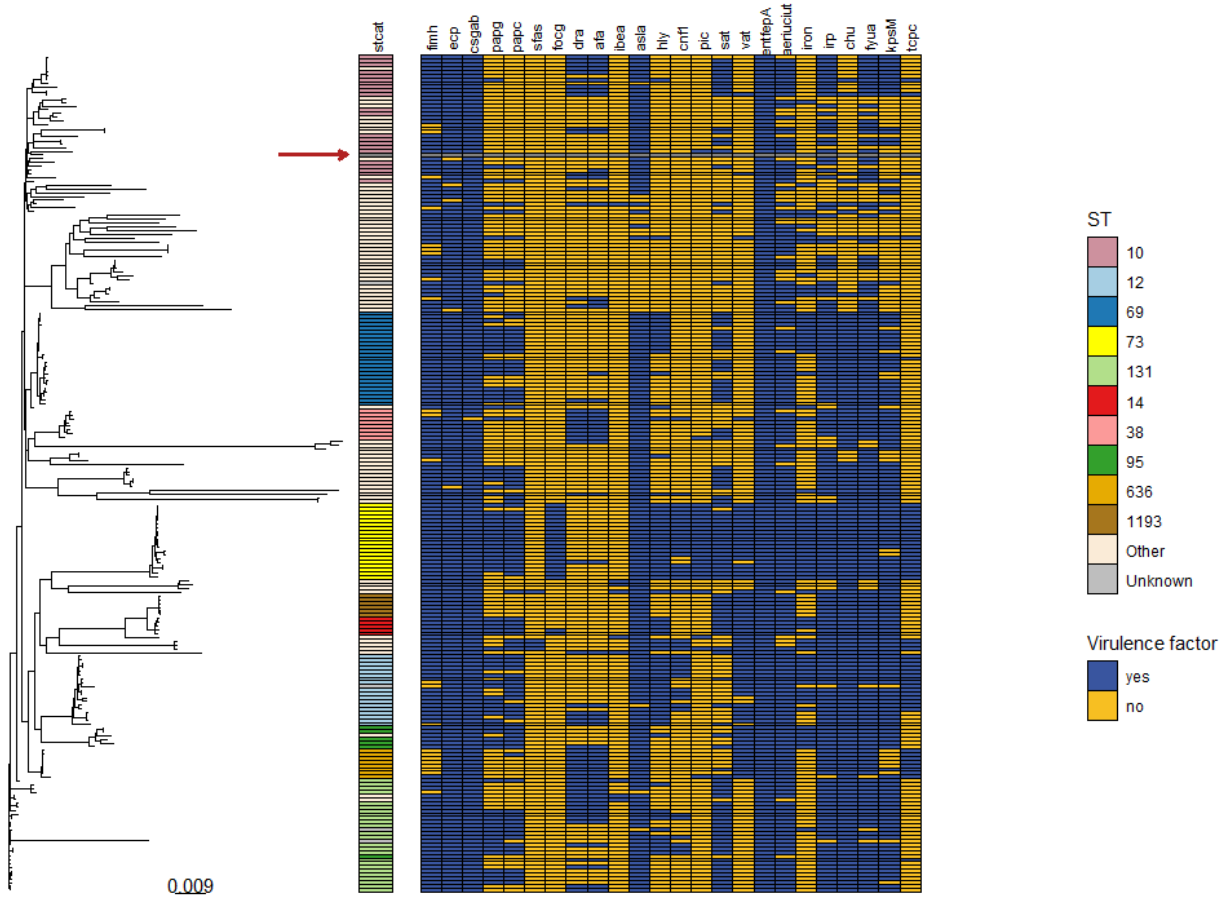
Virulence factors

The *fimH* gene was present in 199 (89%) of isolates while 95 (43%) of isolates carried the *papG* fimbrial adhesin gene. Most ST131 isolates (18/21, 86%) carried *fimH30*. The endothelial brain invasion *ibeA* gene was present in 9 (4%) while the arylsulfatase *aslA* gene in 192 (86%) of isolates. Genes encoding toxins were present as follows: *hly* coding for alpha-hemolysins in 77 (35%), *cnf1* (coding for the cytotoxic necrotizing factor, CNF-1) in 41 (18%) and *satA* coding for a secreted autotransporter in 115 (52%). The prevalence of the autotransporter genes *vac* and *pic* was 70 (31%) and 26 (12%) respectively. Genes involved in iron uptake such as *chu* was present in 162 (72.6%) while genes coding for aerobactin and yersiniabactin were found in 190 (85%) and 187 (84%) isolates respectively. An overview of virulence factors, their pathogenicity mechanisms and associations with STs are presented in Table 1 and Figure 5.

Table 1. Virulence factors of *E. coli* isolates from patients with community acquired UTIs

Virulence factor	**Designation**	**Function**	**Prevalence N (%) Total=223**
ADHESINS			
fimH	Type 1 fimbriae	Binds to alpha-D-mannose residues on bladder urothelial cells and other cell types promoting cell invasion.[2]	199 (89.2)
csgAB	Curli fimbriae	Involved in attachment and invasion of host cells, immune activation, and biofilm formation.[2]	222 (99.6)
papG	P fimbriae	Encoded by the pap (pyelonephritis associated pili) operon;[19] P fimbriae can have proinflammatory effects.[2]	95 (42.6)
sfaS	S fimbriae	Adhesion to intestinal and urothelial cells	4 (1.8)
INVASINS			
ibeA	Endothelial brain invasion	Invasion into host tissues[2]	9 (4.0)
aslA	Arylsulfatase	Endothelial cell invasion[2]	192 (86.1)
TOXINS			
hly	Alpha-hemolysin	Cytotoxic effects on different cell types including leucocytes and renal cells and proinflammatory effects.[2,20] These effects can promote the passage of bacteria into the bloodstream leading to systemic infections.[21]	77 (34.5)
cnf1	Cytotoxic necrotizing factor 1	Causes cell necrosis, inflammation, dissemination of bacteria between cells and may inhibit phagocytosis and induce bacterial persistence in the urinary tract.[2,22]	41 (18.4)
AUTOTRANSPORTERS			
sat	Secreted autotransporter toxin	Cytotoxic effects and can promote the passage into the bloodstream.[2]	115 (51.6)
vat	Vacuolating autotransporter toxin	Promotes urothelial colonization	70 (31.4)
pic	Serin protease autotransporter	Promotes urothelial colonization	26 (11.7)
IRON UPTAKE SYSTEMS			
ent, fepA	Enterobactin	Siderophores; can capture iron from host transporters.[23]	223 (100)
iuc, iut	Aerobactin		190 (85.2)
iroN	Salmochelin		77 (34.5)
irp	Yersiniabactin		181 (81.2)
chu	ChuA, Hma	Capture systems from iron complexes	162 (72.6)
fyuA			187 (83.9)
PROTECTINS			
kpsMI-neuA	K1 capsule	Inhibits phagocytosis and promotes immune tolerance and intracellular survival; associated with invasive infections and bacteremia.[2]	158 (70.9)
tcpC	Toll-like receptor domain containing protein C	Impairs innate immune responses within macrophages and increases UTI severity.[2]	52 (23.3)

Figure 5. Phylogenetic tree showing the distribution of virulence factors *E. coli* sequence types.



The reference is represented as a dark grey line (red arrow). The phylogenetic tree was constructed using core genome alignment and inferred maximum likelihood in snippy and fasttree and was annotated in R (package ggtree).

Discussion

This is one of the first studies making use of whole genome sequencing to describe the epidemiology of ExPEC *E. coli* from unselected clinical samples in sub-Saharan Africa. This study identified a large diversity of STs among study isolates. Overall, resistance genes and pathogenicity factors were prevalent and diverse.

In other studies from the region, samples undergoing whole genome sequencing were selected, usually based on phenotypic antibiotic susceptibility testing results and had a very small sample size (Table 2). As antibiotic resistance genes are associated with particular STs, these studies may not fully describe the range of *E. coli* ST in the respective setting. ST10, ST69 and ST131

which are common globally distributed ExPEC STs[6] were each found in around 10% of the isolates. Overall, 87% of the isolates from this study had resistance to at least three antibiotic classes. Resistance to multiple classes was particularly frequent among isolates belonging to ST131, ST636 and ST1193 although for the last two the number of isolates was very low. Overall, 87% of isolates harboured resistance genes for at least three classes of antibiotics which is concerning given the limited availability of drugs in sub-Saharan Africa. The high prevalence of resistance to multiple antibiotic classes may be explained by frequent antibiotic exposure in this setting. In addition to antibiotics used for treatment of infections in humans, unregulated use of antibiotics in agriculture and environmental contamination may play an important role.[24] Most isolates harbouring ESBL genes in this study belonged to ST131. As previously described[25], ST131 isolates harbouring $bla_{\text{CTX-M}}$ genes frequently had chromosomal mutations associated with fluoroquinolone resistance. Although ST410 is also a globally distributed ST associated with ESBL and resistance to other antibiotics[26] and reported frequently in Africa, it was rare among our isolates. This is in contrast to the findings of another study from Zimbabwe reporting on ESBL-*E. coli* from urine samples where ST410 was present in 27% of isolates.[27] Similarly, ST617 which is relatively prevalent in other studies from Africa,[6] was identified in only two isolates. A few isolates belonged to ST1193 (n=6) which is a new virulent clone with global distribution which frequently harbours ESBLs and fluoroquinolone resistance genes[28]. Only one ST1193 isolate in this study harboured $bla_{\text{CTX-M-27}}$ but all six had *gyrA* mutations conferring fluoroquinolone resistance. Similar to other studies in the region, $bla_{\text{CTX-M-15}}$ was not strictly associated to ST131 but distributed across a number of other STs.[29] ST131 isolates were frequently containing the *fimH30* gene in line with the recognized global expansion of the ST131-H30 lineage.[25]

This study found that genes encoding resistance to trimethoprim-sulfamethoxazole were very common. This is not surprising given the high prevalence of resistance reported in studies from sub-Saharan Africa. This is likely driven by the extensive use of co-trimoxazole as prophylaxis for opportunistic infection in this setting as well as treatment in non-HIV associated conditions. This is however not the case in studies from high-income countries where co-trimoxazole resistance is less frequent.[30] None of the isolates in this study harboured genes for carbapenem resistance. Carbapenems are used infrequently in Zimbabwe because of high cost and limited availability. This study was conducted in primary care enrolling patients with community acquired infections. Prevalence may be higher in patients with hospital-acquired infections although the overall prevalence is anticipated to be very low.[31]

Pathogenicity factors were common among isolates included in this study. The prevalence of genes encoding adhesins among study isolates was 89% for *fimH*, and 43% for *papG.* All but one isolate had *csg*AB genes for curli fimbriae. Invasion protein *ibe*A gene was identified in 4% of the isolates while the arylsulphatase-like gene *asl*A, which contributes to endothelial cell invasion[2] was present in 86% of isolates. Iron uptake systems are essential for bacterial metabolism and survival. Siderophores such as enterobactin, aerobactin, yersiniabactin and salmochelin can capture iron from host transporters.[23] Overall, iron uptake systems were present in the majority of isolates from this study. In this study virulence factors generally had a higher prevalence than that reported in other studies from Zimbabwe[27], the region,[32,33] and globally for UPEC.[4] This may be due to differences in lineage distributions across different studies and settings.

The study is limited by the lack of a comparison with *E. coli* isolates causing more severe infections among hospitalised patients or ExPEC isolates involved in colonisation which would have provided a better understanding of the interplay between STs and virulence factors in this setting.

This is the first study using whole genome sequencing on unselected ExPEC isolates in Zimbabwe. The study highlights the high diversity of lineages, resistance and virulence genes among *E. coli* causing UTIs and provides insights on the molecular epidemiology of the pathogen in this setting. ST131 was relatively prevalent among study isolates in line with the global expansion of this successful clone.

Contributions to the work: I wrote the standard operating procedures, trained and supervised the DNA extraction which was done by a laboratory technician. DNA sequencing was done commercially at MicrobesNG (Birmingham, UK). I conducted the bioinformatics analysis with support from my advisor (RS). I prepared the data visualisations, analysed and interpreted the results.

Table 2. Overview of whole genome sequencing studies on the epidemiology of ExPEC *E. coli* from clinical samples in sub-Saharan Africa*

Author, year	Years of sample collection	Country	Selection	Specimen type	Setting	Method	Number of isolates tested	Sequence types	Resistance mechanisms	Virulence factors
Yehouenou, 2021[34]	2019	Benin	Yes (ESBL and AST profile)	Surgical site infections	4 hospitals	WGS	19	Yes	Yes	No
Irenge, 2019[35]	2012-2014	DRC	ESBL-*E. coli*	Blood and urine	1 hospital	WGS	21	Yes	Yes	Yes
Wawire, 2021[36]	2016	Kenya	Yes (unclear)	Mixed (urine, wound); wastewater	1 hospital, environment	WGS	14 clinical, 9 wastewater	Yes	Yes (genes not specified)	Yes
Musicha, 2017[37]	1996-2014	Malawi	Yes (AST profile)	Blood, CSF, rectal swab	1 hospital	WGS	94	Yes	Yes	No
Tegha, 2021[29]	2012-2018	Malawi	Yes (unclear)	Mixed (blood, urine, CSF, fluid, other)	1 hospital	WGS	58	Yes	Yes	Yes
Mandomando, 2020[38]	2001-2014	Mozambique	Yes (EAEC)	Blood	1 hospital	WGS, PCR	44 EAEC; 22 others	Yes	Yes	Yes
Olalekan, 2020[39]	2016-2018	Nigeria	Yes, ESBL	Unclear	4 hospitals	WGS	113	Yes	Yes	No
Decano, 2021[40]	2017-2018	Uganda, Kenya	Unclear	Urine	Unclear	WGS	55	Yes	Yes	Yes
Takawira, 2021[27]	2017-2019	Zimbabwe	Yes (ESBL and AST profile)	Urine	Reference laboratory	WGS	48	Yes	Yes	Yes

*Studies in neonates and those investigating colonization were not included

AST: antibiotic susceptibility testing; DRC: Democratic Republic Congo; EAEC: enteroaggregative *E. coli;* ESBL: extended-spectrum beta-lactamase; PCR: polymerase chain reaction; WGS: whole genome sequencing.

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CHAPTER 5

Clinical and bacteriological outcomes in patients with urinary tract infections presenting to primary care in Harare, Zimbabwe: a cohort study

Having described the prevalence and risk factors for AMR, this study examines the clinical and bacteriological outcomes of patients with UTIs presenting to primary care. Although UTIs are not life-threatening and can resolve spontaneously, they can impact on quality of life and lead to economic losses. The findings of this study show that there is a high proportion of patients with UTIs who are prescribed ineffective antibiotics or who are not able to afford treatment. Those who receive ineffective or no treatment were less likely to experience symptom resolution and/or bacterial clearance compared to those who received effective treatment (defined as antibiotics to which the bacteria were susceptible). This study highlights the need for optimizing treatment recommendations based on local AMR data.

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Student ID Number	1805237/RITD	**Title**	
First Name(s)	Ioana Diana		
Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

Student Signature	Ioana Diana Olaru
Date	21-12-2022

Supervisor Signature	Katharina Kranzer
Date	21-12-2022

RESEARCH NOTE

REVISED Clinical and bacteriological outcomes in patients with urinary tract infections presenting to primary care in Harare, Zimbabwe: a cohort study [version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background: Treatment for urinary tract infections (UTIs) is usually empiric and is based on local antimicrobial resistance data. These data, however, are scarce in low-resource settings. The aim of this study is to determine the impact of antibiotic treatment on clinical and bacteriological outcomes in patients presenting with UTI symptoms to primary care in Harare.
Methods: This cohort study enrolled participants presenting with UTI symptoms to 10 primary healthcare clinics in Harare between July 2019 and July 2020. A questionnaire was administered and a urine sample was collected for culture. If the urine culture showed growth of $\geq 10^5$ colony forming units/mL of a uropathogen, a follow up visit at 7-21 days was conducted.
Results: The analysis included 168 participants with a median age of 33.6 years (IQR 25.1-51.4) and of whom 131/168 (78.0%) were female. Effective treatment was taken by 54/168 (32.1%) participants. The urine culture was negative at follow up in 41/54 (75.9%) of participants who took appropriate treatment and in 33/114 (28.9%, p<0.001) of those who did not. Symptoms had improved or resolved in 52/54 (96.3%) of those on appropriate treatment and in 71/114 (62.3%, p<0.001) of those without.
Conclusion: The findings of this study show that effective treatment leads to symptom resolution and bacterial clearance in people presenting with UTIs to primary care. Although UTIs are not life-threatening and can resolve without treatment, they do impact on

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version 2 (revision) 17 Aug 2022	✓ view		
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Any reports and responses or comments on the article can be found at the end of the article.

quality of life, highlighting the need for optimised treatment recommendations.

Keywords
AMR, antibiotic resistance, cystitis, UTI

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REVISED **Amendments from Version 1**

Clarifications on the methods used for sample collection and laboratory processing.

Information on the prevalence of antibiotic resistance.

Comment on the possible severe outcomes of urinary tract infections.

Comment on how antibiotics were prescribed during the initial consultation.

Comment on the low yield of urine cultures in this setting and possible alternative diagnoses

Any further responses from the reviewers can be found at the end of the article

Introduction

Urinary tract infections (UTIs) are very common in women, with half of women reporting having had at least one episode by 32 years of age[1], while they are rare in men under the age of 60 years[2]. Many women with a UTI experience moderate to severe symptoms impacting their daily life[1]. Antibiotic treatment is mainly empiric and management recommendations are usually informed by local antimicrobial resistance (AMR) data collected as part of continuous surveillance[3]. Such data are not widely available in many countries in sub-Saharan Africa[4]. As a result, treatment recommendations are usually not informed by local or regional data, which may result in patients receiving ineffective empiric treatment. The aim of this study is to determine the impact of antibiotic treatment on clinical and bacteriological outcomes in patients presenting with UTI symptoms to primary care in Harare.

Methods

The data were collected as part of the Antimicrobial Resistance in Gram-negative bacteria from Urinary Specimens (ARGUS) study, which enrolled patients presenting with UTI symptoms to ten primary healthcare clinics in Harare between July 2019 and July 2020. Details of the ARGUS study have been described elsewhere[5]. Briefly, adult patients (≥18 years) reporting the current presence of at least two symptoms suggestive of a UTI and who had not been recently discharged from hospital (within the previous 72 hours) and did not have an indwelling urinary catheter were eligible for inclusion into the study. Eligible patients presenting to the participating clinics were consecutively recruited into the study. After provision of informed consent, a questionnaire on demographics, clinical history and treatment was administered. After cleaning the urethral meatus and the perineal region, a mid-stream urine sample was collected for culture. Samples were processed on the same day at the Biomedical Research and Training Institute Laboratory and were transported to the laboratory at 4–8°C within 6 hours of collection. Bacterial culture, identification and antimicrobial susceptibility testing (AST) were performed using conventional microbiology techniques. Urine samples were inoculated on chromogenic agar (Brilliance UTI agar, Oxoid, UK) and incubated for 24 hours at 37°C. Bacterial identification was performed using biochemical tests (API 20E, bioMérieux, France, catalase, coagulase)[5]. AST was interpreted using the EUCAST standards[6]. ATCC reference isolates were used to ensure the quality of bacterial identification and AST.

If the urine culture showed growth of $\geq 10^5$ colony forming units/mL of a uropathogen, a follow up visit at 7–21 days was conducted when another urine sample was collected and a questionnaire on symptoms, treatment and healthcare seeking was administered. Bacteriological cure was defined as a negative urine culture on follow up. A favourable clinical outcome was defined as the resolution or improvement of symptoms at follow up.

Treatment was prescribed by the clinic nurses according to routine practice and followed the national guidelines, which recommend amoxicillin 500 mg thrice daily or fluoroquinolones, usually ciprofloxacin 500 mg twice daily, for 3 days as first-line treatment for cystitis[7]. Participants were considered to have received effective treatment if the antibiotic prescribed was shown to have activity against the pathogen as per the AST result and the participant reported having taken the antibiotic. Participants who had positive cultures on follow up and were symptomatic were prescribed effective treatment according to AST. Pregnant women were treated irrespective of symptoms.

This analysis excluded participants in whom the follow-up visit was conducted late (>21 days post enrolment), if the treatment prescribed was not recorded, and if the sample could not be processed. Statistical analyses were performed in STATA v.15 (StataCorp, TX, USA). Univariable analyses were performed using the χ square test for categorical variables and Mann Whitney U test for continuous variables. Adjusted risk ratios were computed using Poisson regression with robust error variances. The analysis was adjusted for *a priori* confounders (age and sex) and variables associated with the outcome in the univariable analysis at a level of significance of $p < 0.2$. The exposure of interest was effective treatment. The outcomes were bacteriological cure and a favourable clinical outcome (resolution or improvement) on follow up. Ethical approval for the ARGUS study was obtained from the Medical Research Council Zimbabwe (MRCZ/A/2406), the Institutional Review Board of the Biomedical Research and Training Institute in Zimbabwe and the London School of Hygiene and Tropical Medicine Ethics committee (Ref. 16424). The study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. All participants provided written informed consent for participation in the study.

Results

Of 1164 participants enrolled into the study, 245/1164 (21.0%) had a positive urine culture with $\geq 10^5$ colony forming units/mL at baseline and in 199/245 (81.2%), a follow up visit was conducted (Figure 1)[8]. This analysis excluded 31 participants. The reasons for exclusion are shown in Figure 1. The analysis included 168 participants with a median age of 33.6 years (IQR 25.1–51.4), of whom 131/168 (78.0%) were female. Participant characteristics are shown in Table 1. Among the 158 *Enterobacterales* isolates identified, the prevalence of resistance

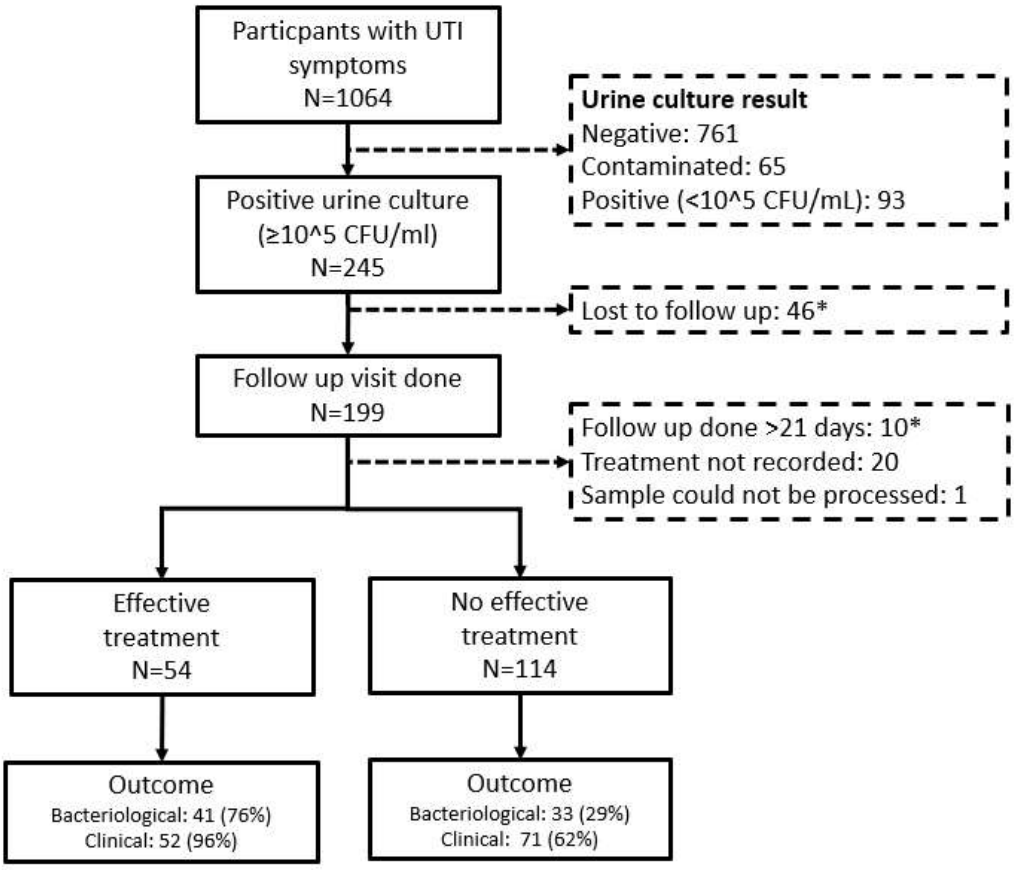

Figure 1. Flow chart of enrolled participants.

was 137/158 (86.7%) for amoxicillin, 132/158 (83.4%) for trimethoprim-sulfamethoxazole, 27/158 (17.1%) for ciprofloxacin, 23/158 (14.6%) for ceftriaxone, and 16/158 (10.1%) for nitrofurantoin. Effective treatment was taken by 54/168 (32.1%) participants, while in 61/168 (36.3%), treatment was ineffective due to resistance, in 35/168 (20.8%) no treatment was prescribed and 18/168 (10.7%) did not take the prescribed treatment. Antibiotic prescriptions were for amoxicillin in 59/168 (35.1%), fluoroquinolones in 51/168 (30.4%) and for other antibiotics in 15/168 (8.9%). Treatment was effective in 13/54 (24%) patients who took amoxicillin and in 41/46 (89%) patients who took a fluoroquinolone.

The urine culture was negative at follow up in 41/54 (75.9%) of participants who took appropriate treatment and in 33/114 (28.9%, p<0.001) of those who did not. Symptoms had improved or resolved in 52/54 (96.3%) of those on appropriate treatment and in 71/114 (62.3%, p<0.001) of those without. Similarly, symptoms had improved or resolved in 67/74 (90.5%) of participants with a negative culture and in 56/94 (59.6%, p <0.001) of those with a positive culture. Participants who took effective treatment were more likely to have favourable clinical and bacteriological outcomes, with adjusted risk ratios of 1.42 (1.20–1.67) and 2.63 (1.77–3.92), respectively, when adjusting for age, sex, HIV status and duration of symptoms (Table 2).

Discussion

This study shows that individuals who receive treatment against which the pathogen is sensitive have a significantly higher chance of experiencing clinical and bacteriological cure on follow up than those given ineffective treatment or not treated at all.

In line with other studies symptom resolution or improvement was common even in those participants who did not take effective treatment. Spontaneous symptom resolution of uncomplicated cystitis occurs in about a third of patients[9]. However, patients without spontaneous cure often experience debilitating symptoms that can persist for several weeks[10], affecting quality of life[11] and leading to economic costs due to loss of income, missed days at work and medical expenses[1,12,13]. Effective empiric treatment increases the speed and likelihood of symptom resolution and therefore is an important intervention at primary care level. In the absence of effective treatment, some lower UTIs may also progress to pyelonephritis

Table 1. Characteristics of study participants according to treatment status.

Characteristic	Total N=168	Effective treatment N=54	No effective treatment N=114	p-value
Age, median (IQR)	33.6 (25.1-51.4)	30.9 (24.2-52.0)	36.6 (25.7-49.5)	0.273
Female sex	131 (78.0)	43 (79.6)	88 (77.2)	0.722
HIV positive	50 (33.8))	9 (18.4)	41 (41.4)	0.005
Education (at least secondary)	129 (76.8)	40 (74.1)	89 (78.1)	0.567
Pregnancy	13 (10.6)	2 (5.3)	11 (12.9)	0.201
Time since the baseline visit	8 (7-11)	8 (7-10)	8 (7-11)	0.203
Duration of symptoms	6 (3-10)	5 (3-7)	7 (4-10)	0.032
Organism isolated (baseline) *E. coli* Coliforms *Enterococcus spp.* Other	 137 (81.6) 21 (12.5) 8 (4.8) 2 (1.2)	 44 (81.5) 6 (11.1) 3 (5.6) 1 (1.9)	 93 (81.6) 15 (13.2) 5 (4.4) 1 (0.9)	-
Symptoms at day 7 Resolved completely Partial resolution Same or worse	 59 (35.1) 64 (38.1) 45 (26.8)	 31 (57.4) 21 (38.9) 2 (0.4)	 28 (24.6) 43 (37.7) 43 (37.7)	<0.001

IQR: interquartile range; 20 participants did not know/disclose their HIV status; eight women did not know if they were pregnant.

Table 2. Association between antibiotic treatment effectiveness and clinical and bacteriological outcomes.

Characteristic	RR (95%CI)	p-value	aRR (95%CI)	p-value
Clinical outcome				
Age (years)	0.99 (0.99-1.00)	0.890	0.99 (0.99-1.00)	0.660
Female sex	0.80 (0.68-0.95)	0.012	0.79 (0.64-0.97)	0.023
HIV infected	0.64 (0.48-0.85)	0.002	0.76 (0.59-0.99)	0.048
Duration of symptoms (days)	0.96 0.93-0.98)	0.001	0.97 (0.94-0.99)	0.014
Received effective treatment	1.55 (1.32-1.80)	<0.001	1.42 (1.20-1.67)	<0.001
Bacteriological outcome				
Age (years)	0.99 (0.99-1.01)	0.931	1.00 (0.99-1.01)	0.632
Female sex	0.82 (0.56-1.19)	0.291	0.76 (0.51-1.14)	0.188
HIV infected	0.64 (0.40-1.03)	0.064	0.87 (0.55-1.39)	0.568
Duration of symptoms (days)	0.93 (0.89-0.98)	0.006	0.96 (0.92-1.01)	0.154
Received effective treatment	2.62 (1.89-3.63)	<0.001	2.63 (1.77-3.92)	<0.001

RR: risk ratio; aRR: adjusted RR; CI: confidence interval; age and duration of symptoms were analysed as continuous variables; duration of symptoms refers to the duration of symptoms prior to the initial clinic presentation (baseline visit).

and bacteraemia which may in turn lead to hospitalisation and severe outcomes.

More than half of participants who took the prescribed antibiotics had infections with organisms that were resistant to the antibiotic prescribed. Resistance to amoxicillin was more frequent (76%) compared to fluoroquinolones (11%), which are recommended as the first-line empiric treatment of UTIs in outpatients in Zimbabwe[7]. The high prevalence of amoxicillin resistance is not surprising and is in line with reports from other countries in sub-Saharan Africa[14–16]. In view of the high prevalence of amoxicillin resistance, recommended use of the drug for empiric treatment of UTI needs to be reviewed. Although fluoroquinolones are safe and resistance is less frequent, they may not be the optimal choice as first-line drugs due to their potential for resistance development[17], particularly when other low-cost alternatives such as nitrofurantoin and fosfomycin are available. Furthermore, fluoroquinolone resistance is increasing in many settings[18], likely driven by selective pressures exerted by their use and the dissemination of successful bacterial clones[19]. This highlights the need to optimise treatment recommendations based on setting-specific AST data.

In this study, effective treatment according to AST resulted in more frequent bacteriological eradication of infection and improved clinical outcomes. While effective treatment has been shown to be associated with symptom resolution and bacteriological clearance in high-income settings[20–22], similar data from sub-Saharan Africa are scarce. Some participants did not fill their antibiotic prescription, potentially due to costs. In addition, while healthcare workers may be aware that amoxicillin is not very effective, it may have been the only inexpensive antibiotic available. Thus, costs and stock outs may impact on antibiotic prescriptions and consequently on the effectiveness of treatment. Healthcare workers did not prescribe antibiotics at the initial consultation in one out of five patients with a positive urine culture. This may be due to uncertainties on the part of the healthcare workers that the patient has an UTI and to suspected alternative diagnoses.

The yield of urine cultures was low in line with findings from other studies from sub-Saharan Africa[23,24]. This can be explained in part by the high prevalence of sexually transmitted infections and antibiotic exposure prior to seeking healthcare among patients included in this study[25]. The availability of rapid diagnostic tests at the point of care such as urinary dipstick or testing for sexually transmitted infections may improve patient care and optimise antibiotic use. Limited availability of diagnostics for sexually transmitted infections does not allow the differentiation between UTIs and sexually transmitted infections. Also, the lack of up to date AMR data can lead to excessive and inappropriate antibiotic use which will further drive the development of AMR. These in turn can only be addressed by sustained investment in laboratory strengthening for obtaining locally-representative surveillance data, and in improving access to diagnostics and to healthcare in general. The study is limited by the relatively high rate of loss to follow up explained by the economic hardships in Zimbabwe, preventing participants from travelling to the clinics for their follow up visits, and the ongoing COVID-19 pandemic, which led to clinic closures and a national lockdown, preventing successful follow-up. An important strength of this study was the follow up of participants using culture to determine the effect of treatment on both clinical symptoms and bacteriological cure. In this study, a high proportion of participants did not receive effective treatment which may not be the case in other settings where antibiotics recommended for UTI treatment reflect the local prevalence of AMR and where effective antibiotics are more available and affordable. However, the impact of effective treatment on patient outcomes would not be different between settings making these results generalizable.

The findings of this study show that there is a high proportion of untreated UTIs, with patients often being prescribed antibiotics that are ineffective or not being able to afford treatment.

Furthermore, the long duration of symptoms prior to presentation suggests that some patients with UTIs may not present to clinics at all. Optimising treatment recommendations could be achieved by conducting sentinel-site surveillance for determining the prevalence of resistance, which would in turn inform empiric treatment. While UTIs have not received a lot of attention, partly because a high proportion resolve without treatment and partly because they are not life-threatening and the long-term morbidity is limited, they do impact on quality of life, particularly in women, and lead to loss of income and economic costs.

Data availability

Underlying data

Dryad: ARGUS clinical and bacteriological outcomes dataset. https://doi.org/10.5061/dryad.v41ns1rwb[8].

This project contains the following underlying data:

- ARGUS_outcome_data.csv
- ARGUS_outcome_codebook.docx

Reporting guidelines

Dryad: STROBE checklist for "Clinical and bacteriological outcomes in patients with urinary tract infections presenting to primary care in Harare, Zimbabwe: a cohort study". https://doi.org/10.5061/dryad.v41ns1rwb[8].

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CHAPTER 6

Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe

As outlined in the first chapter of this work, lacking data on the local epidemiology of AMR and diagnostic uncertainties lead to inappropriate and ineffective antibiotic treatment. Excessive use of antibiotics is one of the major drivers of antimicrobial resistance and globally, most antibiotics are prescribed in the outpatient setting. A higher proportion of clinic presentations in low- and middle-income countries result in antibiotic prescriptions than in high income countries because of the higher burden of infectious diseases and unavailability of diagnostics. In Zimbabwe, primary clinics are nurse-led and represent the first port of call for patients of all ages seeking care for a wide range of conditions.

This study presents attitudes and practices with regards to microbiology tests, antimicrobial and antibiotic prescribing among healthcare providers at public primary health clinics in Harare, Zimbabwe.

This is the first survey among antibiotic prescribers from public primary care clinics in Zimbabwe and overall, few studies have previously focused on midwives or nurses as prescribers. Findings of this survey highlight the need for training on AMR and prescribing and can be used to inform further interventions for improving the use of antibiotics in primary care.

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Student ID Number	1805237/RITD	**Title**	
First Name(s)	Ioana Diana		
Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

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Where is the work intended to be published?	Wellcome Open Research
Please list the paper's authors in the intended authorship order:	Ioana D. Olaru, Rashida A. Ferrand, Shunmay Yeung, Rudo Chingono, Prosper Chonzi, Kudzai P.E. Masunda, Justin Dixon, Katharina Kranzer
Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the principal investigator for the study. I have designed and planned the study, prepared the study protocol, obtained the regulatory approvals, prepared the study forms. I conducted the surveys at the participating clinics, collected and entered the data. I performed the data management, cleaned, and analysed the data, and interpreted the results. I wrote the first draft of the manuscript, revised it following feedback from supervisors and co-authors, prepared and submitted the final draft. I revised the manuscript following peer-review.

SECTION E

Student Signature	Ioana Diana Olaru
Date	21-12-2022

Supervisor Signature	Katharina Kranzer
Date	21-12-2022

RESEARCH NOTE

REVISED Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe [version 2; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background
Overuse of antibiotics is one of the main drivers for antimicrobial resistance (AMR). Globally, most antibiotics are prescribed in the outpatient setting. This survey aimed to explore attitudes and practices with regards to microbiology tests, AMR and antibiotic prescribing among healthcare providers at public primary health clinics in Harare, Zimbabwe.
Methods
This cross-sectional survey was conducted in nine primary health clinics located in low-income suburbs of Harare between October and December 2020. In Zimbabwe, primary health clinics provide nurse-led outpatient care for acute and chronic illnesses. Healthcare providers who independently prescribe antibiotics and order diagnostic tests were invited to participate. The survey used self-administered questionnaires. A five-point Likert scale was used to determine attitudes and beliefs.
Results
A total of 91 healthcare providers agreed to participate in the survey. The majority of participants (62/91, 68%) had more than 10 years of work experience. Most participants reported that they consider AMR as a global (75/91, 82%) and/or national (81/91, 89%) problem, while 52/91 (57%) considered AMR to be a problem in their healthcare

Open Peer Review

Approval Status ? ✓

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version 2 (revision) 29 Apr 2022		✓ view
version 1 30 Mar 2021	? view	? view

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facilities. A fifth of participants (20/91, 22%) were unsure if AMR was a problem in their clinics. Participants felt that availability of national guidelines (89/89, 100%), training sessions on antibiotic prescribing (89/89, 100%) and regular audit and feedback on prescribing (82/88, 93%) were helpful interventions to improve prescribing.
Conclusions
These findings support the need for increased availability of data on AMR and antibiotic use in primary care. Educational interventions, regular audit and feedback, and access to practice guidelines may be useful to limit overuse of antibiotics.

Keywords
AMR, antibiotic resistance, antibiotic use, outpatients

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REVISED **Amendments from Version 1**

Added a more detailed description of non-prescription antibiotic use in Zimbabwe;

Clarified that the survey was conducted prior to result dissemination sessions discussing antimicrobial resistance and diagnosis of infections;

Additional references to studies from Zimbabwe that informed the questionnaire used in this survey;

Highlighted the focus of the survey on nurses and midwives as antibiotic prescribers;

Contrasted findings to those from other studies in sub-Saharan Africa;

Limitation in generalising results to nurses from other settings (rural clinics, private sector);

Rewording of the conclusion to acknowledge that increasing the availability of diagnostics may be difficult to attain however it is important to have data on causative organisms for infections and antimicrobial resistance;

Importance of improving our understanding of how to design training programmes for nurses and midwives and how to communicate antimicrobial resistance.

Any further responses from the reviewers can be found at the end of the article

Introduction

Global antibiotic consumption has increased by more than 65% within the last two decades, driven primarily by an increase in consumption in low- and middle-income countries (LMICs) and reflecting economic growth[1]. Inappropriate antibiotic use is frequent in many settings with at least 30% of all antibiotic prescriptions considered inappropriate[2–4]. This has public health implications since antibiotic overuse is one of the major drivers for antimicrobial resistance (AMR)[5].

In high-income countries, more than 85% of antibiotics are prescribed in the community i.e. in outpatient settings[6]; this is likely similar in LMICs. One in eight and one in two outpatient consultations result in antibiotic prescriptions in high and low-income settings, respectively[3,7]. This difference may be explained by the higher prevalence of infectious diseases and a lack of access to diagnostic testing. In addition, the high workload in low-resource outpatient settings may lead to reduced consultation time and increase the likelihood of antibiotic prescriptions[8,9]. In many low-resource settings, non-prescription antibiotic use is a frequent phenomenon[10]. In Zimbabwe, antibiotic dispensing was historically highly regulated with only 8% of antibiotics issued without a prescription[11]. However, recent economic decline, increasing healthcare utilisation costs and the COVID-19 pandemic, have likely resulted in increased non-prescription antibiotic use[12].

While there are available data particularly on the prescribing practices of doctors working in hospitals, data from outpatient settings in LMICs where nurses are the main antibiotic prescribers are scarce. A better understanding of attitudes and practices of healthcare providers relating to AMR and antibiotic use may allow for the development of strategies to improve prescribing and ultimately curb the increase in AMR. This survey aimed to explore attitudes and practices with regards to microbiology tests, AMR and antibiotic prescribing among healthcare providers (nurses and midwives) at public primary health clinics in Harare, Zimbabwe.

Methods

Setting

Primary health clinics (PHCs) provide nurse-led care for acute and chronic illnesses including HIV and non-communicable diseases as well as antenatal and maternity services for uncomplicated deliveries and well-child clinics for growth monitoring and immunisations. Microbiology diagnostic services beyond rapid testing for malaria and HIV are only available at central laboratories. Pharmacies co-located on PHC premises fill prescriptions at reduced costs compared to independent pharmacies however, stock-outs of medicines are frequent. Unlike in many other countries, in Zimbabwe, most patients have to pay out-of-pocket for healthcare costs such as consultations, diagnostic tests and prescriptions, limiting access to care. In addition, Zimbabwe has been facing considerable hardships in recent years due to economic decline and rapid inflation which impacted on healthcare access and provision.

Study design and participants

This cross-sectional survey was conducted in nine PHCs located in low-income suburbs of Harare between October and December 2020. The PHCs were selected out of 12 facilities if they were serving a low-income population in southern Harare and if they were operational at the time of the survey. Healthcare providers who independently prescribe antibiotics and order diagnostic tests (e.g. nurses, midwives, etc.) were eligible to participate in the survey. The surveys were conducted before dissemination and feedback sessions discussing the results of two studies focusing on viral and bacterial infections and AMR[13,14]. All healthcare workers who were working at the clinic on the day of the survey were invited to participate. The clinic matrons were informed about the dissemination sessions and the plan to conduct the survey and provided their support.

Survey

The survey[15] was developed based on a literature review[8,16–18] and findings from other studies conducted in Zimbabwe[13,14,19]. The studies did not assess the knowledge, attitudes and practices of healthcare workers but rather provided a more comprehensive understanding of the landscape of AMR and prescribing in Zimbabwe. Data on demographics, training and work experience were collected. Main topics addressed by the questions were: availability and use of diagnostic tests that may be used to identify infections with antibiotic resistant pathogens; pathogens encountered in current practice; attitudes and beliefs relating to AMR and antibiotic prescribing; training and sources of information used to improve prescribing. Most questions used a five-point scale with the exception of demographics and questions on the importance of AMR and on sources of information. Questions were answered in terms of importance (very important to very unimportant), helpfulness (very helpful to very unhelpful), and agreement of the survey

taker with a particular statement (strongly agree to strongly disagree) (see extended data for survey and codebook[15]). Knowledge about diagnostic testing and drug susceptibility testing was evaluated using four multiple-choice and free-text questions. The clinical questions were selected to reflect common scenarios that the nurses would encounter in their daily practice and might lead to inappropriate antibiotic use.

Data collection

Data was collected as part of the ARGUS study which evaluates gram-negative resistance and antibiotic usage in primary care[13]. Ethical approval was obtained from the Medical Research Council Zimbabwe (MRCZ/A/2406) and the London School of Hygiene and Tropical Medicine Ethics committee (Ref. 16424).

All prescribers who were working at the clinics on the day of the event were invited to take part in the survey. Each clinic was visited once. The survey contained an information sheet on the purpose of the survey and consent. This section specifically asked the participants to fill in and return the survey if they consented to participate. Data was fully anonymised on collection and no participant identifiers were used. The questionnaires were self-administered using paper-based forms and were filled in prior to the session. Data from the paper questionnaires was entered into electronic forms using Open Data Kit (ODK).

Statistical analysis

Data analysis was performed in R v4.0.3 (The R Project for Statistical Computing). Categorical variables were presented as counts and percentages. A five-point Likert scale was used to determine attitudes and beliefs ranging from 1 point ("very important", "very helpful", "strongly agree") to 5 points ("very unimportant", "very unhelpful", "strongly disagree"). Results were presented aggregated for positive and negative categories (e.g. very important and important formed one category). For these questions, percentages were calculated while excluding questions which were unanswered or where the response was "do not know". To account for non-response, the denominator for the data is reported.

Results

A total of 91 healthcare providers from nine PHCs were approached and all agreed to participate in the survey[15]. Most participants (81/91, 89%) were female and worked in public health facilities only (84/91, 92%), while seven also worked in private health facilities or hospitals. Participants were senior nurses (44/91, 49%), midwives (34/91, 37%), and junior nurses (12/91, 13%). The majority of participants, 62/91 (68%) had more than 10 years of work experience. Figure 1 shows the attitudes and beliefs of healthcare providers related to diagnostic testing, causes of AMR and antibiotic prescribing.

Microbiology test availability and use

Among 69 participants who reported having ordered specific microbiology tests within the previous month, 67/69 (97%) reported ordering a sputum test for tuberculosis with 19/67 (28%) having ordered more than 10 tests for tuberculosis. Urine cultures were ordered by 46/69 (67%) and stool cultures by 31/69 (45%) with 13/46 (28%) and 7/31 (23%) ordering more than five tests in the previous month, respectively. The main challenges in ordering and performing microbiology tests were the lack of access to laboratory testing (78/84, 93%), delays in receiving test results (70/84, 83%), high patient volume (66/80, 83%) and costs of testing (71/83, 86%; Figure 1A and 1B).

Antimicrobial resistance

Most participants reported that they consider AMR as a global (75/91, 82%) and/or national (81/91, 89%) problem, while 52/91 (57%) considered AMR to be a problem in their healthcare facilities. A fifth of participants (20/91, 22%) were unsure if AMR was a problem in their clinics. Among key pathogens, 73/91 (80%), 45/91 (49%), 9/91 (10%) and 8/91 (9%) considered drug resistance to be a problem in *Mycobacterium tuberculosis*, *Salmonella* Typhi, *Staphylococcus aureus* (methicillin-resistant) and gram negatives (presence of extended-spectrum beta-lactamases), respectively. Poor adherence of patients to prescribed antibiotics treatment (87/90, 97%), over-prescription of antibiotics (82/88, 93%) and excessive use of unregulated antibiotics acquired from pharmacies without a prescription (82/88, 93%) or from the informal market (79/87, 91%) were considered very important or important drivers of AMR (Figure 1C).

Antibiotic prescribing

The decision to prescribe antibiotics was mainly influenced by the clinical presentation and laboratory results (87/89, 98%) and severity of illness (79/89, 89%) and was guided by the national guidelines[20] (84/87, 97%; Figure 1D). The decision to prescribe antibiotics was influenced by the patients' or their seniors' expectations in 6/88 (7%) and 13/88 (15%), respectively. Respondents reported prescribing unnecessary antibiotics very often (7/90, 8%), often (8/90, 9%), about half of the times to (29/90, 32%), sometimes (27/90, 30%) and almost never (19/90, 21%). In total, 25 (29%) and 41/90 (46%) of prescribers felt that antibiotic prescriptions should be reduced for inpatients and outpatients, respectively (Figure 1E).

National guidelines were the main source for guiding prescribing in routine practice (85/91, 93%) and as a means to increase knowledge on antibiotic prescribing (88/91, 97%). Other sources of information to support prescribing were textbooks in 64/91 (70%), discussions with colleagues 57/91 (63%) and professional meetings 56/91 (62%). A third of participants (28/91, 31%) reported having received training in antibiotic prescribing in the previous year. Participants felt that availability of national guidelines (89/89, 100%), training sessions on antibiotic prescribing (89/89, 100%) and regular audit and feedback on prescribing (82/88, 93%) were helpful interventions to improve prescribing (Figure 1F).

Prescriber knowledge

Among survey participants, 84/91 (92%) would order a sputum test for tuberculosis in a patient with a prolonged cough and 71/91 (78%) would prescribe appropriate antibiotics in a

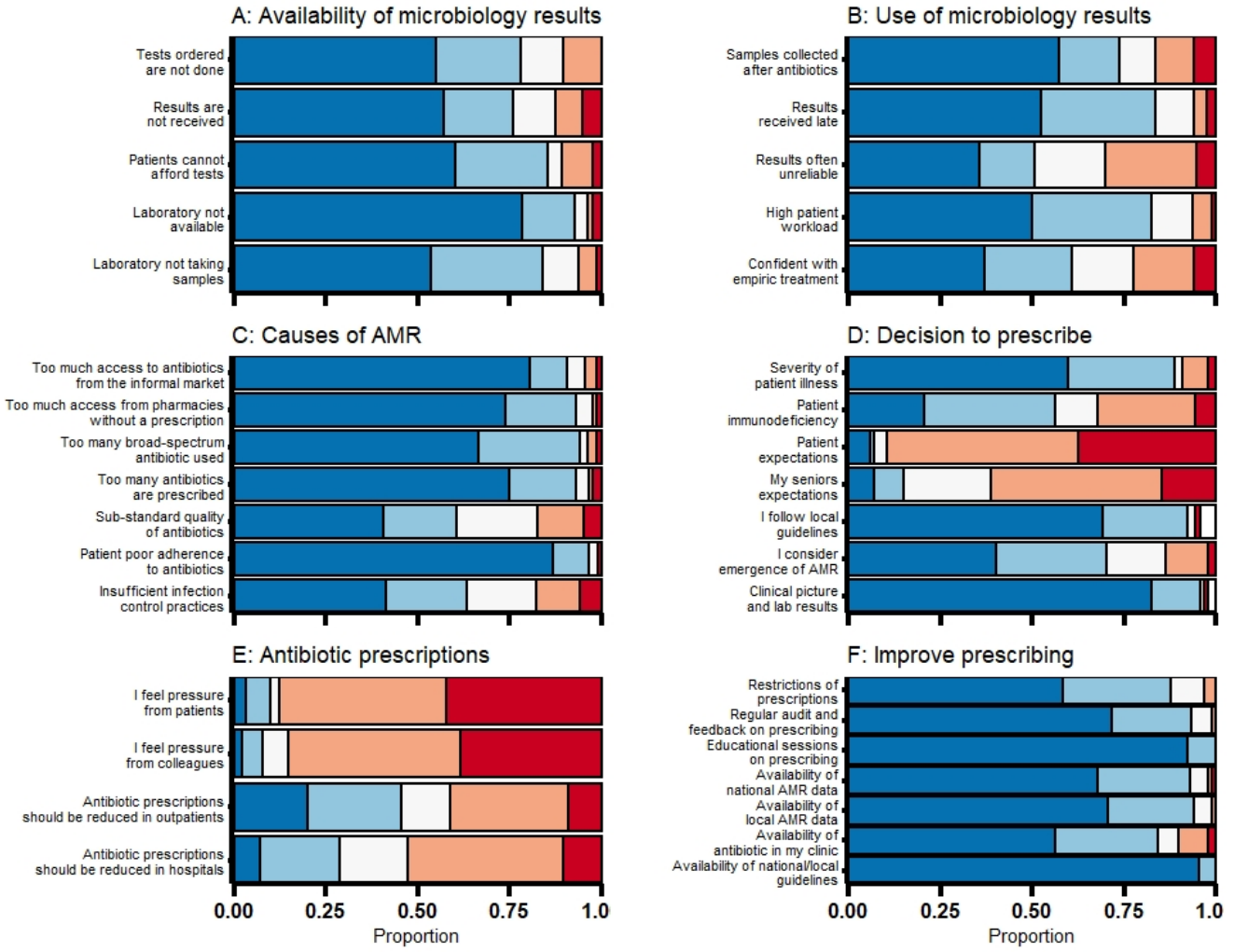

Figure 1. Attitudes and practices relating to microbiology tests, antimicrobial resistance and antibiotic prescriptions. Positive responses are displayed in blues, negative in reds and neutral responses in white. (**A**) affecting the availability of microbiology testing (very important to very unimportant); (**B**) affecting the use of microbiology results (very important to very unimportant); (**C**) causes of AMR (very important to very unimportant); (**D**) guiding the decision to start antibiotics (strongly agree to strongly disagree); (**E**) antibiotic prescriptions (strongly agree to strongly disagree); (**F**) improving antibiotic prescribing (very helpful to very unhelpful).

patient with typhoid fever symptoms. In total, 18 (20%) would prescribe inappropriate antibiotics such as kanamycin and doxycycline to a pregnant patient with symptoms of a sexually transmitted infection. Most participants (81/91, 89%) would prescribe antibiotics in a patient with symptoms suggestive of a viral respiratory tract infection.

Discussion

The study used a new approach by focusing on nurses and midwives from PHCs who are the main prescribers in the outpatient setting in Zimbabwe. This study found that although healthcare providers were aware of the challenges posed by AMR on a global and national level, they considered it less of an issue in their daily practice. Furthermore, while over-prescription of antibiotics was recognized as a problem by most, half of the participants reported that unnecessary prescriptions are infrequent in their current practice. These issues may arise from insufficient knowledge of the prevalence of AMR in their specific setting and from the propensity to attribute it to factors outside their own practice which is also reported by studies elsewhere[21]. This may also come from the perception of futility that their daily practice will impact on AMR on a national or global level[22]. Only one in three participants reported having received formal training on antibiotic prescribing in the previous year.

Limited availability of diagnostics, insufficient laboratory capacity and high costs of diagnostics means that most patients

accessing outpatient departments in sub-Saharan Africa are treated using a "syndromic approach"[23]. This was also reflected by the findings of this survey where healthcare providers reported that there are a number of barriers in accessing microbiological testing such as the lack of access to laboratory testing and high costs which are incurred by the patients. The use of microbiology tests plays an important role in bacterial identification and antibiotic susceptibility testing. Limiting tests to complex cases and patients presenting to private healthcare facilities will lead to data which may not reflect the burden of AMR in the community. Therefore, insufficient laboratory testing results in inadequate and potentially biased surveillance data thus preventing the development of setting-specific treatment recommendations.

Most survey participants were aware of resistance in *M. tuberculosis* likely due to the roll-out and decentralisation of testing using GeneXpert and awareness campaigns on the importance of tuberculosis diagnosis. Resistance in *S.* Typhi was often reported, reflecting the extensive information on the ongoing typhoid fever outbreak[24] provided to healthcare workers by overseeing authorities and non-governmental organizations. Conversely, less than 10% of respondents cited resistance in key pathogens such as methicillin resistance in *S. aureus* and the production of extended-spectrum beta-lactamases in *Enterobacteriaceae*. This may be related to the setting of the survey in outpatient facilities and to limited antibiotic susceptibility testing making the identification of these pathogens infrequent in daily practice. Furthermore, there may be a lack of published and widely disseminated information leading to decreased awareness among healthcare workers.

Most healthcare providers indicated that the decision to prescribe antibiotics is mainly guided by the clinical presentation and the national guidelines and not directly by patient expectations. This is reassuring and contrary to findings from other settings where patients' expectations played an important role in the decision to prescribe antibiotics[3,16,18]. However, there may be indirect pressures on the healthcare worker because they are aware that the patient may not be able to afford accessing the clinic again if symptoms become worse[7]. Furthermore in this study, the national guidelines were described as the main "influencer" in guiding antibiotic prescribing in routine practice. This is in contrast to a study from Gabon showing that prior experience and the opinion of the superior strongly influenced the decision whether or not antibiotics should be prescribed[25].

A total of nine out of ten healthcare workers felt that antibiotics are overused in the formal sector contributing to the increase in AMR. This is a common finding globally[8,17]. Challenges in accessing healthcare such as clinic consultation fees for subsequent visits and potential hospital costs in case of clinical deterioration, promote the prescription of potentially unnecessary antibiotics "just in case"[7]. Generally, healthcare workers will likely prioritise the potential immediate impact of antibiotic prescribing on individual patient outcome over the long-term effects of overuse on AMR on a population-level[22]. Furthermore, in this survey, healthcare workers indicated that antibiotics purchased over the counter from pharmacies or informal vendors may facilitate development of AMR in their communities, highlighting the major challenge of unregulated drug use in LMICs[10,26]. Prescription-drugs in Zimbabwe have historically been very well regulated in comparison to its neighbours, with few non-prescription sales documented in multi-country surveys[10]. However, starting from the economic crisis in 2007, the informal sector grew considerably, including an increase in informal vendors for antibiotics[12].

Regarding strategies to improve antibiotic prescribing, healthcare workers favoured educational and decision support measures such as training and increased availability of guidelines and prescribing data for their setting over restrictive measures for improving prescribing in their daily practice. These may represent effective strategies to improve prescribing as shown in other settings[27,28].

To our knowledge, this is the first survey evaluating the attitudes and practices relating to AMR and antibiotic use among healthcare providers working in PHCs in Zimbabwe. Furthermore, the approach to survey nurses and midwives who are the main antibiotic prescribers in the public sector for outpatients in many settings is innovative. The findings of this survey are of particular importance and can be used to inform the design of future educational activities for this group of healthcare professionals working in PHCs in Zimbabwe and elsewhere. This study has several limitations. As the data were collected within a survey, participants may have given socially-desirable answers. In the attempt to avoid this, data collection was completely annonymous. Only 30% of respondents reported having received training in the previous year and 7% reported that their decision to prescribe antibiotics was based on their seniors' expectations suggesting that responses were not given according to social desirability and supporting the validity of our findings. The study included a relatively small number of participants. However, all prescribers working on the day of the survey across nine PHCs in Harare were invited to participate with no refusals recorded, making the data generalizable to public sector providers of outpatient care in Harare. However, these findings may not be generalizable to healthcare workers working in private clinics or rural settings. Participants may have misunderstood some of the questions however the questions were informed by questionnaires used in other studies from sub-Saharan Africa and responses were generally consistent. Also, responses to some questions may be difficult to interpret because the respondent may have answered in the same way if they agreed with a statement of thought it was important. While increased availability of diagnostics is desirable, roll out is challenged by financial and infrastructural constraints. Also, in reality, turnaround times of microbiological diagnostics is usually too long and hence has limited impact on patient management, specifically in outpatient settings. However, establishing sentinel sites to determine causative organisms in certain settings and generate data on AMR might be a possible solution. In many settings nurses and midwives are the main antibiotic

prescribers. Hence understanding how to design training programmes aimed at nurses and midwives and how to communicate AMR surveillance data to them is important. Surveys such as the one presented in this study conducted in other settings could potentially guide training and teaching programmes.

Data availability

Underlying data

DRYAD: Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe. https://doi.org/10.5061/dryad.66t1g1k1s.

This project contains the following underlying data:

- Raw answers to survey

Extended data

DRYAD: Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe. https://doi.org/10.5061/dryad.66t1g1k1s.

This project contains the following extended data:

- Data codebook
- Survey questionnaire
- STROBE checklist

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Supplementary materials for the above publication *"Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe"* are included in the Appendix 3.

Contents of Appendix 3

Questionnaire used for the survey

STROBE Statement: Checklist of items that should be included in reports of cross-sectional studies

CHAPTER 7

Sexually transmitted infections and prior antibiotic use as important causes for negative urine cultures among adults presenting with urinary tract infection symptoms to primary care clinics in Zimbabwe: a cross-sectional study

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After exploring the healthcare provider perspective on antibiotic prescriptions and overuse, this chapter evaluates exposure to antibiotics in the community prior to accessing healthcare. This study was also undertaken to better understand the much lower yield of urine cultures in studies from LMIC as compared to studies from high-income settings. Specifically, while studies in patients with UTIs from Europe and North-America report that 75-80% of cultures show growth of a uropathogen, culture positivity is much lower in studies from sub-Saharan Africa. Exposure to antibiotics prior to clinic presentation was evaluated using a simple urine bioassay. In addition, the study determined other causes of negative urine cultures among patients presenting with symptoms of UTI to primary care in Harare, Zimbabwe. Excessive antibiotic use is a recognised driver for AMR. This study found that only one in five participants with symptoms of a UTI had a positive urine culture. There was a high prevalence of prior use of antibiotics highlighting the need for improving regulations around antibiotic access to prevent the increase in antimicrobial resistance. In addition, sexually transmitted infections were very common in this patient population.

For this sub-study, participants were recruited consecutively between March and July 2020.

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Student ID Number	1805237/RITD	**Title**	
First Name(s)	Ioana Diana		
Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMJ Open		
When was the work published?	August 2021		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the principal investigator of the study. I have designed and planned the study, prepared the study protocol, obtained the regulatory approvals, prepared the study forms and the standard operating procedures. Patient enrolment and data collection was done by research assistants which I trained and supervised. I prepared the laboratory standard operating procedures, trained the laboratory technicians and oversaw the laboratory work. I ensured that laboratory testing is conducted in accordance to quality standards. I conducted the antibiotic assays and ensured that participants with sexually transmitted infections were dispensed appropriate antimicrobial theapy. I performed the data management, cleaned, and analysed the data, and interpreted the results. I wrote the first draft of the manuscript, revised it following feedback from supervisors and co-authors, prepared and submitted the final draft. I revised the manuscript following peer-review.

SECTION E

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Date	21-12-2022

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Date	21-12-2022

BMJ Open

Sexually transmitted infections and prior antibiotic use as important causes for negative urine cultures among adults presenting with urinary tract infection symptoms to primary care clinics in Zimbabwe: a cross-sectional study

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ABSTRACT

Objective Urinary tract infections (UTIs) are common in primary care. The yield of urine cultures in patients with UTI symptoms can be considerably different between high-income and low-income settings. This study aimed to explore possible causes of negative urine cultures in patients presenting with symptoms of UTI to primary health clinics in Harare.

Design Cross-sectional study.

Setting Nine primary health clinics in Harare, Zimbabwe.

Participants Adults presenting with symptoms of UTIs between March and July 2020.

Primary outcome measures Urine samples underwent dipstick testing, microscopy, culture, and testing for sexually transmitted infections (STIs) using GeneXpert and for the presence of antibiotic residues using an antibiotic bioassay. The primary outcomes were the number and proportion of participants with evidence of STIs, prior antibiotic exposure, leucocyturia and UTIs.

Results The study included 425 participants with a median age of 37.3 years, of whom 275 (64.7%) were women. Leucocyturia was detected in 130 (30.6%, 95% CI 26.2% to 35.2%) participants, and 96 (22.6%, 95% CI 18.7% to 26.9%) had a positive urine culture for a uropathogen. *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* were detected in 43/425 (10.1%, 95% CI 7.4% to 13.4%), 37/425 (8.7%, 95% CI 6.2% to 11.8%) and 14/175 (8.0%, 95% CI 4.4% to 13.1%) participants, respectively. Overall, 89 (20.9%, 95% CI 17.2% to 25.1%) participants reported either having taken prior antibiotics or having had a positive urine bioassay. In 170 (40.0%, 95% CI 35.3% to 44.8%) participants, all of the tests that were performed were negative.

Conclusions This study found a high prevalence of STIs and evidence of prior antimicrobial use as possible explanations for the low proportion of positive urine cultures.

Strengths and limitations of this study

- This is the first study exploring the causes for negative urine cultures among adults presenting with urinary tract infection (UTI) symptoms to primary care in a low-income setting.
- Antibiotic exposure was assessed using two different methods.
- Testing for sexually transmitted infections (STIs) was limited to three major pathogens, which may have led to an underestimation of the prevalence of STIs.
- The prevalence of STIs is not generalisable to all primary healthcare attendees, as the participants were enrolled if they reported the presence of UTI symptoms.

INTRODUCTION

Urinary tract infections (UTIs) are a common reason for presentation to primary care with 10% of adult women experiencing at least one episode per year.[1] Dysuria, which is one of the most common symptoms suggestive of a UTI, is reported in 2%–5% of presentations in general practice.[2] While dysuria and other urinary symptoms such as frequency and urgency may be attributed to acute bacterial cystitis, they are by no means specific.[3] Symptoms can be due to a wide range of infectious agents as well as to other non-infectious causes (table 1). In high-income settings, acute bacterial cystitis is the most common cause of UTI symptoms in women presenting to primary care.[2] Thus, the symptom complex is treated empirically with antibiotics, and diagnostic tests are not deemed routinely necessary. Patients whose symptoms do not respond to first-line antibiotics are reassessed

Table 1 Causes of symptoms with acute onset that may be suggestive of a urinary tract infection in adults

Category		Specific cause
Infectious	Acute bacterial cystitis	*Escherichia coli* *Klebsiella pneumoniae* *Staphylococcus saprophyticus* *Enterococcus* spp Unidentified (partially treated)
	Prostatitis	*E. coli* *K. pneumoniae* *Proteus mirabilis* *Pseudomonas aeruginosa* *Enterococcus* spp
	Sexually transmitted infections	*Chlamydia trachomatis* *Neisseria gonorrhoeae* *Trichomonas vaginalis* *Mycoplasma hominis* *Ureaplasma urealyticum* *Herpes simplex* *Candida* spp
	Other infections	*Mycobacterium tuberculosis* (urogenital) *Schistosoma haematobium*
Inflammatory	Dermatological	Irritant or contact dermatitis Stevens-Johnson syndrome
	Non-infectious	Foreign body (eg, stone) Reactive arthritis with urethritis
Non-inflammatory	Drug or food related	Spermicides, topical hygiene products, drugs, certain foods
	Traumatic	Urogenital instrumentation/surgery Horseback or bicycle riding

for other diagnoses or infections with organisms resistant to first-line antibiotics.[4]

The prevalence of positive urine cultures in patients presenting with presumed UTIs varies widely across different settings. This is particularly true when comparing studies from high-income and low-income settings.[2 5] Possible explanations include (1) differences in prevalence of other conditions causing similar symptoms, (2) unregulated access to antibiotics leading to partially treated infections or (3) differences in laboratory techniques affecting the limit of detection.

This study aimed to evaluate possible causes of negative urine cultures in patients presenting with symptoms of UTI to primary health clinics in Harare.

METHODS

Adults (aged 18 years and above) presenting with symptoms of UTIs to nine primary health clinics in Harare were enrolled into the Antimicrobial Resistance in Gram-negative Bacteria from Urinary Specimens (ARGUS) study. In Zimbabwe, primary health clinics are nurse-led and serve the population living in their catchment area. Patients accessing the acute medical services have to pay a user fee. Access to diagnostic tests is limited beyond HIV, tuberculosis and malaria. The eligibility criteria and enrolment procedures for the ARGUS study were previously described.[6] Patients were included in the study if they presented with at least two symptoms suggestive of UTI (dysuria, urgency, frequency, suprapubic pain and/or flank pain).

This is a cross-sectional study reporting on a subset of participants from the ARGUS study who were consecutively enrolled between March and July 2020. Mid-stream urine samples were collected from study participants and underwent dipstick testing, microscopy and culture using conventional microbiology methods.[6] Leucocyturia was considered to be present if the sample had $\geq 10^6$ white blood cells/L on microscopy or a positive dipstick for leucocytes. Antibacterial activity of urine indicating possible prior antibiotic exposure was ascertained using a urine bioassay. Briefly, 20 µL urine was inoculated on a 6 mm filter paper disc that was incubated on Mueller-Hinton agar and using *Escherichia coli* (ATCC25922) as an indicator organism.[7] Antibacterial activity was considered to be present in the sample if a growth inhibition zone of any size around the disc was observed following incubation for 24 hours at 37°C.

Urine samples were tested for sexually transmitted infections (STIs), specifically *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. For women only, urine samples were also tested for *Trichomonas vaginalis*. STI testing was done using the GeneXpert platform (Cepheid, Sunnyvale, California, USA) according to the manufacturer's instructions. Treatment for STIs was prescribed during the initial

visit by the clinic nurse according to routine practice following the Zimbabwean national guidelines[8] and independent of study procedures. Participants with positive tests were notified of their result and offered treatment for themselves and their partners if they were not treated on their initial appointment. To ascertain if symptoms had resolved, a follow-up phone call was conducted 28 days postenrolment.

Statistical analysis

Data were analysed using Stata V.15. Categorical data were described using counts and percentages, and continuous data were described using medians and IQRs. Differences between categorical and continuous variables were evaluated using the χ^2 and Mann-Whitney U tests, respectively. The level of significance was set at a p value of ≤0.05.

Patient and public involvement

There was no patient and public involvement in the study design. Results from this study were disseminated to nurses working in the polyclinics from where the study participants were recruited in order to improve patient care.

RESULTS

The study included 425 participants with a median age of 37.3 years (IQR 26.8–48.0), of whom 275 (64.7%) were women and 172 (40.5%) were HIV-positive. All samples were tested for the presence of leucocytes, underwent conventional urine culture and were tested for *C. trachomatis* and *N. gonorrhoeae*. Samples from 175 women were tested for *T. vaginalis*. Of 425 urine samples collected, 420 were stored for the antibiotic bioassay; volume was insufficient for storage for the remaining 5 samples. In 255 (60.0%) of the participants, at least one of the tests was positive, while in 170 (40.0%) participants, all of the tests that were performed were negative, and the cause of their symptoms could not be determined or suspected. Figure 1 shows the distribution of positive test results (STI positive 83 (19.6%), urine culture positive 96 (22.6%), leucocyturia 130 (30.6%) and prior antibiotics 89 (20.9%)). Haematuria was detected in two (0.5%) participants.

Urine cultures were positive in 96 (22.6%), negative in 314 (73.9%) and contaminated in 15 (3.5%) participants. A higher proportion of urine cultures were positive in women (n=73, 26.6%) compared with men (n=23, 15.3%). Urine culture positivity was higher in participants with more recent symptom onset prior to presentation (58/225, 25.8% in those with a symptom onset of ≤7 days, vs 37/197, 18.8% for those with symptoms >7 days, p=0.086).

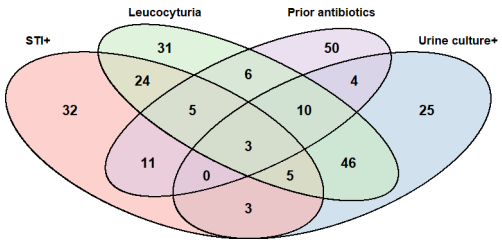

Figure 1 Distribution of positive tests among study participants (170/425 samples; 40% were negative for all tests). STI, sexually transmitted infection.

Overall, 83/425 (19.5%, 95% CI 15.9% to 23.6%) participants had an STI including 11 who also had a positive urine culture. The prevalence of *C. trachomatis* was 43/425 (10.1%, 95% CI 7.4% to 13.4%); that of *N. gonorrhoeae* was 37/425 (8.7%, 95% CI 6.2% to 11.8%); and that of *T. vaginalis* was 14/175 (8.0%, 95% CI 4.4% to 13.1%). Eleven (2.6%) participants had a positive STI test result for two pathogens. The prevalence of *C. trachomatis* was 26/275 (9.5%, 95% CI 6.3% to 13.5%) among women and 17/133 (12.8%, 95% CI 7.6% to 19.7%) among men. For *N. gonorrhoeae*, prevalence was 18/275 (6.5%, 95% CI 3.6% to 10.1%) among women and 19/150 (12.7%, 95% CI 7.8% to 19.1%, p=0.032) among men. STIs were more common in those with recent symptom onset (52/225, 23.1% among those with an onset of symptoms of ≤7 days, and 31/197, 15.7% for those with symptoms >7 days, p=0.057). Participants who tested positive for an STI were younger and were less likely to have HIV infection than those without an STI (table 2).

The median duration of symptoms was 7 days for participants with positive urine cultures and those who had an STI. Among participants who did not have an STI or a positive urine culture, the median duration of symptoms was 8 days (IQR 5–12). The most common presenting symptoms were dysuria (337/425, 79.3%), frequency (310/425, 72.9%) and suprapubic pain (272/425, 64.0%). Overall presenting symptoms were similar between individuals with STIs and UTIs and those with negative cultures (figure 2).

Antimicrobial treatment prior to clinic presentation was reported by 30 (7.1%), while the antibiotic bioassay was positive in 73 (17.4%) including 26 participants with HIV infection who were taking co-trimoxazole prophylaxis. Overall, 89 (20.9%) of the participants had either reported taking antibiotics or had a positive urine bioassay.

STI treatment was prescribed by the clinic nurses at the initial visit in 38 participants, of whom 20 (53%) had a positive STI test, while 63 (76%) of the 83 patients who had an STI were not prescribed STI treatment by the primary healthcare nurse.

A follow-up phone call was made with 393 (92.5%) of the participants. Most (334/393, 85.0%) reported that the symptoms had resolved or significantly improved since enrolment. Among the 59 participants whose symptoms did not improve or resolve, 15 had a positive urine culture and 6 had an STI, while 32 (54.2%) did not have any positive tests. Symptom resolution or improvement was reported by 70/76 (92.1%) participants with an STI, by 74/89 (83.1%) of those with a positive urine culture and by 127/159 (79.9%) with negative results in all tests. Among participants with positive urine cultures, symptoms improved or resolved in 65/69 (94.2%) of those

Table 2 Characteristics of the study participants according to the test result

Characteristic	Total N=425 (%)	STI positive n=83 (%)	Urine culture positive n=96 (%)	Leucocyturia n=130 (%)	Prior antibiotics n=89 (%)	All tests negative n=170 (%)
Age (years) median (IQR)	37.3 (26.8–48.0)	28.4 (23.5–39.2)	39.8 (26.0–50.0)	34.2 (24.8–47.0)	37.6 (27.6–49.4)	39.4 (29.6–49.6)
Female sex	275 (64.7)	51 (61.5)	73 (76.0)	88 (67.7)	51 (57.3)	107 (62.9)
Completed at least secondary education	359 (84.5)	71 (85.5)	75 (78.1)	108 (83.1)	77 (86.5)	141 (82.9)
HIV-positive*	172 (42.4)	20 (25.6)	42 (47.2)	53 (43.4)	38 (44.2)	76 (46.1)
Pregnancy*	35 (13.0)	4 (7.8)	7 (9.7)	15 (17.2)	5 (9.8)	16 (15.5)
Married/have a partner	325 (76.5)	66 (79.5)	74 (77.1)	91 (70.0)	67 (75.3)	132 (77.7)
Duration of symptoms prior to presentation	7 (5–12)	7 (4–12)	7 (4–11)	7 (5–10)	7 (5–12)	9 (6–12)
Symptoms resolved on follow-up*	334 (85.0)	70 (92.1)	74 (83.2)	105 (89.0)	73 (89.0)	127 (79.9)

*19 participants did not know their HIV status; 5 women did not know if they were pregnant; follow-up information available for 393 participants.
STI, sexually transmitted infection.

Figure 2 Presenting symptoms in study participants according to urine culture and STI test result. Severe symptoms were defined as symptoms affecting daily activities. STI, sexually transmitted infection.

who reported having taken their prescribed treatment and in 9/20 (45%) of those who had not.

DISCUSSION

This study found that only one in five people presenting to primary care in Harare with UTI symptoms had a positive urine culture with a uropathogen explaining their presenting symptoms. There was a high prevalence of STIs and evidence of prior antimicrobial use among study participants as possible explanations for the low proportion of positive urine cultures. However, in 40% of patients, symptoms remained unexplained.

Only 20% of study participants had a positive urine culture, which is similar to findings from studies conducted in other sub-Saharan African countries where culture positivity was found to be 11% and 36% among outpatients.[5 9] In contrast, studies conducted among women with cystitis in high-income countries, mostly from Europe and the Americas, report that 75%–80% of urine cultures reveal significant growth of uropathogens.[2 10 11] This may be explained by differences in access to healthcare, unregulated antibiotic use and prevalence of other conditions with similar symptoms between settings.[5 12–14]

In our study, the median time to presentation was 7 days, while studies from high-income settings report a much shorter time between symptom onset and clinic presentation of 2–3 days.[10 11] Thus, the lower yield of urine cultures in this study may be due to spontaneous resolution of infection because of the delays in accessing healthcare or self-treatment with antibiotics.[15] This may particularly be the case in patients with leucocyturia where symptoms can be explained by residual inflammation. Dysuria and leucocyturia in the absence of positive urine cultures may be due to other causes such as urogenital tuberculosis, schistosomiasis and other inflammatory conditions.[3] However, other infections are unlikely to be the cause of symptoms in our patient population, given that for the vast majority, the symptoms resolved or improved on follow-up and that haematuria was present in less than 1%.

Antimicrobial use prior to clinic presentation may also have contributed to a lower urine culture yield, with one in five study participants having evidence of taking

antibiotics prior to presentation to primary care. While only 7% of the study participants reported antimicrobial use in the previous 2 weeks, 17% had evidence of antimicrobial activity in their urine. Although antibiotic access from pharmacies is highly regulated in Zimbabwe, antibiotics can be acquired from the informal market.[16] Patients may not report on self-treatment at clinic presentation due to social desirability bias. Prior antimicrobial use was similar to that reported by patients from Senegal,[17] which had a culture positivity of 27% but lower than that in studies from the USA.[18] This suggests that asking patients about antimicrobials may not be sufficient in determining prior exposure as shown in findings from Ghana.[19] On the other hand, the antibiotic bioassay may overestimate antimicrobial use for treatment of infections by also detecting antimicrobials consumed unintentionally in food and water.[20] Alternative explanations for symptoms suggestive of UTIs such as dysuria include irritant hygienic products, spermicides and certain foods.[3]

STIs are highly prevalent in some sub-Saharan African settings,[21] and there is some symptom overlap between STIs and UTIs. A meta-analysis of studies from sub-Saharan Africa reported a pooled prevalence for *C. trachomatis* of 7.8% in women of reproductive age,[22] while a study from Zimbabwe testing mainly asymptomatic adolescents and young people found a prevalence of 17% of *C. trachomatis* and/or *N. gonorrhoeae*.[23] While STIs may be asymptomatic particularly in women, patients with symptoms due to STIs frequently experience diagnostic and treatment delays leading to prolonged symptoms prior to presentation.[24] One of the study eligibility criteria included recent symptom onset (within 2 weeks). This was aimed at increasing the likelihood of UTIs compared with STIs. Despite this, STI prevalence in our study was almost 20%. Importantly, 1 in 15 women and 1 in 8 men tested positive for *N. gonorrhoeae*. The high prevalence of STIs may also explain the large proportion of men enrolled into the study who are generally more likely to experience symptoms from STIs which are similar to symptoms of UTIs.[24] The study may have missed other STIs because testing was limited to three major pathogens but did not include other organisms that may cause urethritis and urinary tract symptoms such as *Mycoplasma genitalium*, leading to an underestimation of STI prevalence. Furthermore, STIs may have been missed because the study used mid-stream urine samples, while first-catch samples are recommended for the diagnosis of STIs for optimal yield on urine samples. Genital ulcers caused by herpes simplex and vaginitis due to *Candida spp.* may explain symptoms in some patients. Also, men were not tested for *T. vaginalis* as this is less frequent in men.[25] Potential participants who did not seek care because of not being able to afford the clinic fees would have been missed.

Only a quarter of participants who tested positive for an STI underwent STI treatment on the initial clinic presentation because the attending nurse did not suspect an STI. This highlights the difficulties in differentiating the two conditions on clinical grounds. Access to diagnostic testing at primary care level is thus urgently needed.

The prevalence of STIs is not generalisable to all primary healthcare attendees, as the participants were selected on the basis of STI/UTI symptoms.

CONCLUSIONS

This is the first study exploring the causes for negative urine cultures among adults presenting with UTI symptoms to primary care in a low-income setting. The findings of this study highlight the high STI burden in this patient population. The overlap in symptoms in patients diagnosed with UTIs and STIs, respectively, results in underdiagnosis and undertreatment of STIs. Diagnostic testing is paramount to ensure appropriate treatment. Very few participants who had an STI received appropriate treatment on clinic presentation because STIs were not suspected, emphasising the missed opportunities for diagnosis and treatment. The high prevalence of prior antibiotic exposure in patients presenting to primary care should prompt an improvement of regulations around antibiotic access to prevent development of antimicrobial resistance.

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Contributors IDO and KK conceived the study idea and planned the study. RAF contributed to the study idea. PC, KPEM and AM provided support with the resources needed for the study. SY and RAF provided support with conducting the study. IDO and MC conducted the laboratory testing and the interpretation of the results. IDO and KK analysed and interpreted the data and drafted the initial manuscript. MC, SY, DM, MM, PC, KPEM, AM and RAF reviewed and provided feedback on the initial manuscript. All authors read and approved the final manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The study was approved by the London School of Hygiene and Tropical Medicine ethics committee (ref. 16424) and the Medical Research Council of Zimbabwe (MRCZ/A/2406). All study participants provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data relevant to the study are included in the article. Data availability statement: the data

underlying this study can be obtained upon request by emailing the corresponding author.

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CHAPTER 8

Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe

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Increasing access to diagnostics in LMICs is key to obtaining AMR data for surveillance and clinical care that will ultimately improve patient management. While the previous chapters presented data on the prevalence of AMR and on antibiotic use, the following two chapters provide potential diagnostic solutions for broadening our understanding of AMR in LMICs.

The study in this chapter explores the use of two novel culture methods (InTray COLOREX Screen/ESBL and Compact Dry EC) for pathogen identification and detection of resistance in patients from the ARGUS study who presented with UTI symptoms to PHCs in Harare. The results show good performance of the novel culture systems. Both systems have several advantages over conventional culture methods and have the potential to expand and decentralize laboratory testing which would be particularly valuable in low-resource settings. Specifically, they are pre-prepared, have a relatively long shelf-life, and are easy to use and interpret. The InTray COLOREX ESBL can be used to detect resistance to third-generation cephalosporins reducing turnaround times while Compact Dry EC can be stored at room temperature for up to 18 months prior to use.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

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First Name(s)	Ioana Diana		
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Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the principal investigator for the study. I have designed and planned the study, prepared the study protocol, obtained the regulatory approvals, prepared the study forms and the standard operating procedures. Patient enrolment and data collection was done by research assistants which I trained and supervised. I ensured that the InTrays are available in the field on a daily basis. I prepared the laboratory standard operating procedures, trained the laboratory technicians and oversaw the laboratory work. I read and documented InTray results following incubation, prepared the dilutions for Compact Dry, inoculated the plates and read them after incubation. I ensured that laboratory testing is conducted in accordance to quality standards. I performed the data management, cleaned, and analysed the data, and interpreted the results.

SECTION E

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ORIGINAL ARTICLE

Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe

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Abstract

Antimicrobial resistance surveillance data is lacking from many resource-limited settings mainly due to limited laboratory testing. Novel culture systems may address some of the limitations of conventional culture media and expand the availability of microbiology services. The aims of this study were to evaluate the performance of InTray COLOREX Screen/ESBL and Compact Dry for the detection of uropathogens and of extended-spectrum beta-lactamase (ESBL)-producing organisms from urine samples. Urines samples were collected from patients presenting with symptoms of urinary tract infection to primary care clinics in Harare. Performance of the InTray COLOREX Screen, ESBL and Compact Dry chromogenic media were compared to the reference of culture using Brilliance UTI agar and conventional antimicrobial susceptibility testing. A total of 414 samples were included in the analysis. Of the included samples, 98 were positive on Brilliance UTI agar and 83 grew *Enterobacterales*. The sensitivities and specificities for *Enterobacterales* were 89.2% (95% CI 80.4–94.9) and 98.2% (95% CI 96.1–99.3) for InTray Screen and 95.2% (95% CI 88.1–98.7) and 99.7% (95% CI 98.3–100) for Compact Dry. Extended-spectrum beta-lactamases were present in 22 isolates from the Brilliance UTI agar. The sensitivity of the InTray COLOREX ESBL culture plates for the detection of ESBL-producing organisms was 95.5% (95% CI 77.2–99.9) and specificity was 99.5% (95% CI 98.2–99.9%). Our findings show good performance of the novel culture systems for the detection of uropathogens and ESBL-producing organisms. Both systems have several advantages over conventional media and have the potential to expand and decentralize laboratory testing.

Keywords AMR · Antibiotic resistance · *Enterobacterales* · ESBL

Introduction

Antimicrobial resistance (AMR) represents a major threat to human health by impeding effective treatment of serious infections and leading to increased morbidity, mortality and healthcare costs [1]. Acknowledging the implications of rising AMR, in 2015, the World Health Assembly adopted the Global Action Plan on AMR which outlined specific actions to address the increase in AMR including strengthening AMR surveillance and global data sharing

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[2]. In response to the Global Action Plan, the World Health Organization launched the Global AMR Surveillance System (GLASS).

The 2020 GLASS report emphasizes the persisting geographic gaps in AMR surveillance with health facilities and laboratories from Europe and the Americas contributing the majority of data. Data from low- and middle-income countries (LMIC) in Africa and Southeast Asia are scarce due to limited laboratory capacity and availability of microbiological diagnosis [3]. The main challenges restricting bacterial identification and detection of resistance are insufficient laboratory scientists and technicians, stock-outs of laboratory consumables, short shelf life of some key reagents, difficulties in supply chains management and prohibitive costs [4, 5]. In most LMICs, both availability and cost of diagnostic tests limit access for a large majority of patients who have most infectious conditions treated empirically and with the use of case definitions rather than laboratory confirmation. Blood cultures for example are usually restricted to tertiary referral hospitals and are not available at lower tier hospitals (i.e. district hospitals) and peripheral healthcare facilities [6].

Even at the levels where these tests are available, the turnaround time is such that antimicrobials are prescribed and consumed before the results are available to influence the choice. Limited availability of diagnostics results in a lack of AMR surveillance data and non-adaptation of empirical treatment to local pathogens and resistance profiles. Available data are usually biased towards more complex cases treated at referral centres or in private healthcare settings, and are not representative of the overall burden of resistance, challenging the development of locally adapted treatment recommendations.

Two novel ready-to-use culture systems, InTray and Compact Dry, may facilitate processing of urine samples in low-resource settings particularly in regional and district healthcare facilities where fully equipped microbiology laboratories may not be available. These systems have particular advantages that may allow for their use in lower tier facilities, thus expanding access to microbiology services. Urinary tract infections (UTIs) are common Gram-negative infections in outpatient settings. Organisms causing UTIs may provide valuable information on community-level Gram-negative resistance. Urine samples are non-invasive and considered priority specimens for AMR surveillance by the WHO-GLASS [3].

The aims of this study were to evaluate the performance of InTray COLOREX Screen/ESBL and Compact Dry for the detection of uropathogens and of extended-spectrum beta-lactamase (ESBL)-producing organisms in urine samples from patients presenting with UTI symptoms to primary care clinics in Harare.

Methods

Study participants were recruited into the antimicrobial resistance in Gram-negative bacteria from Urinary Specimens (ARGUS) study which is an observational cross-sectional study enrolling adult patients who present with suspected UTIs to one of nine participating primary health clinics in Harare. The procedures and eligibility for the ARGUS study have been described in detail elsewhere [7]. Briefly, after obtaining informed consent, demographic and medical history was collected using a questionnaire and entered directly in an electronic form using the Open Data Kit (ODK, www.opendatakit.org). A midstream urine sample was collected from each of the study participants. If the transportation time to the laboratory was anticipated to exceed 4 h, the urine sample was placed in a thermally insulated bag containing ice packs.

Culture media

This study used two novel culturing techniques—(1) InTrays COLOREX Screen and InTray COLOREX ESBL (Biomed Diagnostic, White City, OR, USA) and (2) Compact Dry EC (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan)—and compared them to culture on Brilliance UTI agar (Oxoid, UK) which is considered the reference standard [8].

InTrays are ready-to-use commercially available small-sized agar plates (5-cm diameter for the agar) with chromogenic substrates to differentiate between multiple bacterial species based on colony colour. InTray COLOREX ESBL plates contain, in addition, antimicrobial compounds for selective identification of extended-spectrum beta-lactamase (ESBL)-producing organisms. InTrays have to be stored at 2–8 °C and have a shelf life of 6 (InTray COLOREX ESBL) to 12 months (InTray COLOREX Screen) [9].

Compact Dry EC is a dehydrated ready-to-use chromogenic medium designed for quantifying *Escherichia coli* and coliforms from food products. The media is hydrated by sample inoculation and capillary action allows for diffusion of the sample across the plate. The media contains two chromogenic enzyme substrates, Magenta-Gal and X-Gluc, which enable the differentiation between *E. coli* (blue) and other coliforms (pink). The plastic casing has a grid with large and small squares for ease of colony counting. For optimal colony counting, the manufacturer recommends diluting samples to concentrations of 100 colony forming units (CFU)/mL. The media is heat, light and moisture-sensitive and is supplied in small opaque pouches containing desiccant. The plates are stored at room temperature up to 30 °C and have a shelf life of 18 months post-manufacture [10].

Colony appearance and characteristics of the different media are described in Table 1 and additional Figures S1 and S2.

Sample processing

Following collection, the urine samples were inoculated by trained research assistants at the point of care onto the InTray COLOREX Screen and ESBL culture plates using a cotton swab (one tray per sample). For the Compact Dry and Brilliance UTI agar, urine samples were processed at the Biomedical Research and Training Institute research laboratory. The inoculated culture plates were then transported to the laboratory where they were incubated at 37 °C for 24 h. The InTrays were read after 24 h by a trained microbiologist. Information on the number of CFU, morphology, colour and growth was recorded using a standardized form. Because InTrays require refrigeration, uninoculated plates had to be transported at the end of each working day to the laboratory and dispatched again to the clinics on the following day.

For Compact Dry, it was assumed that positive urine cultures would have a bacterial concentration of at least 10^3 CFU/mL [11]. To perform colony counts, serial dilutions of 1:10 were performed on the day of sample collection. The urine samples were diluted up to 10^6 in sterile phosphate-buffered saline and 1 mL each of the 10^3 and 10^6 dilutions was inoculated on the Compact Dry and incubated for 24 h at 37 °C. The optimal dilutions were established during a pilot phase and previous use of the culture system in our laboratory (unpublished). The plates were read by a trained microbiologist and the number of CFU and colony appearance was recorded. If colonies could not be counted, growth was categorized semi-quantitatively into semi-confluent (some individual colonies still visible) and confluent growth (individual colonies not visible with a change in substrate colour).

Brilliance UTI agar was inoculated at the laboratory using 1-µL sterile loops and incubated at 37 °C for 24 h. Growth was reported semi-quantitatively into three categories: 10^3–10^4 CFU/mL, 10^4–10^5 CFU/mL and $> 10^5$ CFU/mL. All cultures showing growth of $> 10^3$ CFU/mL with the predominance of uropathogens were considered positive.

Antimicrobial susceptibility testing (AST) was done by disc diffusion and interpreted according to the EUCAST standards [12]. ESBL testing of *Enterobacterales* was performed according to the EUCAST recommendations [13]. Briefly, isolates were screened for the presence of ESBLs using cefpodoxime. Isolates positive on the screening test underwent confirmation by synergy testing with amoxicillin/clavulanic acid and ceftazidime. Quality control for bacterial identification and AST was performed using ATCC reference isolates.

Table 1 Colony appearance and product characteristics for the three culture systems used in the study [8–10]

	Brilliance UTI agar	InTray COLOREX	Compact Dry EC
E. coli	Pink	Pink	Blue
KESC*	Dark blue	Blue/turquoise	Red-violet (pink)
Proteus group	Brown halo	Brown halo	Yellow–brown
Pseudomonas spp.	Brown/green	Cream, translucent	-
Enterococcus spp.	Turquoise	Blue/turquoise	Inhibited
S. aureus	White/cream	Golden	Inhibited
S. saprophyticus	Pink, small	Pink, small	Inhibited
Other streptococci/staphylococci	Non-pigmented/white	White	Inhibited
Product and use characteristics			
Storage temperature	Dehydrated: RT Prepared: 2–8 °C	2–8 °C	RT (< 30 °C)
Shelf life	Dehydrated: ~ 2 years Prepared: 2 weeks	6–12 months	18 months
Size/format (mm)	Dehydrated: container Prepared: 85 × 85 × 14	103 × 75 × 8 Agar: 5 cm	Culture media: 5.5 cm
Number of samples	Multiple	Single	Single
Preparation required	Yes (dehydrated)	No	No
Number of samples inoculated	1–6/ plate	1	1
Sample and volume inoculated	Neat urine, 1 µL	Neat urine, using a swab	Diluted urine 10^3 and 10^6; 1 mL
Location of sample inoculation	Laboratory	Point of care (clinic)	Laboratory

KESC Klebsiella, Enterobacter, Serratia, Citrobacter; *RT* room temperature. Dehydrated media, supplied in powder form requiring preparation; prepared, pre-poured plates as supplied by the manufacturer

Experience in using the tests

Research assistants and laboratory staff involved in sample processing were asked about the perceived advantages and shortcomings of the test systems. The number of times the InTray plates were removed from the fridge and dispatched to the clinics was recorded to determine if multiple exposures to high temperatures affect the test performance.

Statistical analysis

Descriptive analyses were performed in STATA v.15 (StataCorp, TX, USA). To evaluate test performance, sensitivity and specificity with 95% confidence intervals (95% CI) were calculated. The correlation between CFUs on Compact Dry and growth on Brilliance UTI agar categorized semi-quantitatively was evaluated using Spearman's correlation test.

The study was granted ethics approval by the London School of Hygiene and Tropical Medicine (ref. 16,424) and by the Medical Research Council of Zimbabwe (MRCZ/A/2406).

Results

A total of 431 urine samples were tested using the three culture systems. Of those, 17 (3.9%) were contaminated on Brilliance UTI agar and were excluded leaving 414 samples for the final analysis. The median age of study participants was 36 years (IQR 26–46), 263 (63.5%) were female and 169 (42.7%) were HIV positive. Participant characteristics are shown in additional Table S1.

InTray COLOREX Screen for uropathogen detection

Using the Brilliance UTI agar, 98 urine cultures were positive. Uropathogens identified were 72 *E. coli* (73.5%), 11 other *Enterobacterales* (11.2%), 13 *Enterococcus spp.* (13.3%) and two *Staphylococcus aureus* (2.0%). Of the 72 cultures growing *E. coli* on the Brilliance UTI agar, 64 showed growth of pink colonies (*E. coli*) on the InTray COLOREX Screen culture plates while eight were positive on Brilliance only and six on InTrays only. For other coliforms, ten cultures were positive on both Brilliance and InTrays and an additional sample was positive on the Brilliance UTI agar only (Table 2).

Of the 13 samples that were positive on the Brilliance UTI agar for enterococci, nine were also positive for enterococci on the InTray COLOREX Screen cultures. The four discordant samples that were positive for enterococci on the Brilliance UTI agar had growth of enterococci on the InTrays but enterococci were not the dominant organism. Conversely, 15 cultures had predominant growth of enterococci on the InTray but were negative on the Brilliance UTI agar. The two cultures with growth of *S. aureus* were positive on both culture media.

Using the Brilliance UTI agar as the reference standard and a threshold for positivity of 10^3 CFU/mL, the sensitivity of InTray COLOREX Screen for detecting *Enterobacterales* was 89.2% (74/83; 95% CI 80.4–94.9) and the specificity was 98.2% (325/331; 95% CI 96.1–99.3). Positive and negative predictive values were 92.5% (74/80; 95% CI 84.4–97.2) and 97.3% (325/334; 95% CI 94.9–98.8), respectively. Test performance according to different thresholds for culture positivity is shown in Table 3.

Compact Dry EC for uropathogen detection

Of the 83 samples which had growth of *Enterobacterales* on Brilliance UTI agar, 79 had growth on the Compact Dry EC of *Enterobacterales* at the $1{:}10^3$ dilution and 58 at the $1{:}10^6$ dilution. Four samples which were positive for *E. coli* on Brilliance were negative on Compact Dry and one sample with *Proteus spp.* was positive on

Table 2 Comparison between culture results for InTray Screen and Brilliance UTI agar for *Enterobacterales*

		Brilliance UTI agar				Total
		Negative for coliforms[#]	10^3–10^4 CFU/mL	10^4–10^5 CFU/mL	$>10^5$ CFU/mL	
InTray COLOREX Screen	Negative (or 1–4 colonies)[*]	325	4	3	2	334
	Positive					
	5–49 colonies	4	5	3	0	12
	50–100 colonies	0	1	0	1	2
	Confluent growth	2	0	8	56	66
	Total	331	10	14	59	414

[*]Negative cultures, one colony on InTray ESBL but not on Screen ($n=2$); mixed growth of organisms with colonies on InTray but < 5 colonies ($n=3$); [#]3/6 discordant cultures with a positive InTray also had growth with low colony count on Compact Dry. Gram-positive organisms (*Enterococcus* spp., $n=13$ and *S. aureus*, $n=2$) were not included in the table as their growth is inhibited on the Compact Dry media

Table 3 Performance for Compact Dry EC and InTray Screen in the detection of *Enterobacterales* compared with Brilliance UTI agar and using different thresholds for culture positivity on Brilliance agar

	Cut-off on Brilliance agar	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Compact Dry at $1:10^3$ dilution	10^3	95.2 (88.1–98.7)	99.7 (98.3–100)	98.8 (93.2–99.9)	98.8 (97.0–99.7)
	10^4	98.6 (92.6–100)	97.7 (95.4–99.0)	90.0 (81.2–95.6)	99.7 (98.3–99.9)
	10^5	100 (93.9–100)	94.1 (91.1–96.3)	73.8 (62.7–83.0)	100 (98.9–100)
InTray COLOREX Screen	10^3	89.2 (80.4–94.9)	98.2 (96.1–99.3)	92.5 (84.4–97.2)	97.3 (94.9–98.8)
	10^4	93.2 (84.7–97.7)	96.5 (93.9–98.2)	85.0 (75.2–92.0)	98.5 (96.5–99.5)
	10^5	96.6 (88.3–100)	93.5 (90.4–95.8)	71.3 (60.0–80.8)	99.4 (97.9–99.9)

NPV negative predictive value; *PPV* positive predictive value

Compact Dry only (Table 4). The sensitivity and specificity for uropathogen detection were 95.2% (79/83; 95% CI 88.1–98.7) and 99.7% (330/331; 95% CI 98.3–100), respectively, using a threshold for urine culture positivity of 10^3 CFU/mL on the Brilliance UTI agar (Table 3). Positive and negative predictive values were 98.8% (79/80; 95% CI 93.2–99.9) and 98.8% (330/334; 95% CI 97.0–99.7). Using the $1:10^3$ dilution, 55/80 (69%) of positive samples on Compact Dry had semi-confluent or confluent growth and therefore colony counts could not be performed. While the $1:10^3$ dilution had a better sensitivity, the higher dilution allowed for colony counts. There was a strong correlation between CFUs determined using the Compact Dry and the semi-quantitative assessment on Brilliance UTI agar (Spearman's rho 0.924, $p < 0.001$; Fig. 1).

InTray COLOREX ESBL for the diagnosis of cephalosporin-resistant organisms

InTray COLOREX ESBL culture results were available for 413 samples. Synergy testing for the presence of ESBLs was positive in 22/24 isolates tested (*E. coli*, $n = 20$; other coliforms, $n = 2$). Of the 22 ESBL-positive organisms using conventional methods, 21 were positive on InTray COLOREX ESBL. Two samples were positive on InTray COLOREX ESBL only: negative on Brilliance UTI agar ($n = 1$); negative screening test for ESBL using cefpodoxime ($n = 1$). One sample which was ESBL positive using conventional methods and negative on InTray had growth of a single colony on InTray. The sensitivity of the InTray COLOREX ESBL culture plates for the detection of ESBL-producing organisms was 95.5% (21/22; 95% CI 77.2–99.9) and specificity was 99.5% (400/402; 95% CI 98.2–99.9%).

Table 4 Comparison between culture results for Compact Dry and Brilliance UTI agar for *Enterobacterales*

		Brilliance UTI agar				Total
		Negative for coliforms	10^3–10^4 CFU/mL	10^4–10^5 CFU/mL	$>10^5$ CFU/mL	
Compact Dry EC at $1:10^3$ dilution	Negative (or 1–4 colonies)	330	3	1	0	334
	5–49 colonies	0	6	3	2	11
	50–250 colonies	0	1	4	2	7
	Semi-confluent growth or > 250 colonies	0	0	6	18	24
	Confluent growth	1*	0	0	37	38
Compact Dry EC at $1:10^6$ dilution	Negative	331	8	11	6	356
	1–49 colonies[#]	0	2	3	32	37
	50–250 colonies	0	0	0	18	18
	Semi-confluent growth or > 250 colonies	0	0	0	2	2
	Confluent growth	0	0	0	1	1
	Total	331	10	14	59	414

Cultures which showed contamination on Brilliance UTI agar were excluded; *this culture had mixed growth and one of the organisms was Proteus which had confluent growth on the Compact Dry due to swarming; [#]for the $1:10^6$ dilution, this category included growth of 1–49 colonies

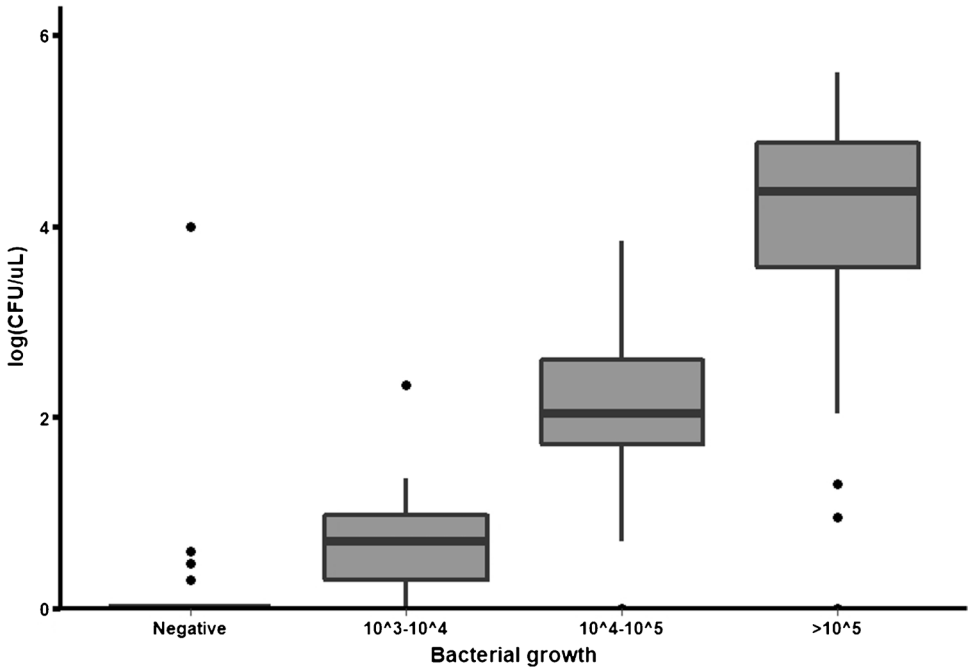

Fig. 1 Comparison of semi-quantitative bacterial growth on Brilliance UTI agar and colony counts using Compact Dry

Reported experience of staff using the tests

Research assistants who inoculated the urine samples onto InTrays at point of care ($n=9$) reported that overall the diagnostic device was easy to use, and they reported no challenges inoculating the agar in the field. The procedure was simple and was not time-consuming. The main challenge was the requirement for the InTrays to be refrigerated and thus InTrays not used on a particular day had to be transported daily from the laboratory to the clinics. In order to decrease the number of times the InTrays were transported between the laboratory and clinics, a limited number of InTrays was issued to each clinic every day. This meant that research assistants sometimes ran out of InTrays if the number of participants enrolled outnumbered the number of InTrays sent to the clinic. The median number of times InTrays were sent to the clinics was 2 (IQR 1–4). The number of times an InTray was transported to a clinic did not affect sensitivity of the culture system.

Laboratory staff ($n=2$) reported on the processing, reading and interpretation of the test results. Both InTray and Compact Dry plates are relatively small in size minimizing incubator space requirements. For both systems, cultures were very easy to read and interpret. A major advantage compared to the UTI Brilliance agar was that InTrays and Compact Dry were ready to use and did not require media preparation and autoclaving. In LMICs, electricity supplies can be unreliable and generators cannot always provide a sufficient power supply for high-energy processes such as autoclaving. In the study setting, media preparation and autoclaving could not be done during power-cuts. However, Compact Dry was more difficult to inoculate because they required sequential dilutions which were performed in the laboratory.

Discussion

Both novel culture systems performed well in detecting uropathogens compared to Brilliance UTI chromogenic media, the reference standard. The diagnostic systems were easy to use and study staff required minimal training for inoculation and reading. Furthermore, InTray COLOREX ESBL plates showed a high sensitivity and specificity for the detection of ESBL *Enterobacterales* making them an attractive tool for AMR surveillance in LMICs. Because the system selectively identifies ESBL-producing organisms from primary specimens, it can reduce time to results when used for diagnosis, which in turn may reduce time to appropriate therapy.

Conventional culture media have to be prepared frequently and require refrigeration which may be challenging in LMICs due to unreliable electricity supplies and equipment maintenance. Preparation of dehydrated media requires autoclaving and quality control of every prepared batch. InTray and Compact Dry are pre-prepared culture plates which are smaller than conventional plates and have an extended shelf life bypassing some of the shortcomings associated with conventional cultures. Both systems are based on chromogenic identification. Although chromogenic media for bacterial identification are not novel and have been in use for more than two decades [14], media

prepared in-house from dehydrated powder usually expire within 2 weeks [8]. InTray COLOREX Screen and ESBL are stable for 6–12 months and Compact Dry EC for 18 months post-manufacture. Furthermore, Compact Dry media does not require refrigeration and can be stored at temperatures up to 30° which is an added advantage when used in LMICs.

The Compact Dry system has been designed for determining bacterial contamination of food [15]. One previous study from Japan reported good sensitivity of the Compact Dry when used to investigate UTIs in humans, but the sample size was small ($n=25$) [16]. Because the Compact Dry is a highly sensitive culture system and positive urine cultures usually have high bacterial loads, the urine samples were diluted prior to inoculation. For the purpose of this study, serial dilutions were performed. However, the procedures could be simplified by performing a single dilution at a higher factor (for example by diluting 1-µL sample into a 1 mL of sterile solution). A volume of 1 mL is required in order to rehydrate the dry culture media. Although of limited clinical significance, Compact Dry allows for colony counts which can be used to estimate bacterial load in samples. In this study, colony counts using the Compact Dry correlated well with the semi-quantitative results on Brilliance UTI agar.

Gram-positive pathogens such as *Enterococcus* spp. and *S. saprophyticus* account for 15% of uncomplicated UTIs [17]. While InTrays can detect these pathogens, Compact Dry ECs can only be used for the detection of *Enterobacterales* and do not support growth of Gram-positive organisms. Enterococci were detected more frequently using InTrays than on conventional media. This may suggest an under-diagnosis of enterococcal infections using conventional media although growth of enterococci from midstream urine samples may not necessarily reflect the presence of enterococci in the urinary bladder [18] and therefore results should be interpreted with caution considering the patient's medical history.

Cultures that were positive on InTray only may be explained by inoculation of a larger sample volume using swabs compared to 1-µL inoculation on Brilliance UTI agar. Furthermore, immediate inoculation of the sample may have prevented loss of bacterial viability during transport. False-negative Compact Dry results were only observed for urine samples with lower bacterial load classified as 10^3–10^4 CFUs on the Brilliance UTI agar. These urine samples may have shown growth on Compact Dry if the urine had been less diluted ($<10^3$). One sample showed confluent growth for *Proteus* spp. on the Compact Dry but was negative on Brilliance UTI agar. This was due to the inhibition of swarming on the Brilliance agar but not on Compact Dry.

This is the first study evaluating the performance of the InTray and Compact Dry culture systems for the diagnosis of UTI. We acknowledge that the study is limited by its relatively small sample size and low proportion of positive urine cultures.

Our findings show good performance of the novel culture systems for the detection of uropathogens and ESBL-producing organisms. These systems may simplify laboratory workflow, reduce technician processing time and facilitate procurement and stock management. Both systems have potential to expand and decentralize laboratory testing. Use of the systems in sentinel clinical sites may enhance understanding of pathogens and AMR burden in LMICs. Further research is needed to demonstrate cost-effectiveness and feasibility of wider implementation of these systems for AMR surveillance, and potentially impact on patient outcomes in LMICs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-021-04312-4.

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Author contribution IDO and KK conceived the study idea and planned the study. WE, NM, JP and CF contributed to the study idea. NM, JP, CF, PC and KPEM provided support with the resources needed for the study. SY, RAF and HH provided support with conducting the study. IDO and MC conducted the laboratory testing and interpretation of results. IDO and KK analysed and interpreted the data, and drafted the initial manuscript. WE, MC, NM, JP, SY, RAF, HH, PC, KPEM and PM reviewed and provided feedback on the initial manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and/or analysed during the current study are available in the Data Compass (LSHTM) repository, https://datacompass.lshtm.ac.uk/1997/. All data generated or analysed during this study are included in this published article and its supplementary information files.

Code availability Not applicable.

Declarations

Ethics approval The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was granted ethics approval by the London School of Hygiene and Tropical Medicine (ref. 16424) and by the Medical Research Council of Zimbabwe (MRCZ/A/2406).

Consent to participate All study participants provided written informed consent prior to study participation.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Supplementary materials for the above publication *"Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe"* are included in the Appendix 4.

Contents of Appendix 4

CHAPTER 9

Evaluation of a novel culture system for rapid pathogen identification and detection of cephalosporin resistance in neonatal Gram-negative sepsis at a tertiary referral unit in Harare, Zimbabwe

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This study investigated the use of the InTray COLOREX Screen/ESBL media for pathogen identification and detection of resistance in neonates with suspected sepsis. The study was conducted at the neonatal unit of Harare (Sally Mugabe) Central Hospital in 2020 which is the largest unit of this type in the country.

The use of InTrays in this study allowed for an important reduction in turnaround time to result from 3-5 days for positive blood cultures when using conventional laboratory methods to 28 hours from the time of blood culture collection. This method has the potential to simplify laboratory procedures while scaling-up the availability of diagnostics for severe infections in low-resource settings. The study also found a very high burden of *Klebsiella*-sepsis in neonates which is a common finding of studies on causes of neonatal sepsis from LMICs, where units are overcrowded and the implementation of effective infection control measures is limited.

The clinical study was led by Dr Gwendoline Chimhini who is a senior paediatrician and neonatologist at Harare Central Hospital. The laboratory work was conducted at the Biomedical Research and Training Institute under my supervision. It was my idea to evaluate the InTrays within the neonatal study which was about to start. I also planned the working flow for laboratory testing including the introduction of a modified rapid AST technique for reducing turnaround times. I was responsible for data quality assurance and analysis, I prepared the first draft of the manuscript with input from Dr Chimini. Dr Chimhini and myself are joint first authors for this work.

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SECTION A – Student Details

Student ID Number	1805237/RITD	**Title**	
First Name(s)	Ioana Diana		
Surname/Family Name	Olaru		
Thesis Title	Understanding Gram-negative infections and antimicrobial resistance in Zimbabwe		
Primary Supervisor	Katharina Kranzer		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Paediatric Infectious Diseases Journal		
When was the work published?	September 2021		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	NA		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am a joint first author for this work. I had the idea of using the InTrays in this study and of implementing rapid antimicrobial susceptibility testing and early reading for reducing turnaround times. I planned and supervised the laboratory work, designed the laboratory forms, wrote the standard operating procedures, and trained the laboraory technicians. I verified all positive blood cultures on a daily basis (daily laboratory rounds) and provided real-time feedback and interpretation to the neonatologists. I designed the forms for the electronic data collection and issued the results. I was also responsible for data cleaning and analysis. Together with Dr Chimhini who led the clinical study on neonatal sepsis, we interpreted the results, drafted the first version of the manuscript and revised it following the reviewers' comments.

SECTION E

Student Signature	Ioana Diana Olaru
Date	21-12-2022

Supervisor Signature	Katharina Kranzer
Date	21-12-2022

Article type: Original study

Abbreviated title: Rapid diagnosis for neonatal sepsis in a low-resource setting

Running head: Rapid diagnosis for neonatal sepsis in Zimbabwe

Title: Evaluation of a novel culture system for rapid pathogen identification and detection of cephalosporin resistance in neonatal Gram-negative sepsis at a tertiary referral unit in Harare, Zimbabwe

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ABSTRACT

Background: Neonatal sepsis accounts for a large proportion of neonatal deaths in sub-Saharan Africa. The lack of access to diagnostic testing and excessively long turnaround times to result contribute to delays in sepsis identification and initiation of appropriate treatment. This study aims to evaluate the novel InTrays COLOREX Screen and ESBL for rapid identification of bacterial pathogens causing sepsis and detection of resistance.

Methods: Neonates with suspected sepsis admitted to the Harare Central Hospital were prospectively enrolled. One blood culture was collected and incubated using the BacT/ALERT automated system. Positive blood cultures with potential pathogens identified by Gram-stain were inoculated on the InTray COLOREX Screen and ESBL culture plates.

Results: A total of 216 neonates with suspected sepsis were recruited. Pathogens were isolated from blood cultures in 56 (25.9%) neonates of which 54 were *K. pneumoniae*. All *K. pneumoniae* were resistant to ceftriaxone and 53 (98%) were resistant to gentamicin. Sensitivity and specificity for ceftriaxone-resistant *K. pneumoniae* detection using InTrays were 100%. InTrays results were interpretable as early as 5-10 hours (median 7 hours, IQR 7-7) post blood culture positivity enabling rapid identification and notification of result and leading to a 60% reduction in time to result from blood culture collection.

Conclusions: This study shows that the implementation of a novel culture method was feasible and reduced turnaround times for results by 60% compared to standard microbiological techniques. Impact on patient outcomes and cost-effectiveness of this method need to be demonstrated.

INTRODUCTION

The sustainable developmental goals (SDG) call for the end of preventable deaths of newborns, with all countries aiming for a neonatal mortality rate of <12 deaths per 1000 live births. The sub-Saharan African region has the highest neonatal mortality globally (27 deaths per 1000 live birth) with 42 of 48 countries at risk of missing the SDG target.(1) Globally, 15% of neonatal deaths are attributable to sepsis, but the proportion is likely to be higher in sub-Saharan Africa. A recent study estimated the number of neonatal sepsis cases in sub-Saharan Africa to be 355,500-605,750 annually leading to 177,500–302,870 deaths.(2)

While neonatal sepsis is a life threatening condition, nosocomial neonatal sepsis may be reduced by adhering to stringent infection prevention and control practices.(3) Effective treatment of neonatal sepsis relies on early and rapid diagnosis and appropriate antibiotic treatment.(4) Ideally empiric antibiotics should take into account the most frequently isolated organisms and antimicrobial susceptibility profiles in a specific setting.(5) Targeted antibiotic treatment should be informed by antimicrobial susceptibility of the bacterial isolate cultured from the infant's blood.(6) Unfortunately, antimicrobial susceptibility data are unavailable in most resource-limited settings due to limited laboratory capacity especially in lower-level facilities such as district and provincial hospitals.(7)

A systematic review found that the most common Gram-negative bacterial species representing 21% of all blood culture isolates in neonates in sub-Saharan Africa were *Klebsiella spp*. and that the majority were resistant to ceftriaxone and gentamicin.(7) The mechanism of resistance against third generation cephalosporins is usually extended-spectrum beta-lactamase (ESBL) production. Both first and second-line neonatal sepsis treatment regimens recommended by the World Health Organization (WHO) are ineffective in treating ESBL-*Klebsiella*.(8) In the absence of antimicrobial susceptibility results, it is thus not surprising that case fatality rates of neonatal ESBL-*Klebsiella* sepsis are extremely high exceeding 35-70% in many low- and

middle-income countries.(9-12) Even if microbiological diagnostics are available, results are often delayed or not returned. A recent audit conducted in one of the busiest neonatal care units in Zimbabwe reported that 67% of blood culture results were never received by the responsible clinician. Only 4% of blood culture results arrived in time to impact on clinical decision-making.(13) Similar to other settings in sub-Saharan Africa, ESBL-*Klebsiella pneumoniae* was common both on admission to the unit and during hospitalization. However, first and second-line treatment for neonatal sepsis remained aligned with WHO recommendations due to high cost and limited availability of carbapenems.(13) InTrays are commercially available, ready-to-use agars containing chromogenic substrates that enable the differentiation between multiple bacterial species based on colony colour. InTray COLOREX ESBL plates contain in addition to the chromogenic substrates, antimicrobial compounds for selective identification of ESBL producing organisms. They have a series of advantages over conventional culture media which may lead to shorter turnaround time (TAT) to result. Using conventional microbiology techniques, blood culture results are available after at least 72 hours from blood culture collection.

We conducted a study to evaluate the novel InTrays COLOREX Screen and ESBL for the work-up of positive neonatal blood cultures in Zimbabwe and investigated whether an algorithm including the chromogenic agar would reduce time to results.

METHODS

Study design and setting

This study included babies admitted to the neonatal unit at Harare Central Hospital (HCH) between March 4th and June 29th 2020 in Zimbabwe. HCH is a tertiary teaching hospital. The neonatal care unit has the capacity to admit 100 neonates, but often operates at 150% capacity

leading to unit overcrowding and difficulties in implementing effective infection prevention and control measures. Monthly admissions before the COVID-19 pandemic averaged 400. The HCH guidelines for managing neonatal sepsis were adapted from the NICE guidelines and the World Health Organization Pocket book of hospital care for children on management of neonatal sepsis.(6, 14) First-line treatment for neonatal sepsis is benzyl-penicillin and gentamicin and ceftriaxone is used as second-line.

Any neonate with suspected sepsis based on the presence of one major risk factor or two or more minor risk factors for sepsis was eligible for inclusion (Table S1). Extremely low birth weight neonates (birth weight <1000 g) were excluded. Mothers of eligible neonates provided written informed consent. Skin disinfection prior to blood culture collection was performed using 70% alcohol wipes. Blood cultures aiming for a volume of 2 mL were collected and sent to the laboratory at the Biomedical Research and Training Institute within 4 hours of the blood draw. Sample volume was assessed by weighing the blood culture bottles before and after filling. Only one sample was included per neonate. Repeat samples were not analysed in the study.

An interviewer administered questionnaire was used to collect information about the antenatal and perinatal period.

Microbiological investigations

Blood culture bottles were incubated using the automated BacT/ALERT (bioMerieux, Marcy-l'Etoile, France) system with continuous monitoring for growth. Positive blood cultures were processed using conventional methods. Samples showing Gram-negative bacilli were also inoculated on the InTray COLOREX Screen and ESBL chromogenic media (Biomed Diagnostic, White City, Oregon, USA; see methods section in the supplement). underwent Gram staining, one drop of the positive blood culture was inoculated on conventional media (MacConkey, blood and chocolate agar (HiMedia, Bombay, India)) on day

0. If the Gram stain showed Gram-negative bacilli or Gram-positive cocci in chains, the sample was also inoculated on the InTray COLOREX Screen and ESBL chromogenic media (Biomed Diagnostic, White City, Oregon, USA; Figure S1). InTray cultures were incubated at 37°C and read at the end of the working day (6-8 hours post-inoculation) and at 24 hours post-inoculation. For Gram-negative bacilli, direct antimicrobial susceptibility testing (AST) from the positive blood culture was performed and read on day 1. *Enterobacteriaceae* were identified using biochemical tests on sub-cultured isolates (Analytical Profile Index, API 20E, bioMerieux, Marcy-l'Etoile, France) with results available on day 2. Other organisms were identified by colony morphology and additional conventional microbiology tests including catalase, tube coagulation, appearance on bile-aesculin agar. AST ("indirect AST") was repeated using a 0.5 McFarland inoculum for all isolates which underwent AST directly from the sample. Antimicrobial susceptibility testing (AST) was performed by disc diffusion for ampicillin, ceftriaxone, imipenem, ciprofloxacin, gentamicin, amikacin, chloramphenicol and by determining minimum inhibitory concentration using Etests (bioMerieux, Marcy-l'Etoile, France) for vancomycin. AST results were interpreted using the EUCAST standards.(15) Figure 1 shows the testing algorithm for positive blood cultures using the InTray system. Quality control of laboratory tests for media growth support, bacterial identification and AST was performed using ATCC strains.

Result reporting

Positive results were reported directly to the attending clinicians using an encrypted smartphone-based messaging platform as soon as they were available. These included Gram-stain results for potential pathogens, growth of a suspected resistant organisms on the InTray ESBL plates at 6-8 hours post-inoculation, preliminary bacterial identification and AST results from the directly inoculated sample at 24 hours, and final bacterial identification and AST for pathogens at 48 hours post-inoculation.

Data entry and analysis

Statistical analyses were performed in STATA v.15 (StataCorp, TX, USA). Descriptive analysis was performed to characterize the study population and laboratory findings. A Kaplan Meier analysis was performed to compare time to death from blood culture collection in neonates with and without *K. pneumoniae* sepsis. Babies were censored at 28 days or at discharge for those without a day-28 follow-up visit.

Permission and Ethics

Permission to conduct the study was obtained from the Harare Central Hospital ethics committee.

Institutional Review Board (IRB) approval was obtained from the Medical Research Council Zimbabwe (MRCZ/A/2547).

RESULTS

Between March 1 and June 30, 2020, 972 neonates were admitted to the unit of which 451 (46.4%) had an episode of suspected sepsis during their admission (Figure 2). A total of 216 neonates with suspected sepsis were recruited into the study.

Maternal and birth characteristics

Median age for the mothers was 25 years (IQR 22-31), 22 (12.8%) were HIV infected and 90 (41.7%) were primigravidae. The place of delivery was Harare Central Hospital in 136 (63%), another healthcare facility in 51 (23.6%) and 23 (10.6%) babies were born before arrival to health facilities. For 6 babies, the place of delivery was not documented. The majority of babies (164/216, 75.9%) were delivered by normal vertex delivery, while 46 (21.3%) were born by caesarean section. Maternal and neonatal characteristics are described in Table 1.

Neonatal characteristics

This study included 125 (57.9%) boys, 90 (41.7%) girls and one baby with ambiguous genitalia; 23 (10.6%) babies were from multiple pregnancies. Birth weight was very low (1000-1499g) in 54 (25.4%), low (1500g-2499g) in 65 (30.5%), and normal (≥2500g) in 87 (40.8%). Seven (3.3%) babies had a birth weight of >4000g.

Microbiological diagnosis

A blood culture was collected within the first 72 hours of life in 142 (65.1%), between 4 and 7 days in 49 (22.5%) and after the first week in 27 (12.4%; Figure 3). The median filling volume of the blood culture bottles was 1.08 mL (IQR 0.56-1.75). Pathogens were isolated from blood cultures in 56 (25.9%) neonates and further 29 (13.4%) blood cultures were categorized as contaminated (Figure 2). *K. pneumoniae* was isolated from 54 and *Enterococcus spp.* from three neonates (Figure 3). One baby had two pathogens isolated from the blood culture. Coagulase-negative staphylococci were the most common contaminants (n=24) followed by *Bacillus spp.* (n=4) and non-pathogenic viridans streptococci (n=1). The prevalence of resistance in *K. pneumoniae* was 54/54 (100%) for ceftriaxone, 53/54 (98%) for gentamicin, 50/53 (94%) for amikacin, 9/54 (17%) for chloramphenicol, 6/54 (11%) for ciprofloxacin and 0/54 (0%) for imipenem. Appropriate treatment with meropenem was initiated in neonates with ESBL-*K. pneumoniae* as soon as the microbiology results were available. All *K. pneumoniae* isolates showed growth of blue colonies on the InTray Screen and ESBL culture indicating ceftriaxone resistance. *Enterococcus spp.* isolates only had growth on the InTray Screen plate and were all ampicillin-resistant. Sensitivity and specificity for ESBL-*K. pneumoniae* detection using InTrays were 100%. Risk factors for *K. pneumoniae* sepsis and outcomes are described in the Supplement.

Testing times

Testing times and laboratory procedures are shown in Figure 1. The median time from blood culture collection to laboratory receipt was 3 hours (IQR 2-4) and the median time to positivity

for blood cultures was 18 hours (IQR 17-20). A subset of 27 (50%) InTrays were read at 5-10 hours (median 7 hours, IQR 7-7) post-inoculation and the treating clinicians were notified of growth of colonies suggestive of third-generation cephalosporin-resistant *K. pneumoniae* at the time of reading (Figure 4). A further 21 (39%) InTrays were read the next day, after a median of 23 hours (IQR 23-24) post-inoculation. Of those, nine were inoculated during the weekend or were positive late during the working day and therefore reading was not possible on the day of inoculation. For six InTrays, the result was delayed beyond 24 hours. Results of the early InTray reading were available at 28 hours from blood culture collection and those for the final identification and AST at 68 hours leading to a 60% reduction in the total time to result post blood culture collection.

Outcomes of neonates with ESBL-K. pneumoniae sepsis

The median duration of hospital stay was 11 days (IQR 6-21) in neonates with a positive blood culture for *K. pneumoniae* compared to 7 days (IQR 4-12, p=0.004) in babies with either a negative or contaminated blood culture. In-hospital mortality was 25/54 (46%) in neonates with *K. pneumoniae* sepsis and 27/162 (17%, p <0.001, Figure S2) in neonates without a positive blood culture for *K. pneumoniae*. Of the 30 deaths which occurred within 72 hours of the blood culture collection, 15 (50%) were in neonates with confirmed *Klebsiella* sepsis. Follow-up calls were successful for 162/164 neonates; one baby died after discharge.

DISCUSSION

This study found that one in four neonates with suspected sepsis had a positive blood culture with *K. pneumoniae* and isolates were almost universally resistant to first- and second-line antibiotics as recommended by the WHO guidelines for LMICs.(14) The use of a novel culture system and feeding-back laboratory results in real-time led to a time to preliminary pathogen identification and detection of cephalosporin resistance of as little as 28 hours from blood

culture collection (7 hours from blood culture positivity) as compared to three days when using conventional laboratory methods and five days for the HCH laboratory.

The predominance of *K. pneumoniae* among the isolates is not surprising and reflects findings from across sub-Saharan Africa.(7) The high burden of *Klebsiella*-sepsis in neonates is likely related to suboptimal infection prevention and control procedures that enable nosocomial transmission, although the early onset of infection in many neonates also raises the possibility of perinatal transmission.(7) Nosocomial transmission is facilitated by the long survival of the pathogen on dry surfaces (16), unit overcrowding and limited adherence to aseptic techniques and hand hygiene.(17)

Mortality in this study was very high with every second neonate with a positive culture for *K. pneumoniae* dying during the course of admission. The study was conducted in a referral unit admitting babies following high-risk pregnancies, with hypoxic ischaemic encephalopathy, congenital abnormalities, or who were born prematurely. These babies require a high-level of care and frequent handling by healthcare staff increasing the risk of colonization and infection particularly in the absence of stringent infection prevention and control procedures and hand hygiene.

Blood cultures are essential for the identification of pathogens causing sepsis and for determining effective treatment. Furthermore, in an outbreak setting, early detection and implementation of infection prevention and control measures are crucial in preventing neonatal deaths. In many LMICs however, laboratory testing capacity is limited by the unavailability of laboratory consumables and staff.(18) Conventional bacterial identification and AST require frequent media preparation, as well as the presence of reliable supply chains to avoid stock-outs. Use of InTrays may bypass some of these challenges because they are ready-to-use, easy to inoculate and have a relatively long shelf-life of 6-12 months. Their use may result in important reductions in TAT for presumptive pathogen identification and detection of

cephalosporin resistance with an excellent sensitivity and specificity. This is essential given that cephalosporin resistance is often associated with resistance to gentamicin (7) and thus resistant pathogens cannot be effectively treated with the recommended empiric antimicrobials for neonatal sepsis.(14) Other strategies to reduce TAT such as dehydrated chromogenic media for ESBL detection (19) and rapid AST (20) are available but they may be more difficult to implement in LMICs requiring higher-skilled technicians. In addition, dehydrated media has a relatively short shelf life once prepared and requires more stringent quality control.

Prolonged TATs are commonly reported as a challenge in LMICs (21, 22) and rapid communication of preliminary results such as Gram-stains are often insufficient.(23) For blood cultures, a study in Ethiopia reported TATs of seven days although the authors did not differentiate between TATs for positive and negative cultures.(24) The study cited repeated stock-outs, high work-loads and insufficient staffing as the main reasons for delays in TATs for a wide range of laboratory tests.(24) An earlier study conducted in the same neonatal unit in Zimbabwe prior to the implementation of this study's testing and notification algorithm reported TATs of five days for positive cultures. The long TAT was partly due to delays in pre- and post-analytical times and only 4% of culture results arrived early enough to impact on clinical management.(13)The current study showed that TAT from blood culture collection to presumptive pathogen identification and AST could be shortened to as little as 28 hours, or 5-8 hours from blood culture positivity. InTrays were easy to use and required minimal training which also contributed to the short TATs. Presumptive pathogen identification using colony colour and the potential to reduce the requirements for subcultures can be valuable especially in settings where highly-trained laboratory staff may not be always available.

The study is limited by its enrollment of babies from a single facility and by its recruitment restricted to working hours and weekdays due to logistics and research laboratory operating times. As a result, blood cultures becoming positive overnight were only investigated the next

morning. Similarly, blood cultures that became positive late during the working day or during weekends were not read at 6-8 hours. Had the study been done in a routine laboratory shift system, TATs could have further been decreased. The samples were processed in a well-functioning research bacteriology laboratory and testing was supervised by trained clinical microbiologists. Heightened efforts were made to minimize laboratory TAT in order to provide timely results to clinicians and optimize patient care. Thus, these results may not be generalizable and replicated in routine hospital laboratories in LMICs operating under resource and staff constraints. This study used an automated blood culture system with continuous monitoring for growth. These systems are often not available in LMICs despite the added advantage of higher yields and shorted TATs compared with manually-read blood cultures prepared in house.(25) We were only able to evaluate the InTrays for a single Gram-negative pathogen however ESBL-*Klebsiella spp.* is particularly problematic on neonatal units in LMICs. This study was not designed and powered to show an impact of InTray use and rapid result notification on neonatal mortality.

This is the first report evaluating the use of InTrays in reducing TATs for blood cultures in LMICs. These tests have several advantages: prolonged shelf-life and easy inoculation, reading and interpretation requiring less-skilled laboratory staff.

This study highlights the burden of *Klebsiella*-sepsis in a tertiary neonatal care unit in sub-Saharan Africa. The implementation of a novel culture method paired with rapid communication of results was feasible, led to simplified laboratory procedures, and reduced TAT by 60%. Further research is needed to demonstrate cost-effectiveness of these methods and their impact on patient outcomes.

Competing interests

None of the authors have any competing interests.

Supplemental Digital Content 1. Document

Acknowledgments

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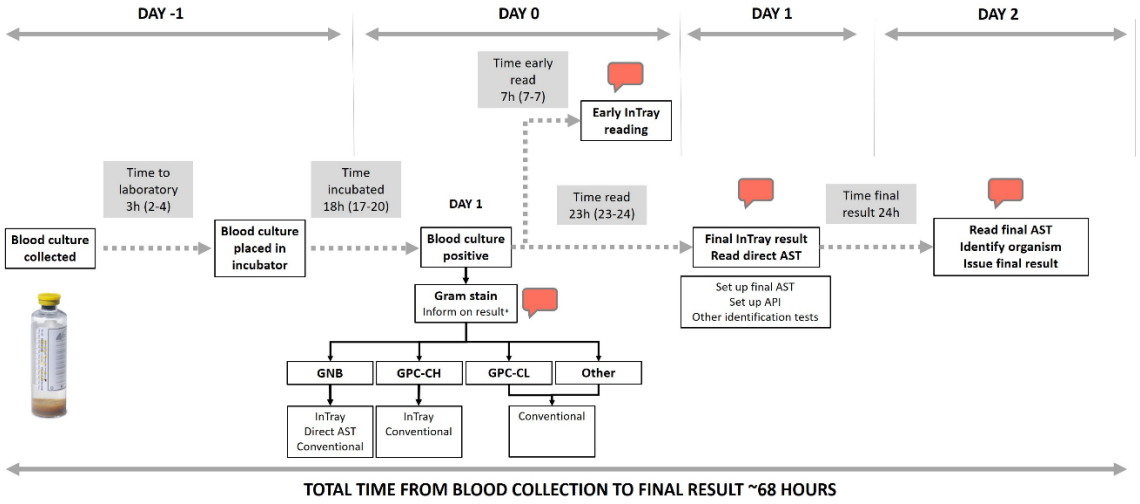
Table 1. Characteristics of mothers and neonates enrolled into the study

Neonatal characteristics	**N=216**
Female sex, n (%)	90 (41.7)
Birthweight, n (%)	
<1500g	54 (25.4)
1500-2499g	65 (31.5)
≥2500g	94 (44.1)
Product of multiple pregnancy, n (%)	23 (10.7)
Maternal characteristics	
Age in years, median (IQR)	25 (22-31)
Number of antenatal clinic visits, n (%)	
None	65 (30.1)
1	55 (25.5)
2	34 (15.7)
3	27 (12.5)
≥4	35 (16.2)
HIV positive*, n (%)	22 (12.8)
Parity, n (%)	
Primigravida	90 (41.7)
Multigravida	126 (58.3)
Prolonged rupture of membranes (18h or more), n (%)	23 (19.2)
Prolonged labour (24h or more), n (%)	7 (3.8)
Caesarean delivery, n (%)	46 (21.3)
Birth outside a healthcare facility, n (%)	23 (10.7)
Meconium-stained amniotic fluid, n (%)	42 (19.4)
Neonatal care variables	
NG/OG-feed, n (%)	86 (39.8)
Oxygen, n (%)	84 (38.9)
CPAP, n (%)	35 (16.2)
Invasive ventilation, n (%)	4 (1.9)
Required surgery, n (%)	8 (3.7)
Duration of hospital stay in days, median (IQR)	8 (5-14)

The following variables had missing data: birth weight (n=3), HIV status (n=44), prolonged rupture of membranes, excluding neonates born by Caesarean section and outside a healthcare facility (n=50); prolonged labor, excluding neonates born outside a healthcare facility (n=23). CPAP: continuous positive airway pressure; NG: nasogastric, OG: orogastric.

Figure 1. Laboratory procedures and result reporting for positive blood cultures

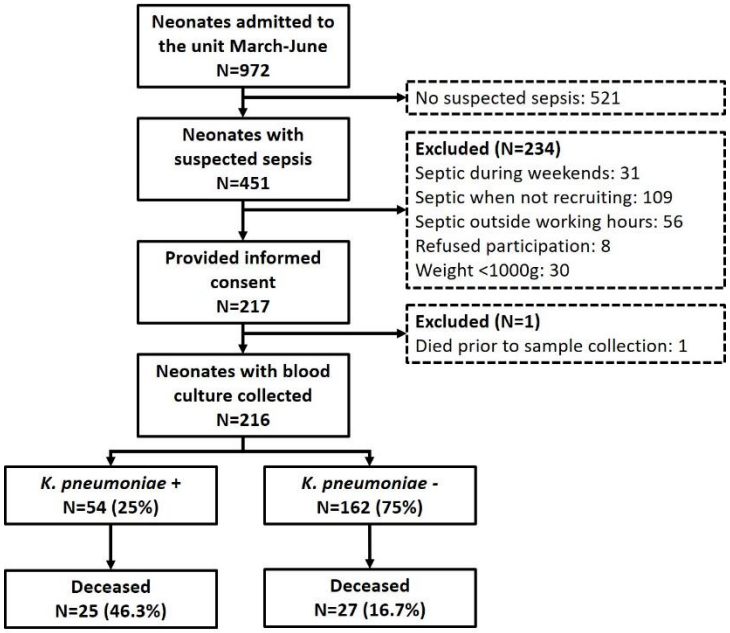


Call outs represent results which were notified in real-time using messaging platforms. The times presented in the shaded boxes represent median times and interquartile ranges.

**The Gram stain results were only notified for GNB and GPC in chains. Conventional methods included inoculation on MacConkey, blood and chocolate agar. InTrays read were InTray COLOREX Screen and ESBL. Time to early bacterial identification and ESBL-detection ("Early InTray reading"): 7 hours from culture positivity. Time to full pathogen identification and AST results using conventional methods ("Read final AST, Identify organism, Issue final result"): 47 hours from culture positivity (68 hours from culture collection).*

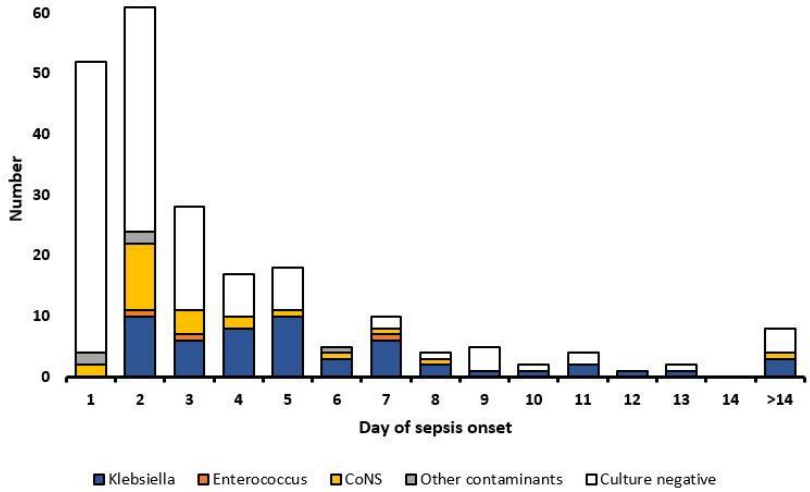
API: analytical profile index; AST: antimicrobial susceptibility testing; GNB: Gram negative bacilli, GPC-CH: Gram positive cocci in chains; GPC-CL: Gram positive cocci in clusters.

Figure 2. Flow chart of study participants



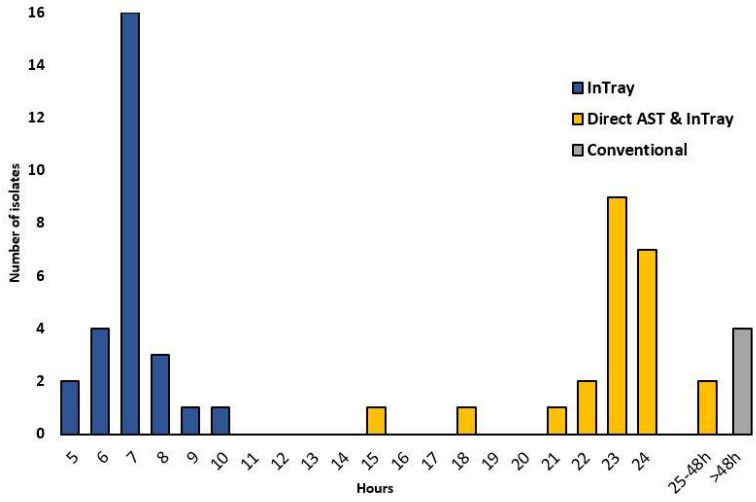
Septic when not recruiting: neonates were diagnosed with sepsis during the national lockdown (April – May) or before the study started recruitment in March and after the study completion in June (total number of neonatal admissions were only available for the complete month). Neonates who were septic outside regular working hours (weekend and at night) could not be included into the study. For these neonates, blood cultures were collected and sent to the routine laboratory. For one neonate who was severely ill, the mother provided informed consent but the baby died before a sample could be collected.

Figure 3. Culture results according to the day of sepsis onset from birth



Cultures were positive for K. pneumoniae starting with day two from birth. The white columns represent negative cultures. Most blood culture were collected within the first five days of life.

Figure 4. Time from blood culture positivity to presumptive identification and determination of third-generation cephalosporin resistance using the different methods for blood cultures positive for *K. pneumoniae.*



InTray method: growth of blue colonies on the InTray and ESBL plate was interpreted as presumptive K. pneumoniae with third-generation cephalosporin resistance; Direct AST and InTray: AST was performed directly from the blood culture bottle and read after ~24 hours. The horizontal axis shows the time in hours from blood culture positivity.

Supplementary materials for the above publication *"Evaluation of a novel culture system for rapid pathogen identification and detection of cephalosporin resistance in neonatal Gram-negative sepsis at a tertiary referral unit in Harare, Zimbabwe"* are included in Appendix 5.

Contents of Appendix 5

CHAPTER 10

Discussion of findings

The studies conducted as part of this PhD provide a comprehensive body of work on Gram-negative AMR in Zimbabwe. They address a number of knowledge gaps related to AMR in Zimbabwe. This chapter discusses the main results of these studies, the evidence derived from this work, and its implications for policy and practice. Finally, it provides some considerations on future research for improving our understanding of AMR in LMICs.

Main findings of this work

The key findings of this PhD are:

1. There is a high prevalence of resistance to antibiotics recommended as first and second line treatment as per the national guidelines for UTIs and neonatal sepsis; the current guidelines are not aligned with the AMR epidemiology locally;
2. *E. coli* isolates causing UTIs are frequently multidrug resistant and resistance mechanisms and sequence types are diverse;
3. PLWH are at increased risk of infections with third-generation cephalosporin-resistant *E. coli* which limits treatment options and is important to consider when PLWH present with severe infections;
4. Antibiotic use in the community, prior to accessing primary care is common, which may be explained by financial constraints to access primary health care;
5. Diagnostic uncertainties, and insufficient training on AMR and antibiotic prescribing among primary care providers may contribute to antibiotic overuse in this setting;
6. Novel culture media have an excellent performance for bacterial identification and detection of resistance and can be used to simplify laboratory procedures, reduce turnaround times, and scale-up AMR surveillance.

The following section discusses in more detail the key findings of this PhD and their implications.

The burden of AMR in sub-Saharan Africa is not well described, routine surveillance is largely non-existent and most research studies are from single centre referral hospitals.[1] Prior to this work, only three studies investigating AMR in Zimbabwe had been published: two focused on carriage of resistant Gram-negatives and one summarised routine data from a private laboratory.[2-4] The ARGUS study showed that the prevalence of Gram-negative resistance to first-line antibiotics as recommended by the national guidelines[5] was high in community acquired UTIs. Four in five and one in five *E. coli* isolates were resistant to amoxicillin and quinolones, the two antibiotic classes recommended as first line treatment for UTIs by the national guidelines. In contrast, resistance to nitrofurantoin which is inexpensive and available in Zimbabwe was less than 10%. Consequently, nitrofurantoin should be included in the treatment recommendations for uncomplicated UTIs in primary care.

Patients who received ineffective treatment for UTIs were at increased risk of clinical and bacteriological failure. Overall only a third of patients took antibiotics that were effective according to AST results. Among those taking effective treatment symptom improvement and bacterial clearance from urine culture were high at 96% and 76% respectively. However, among those who did not receive effective treatment only 62% had improved symptoms and 30% a negative culture at follow-up.

Third-generation cephalosporin resistance, particularly among patients with HIV, was common with almost 27% of PLWH having infections with ESBL-*E. coli* compared to only 13% among participants without HIV. Of note, a significant proportion of UTIs are self-limited even without treatment; most UTIs affect the lower urinary tract only and are non-severe and do not result in systemic infections. However, the high prevalence of resistance among *E. coli* in the community points to a more serious problem. *E. coli* is a frequent cause of severe community-acquired infections and death.[6] Resistance to third-generation cephalosporins in *E. coli* dramatically reduces treatment options in low-income settings as alternatives such as carbapenems are often not available or if available unaffordable. Furthermore, guidelines which are not informed by the local epidemiology will lead to ineffective treatment. The same holds true for neonatal sepsis due to *K. pneumoniae* which were almost universally resistant to third-generation cephalosporins and aminoglycosides.

This PhD also provides novel data on the genomic epidemiology and underlying molecular mechanisms for resistance of ExPEC *E. coli*. ST131, a globally disseminated lineage associated with AMR, is relatively common in the isolates collected as part of this study. However, other lineages frequently reported in other studies in sub-Saharan Africa[7] and in Zimbabwe[8], such as

ST410 and ST617, were less common. Furthermore, this study highlights a high diversity of STs and resistance mechanisms with isolates frequently harbouring resistance genes to multiple antibiotic classes. More data on the genomic epidemiology of *E. coli* and other pathogens from sub-Saharan Africa would be needed for better understanding the molecular mechanisms of AMR and for outbreak investigation and control. The high costs of whole genome sequencing and the specialised skills and infrastructure required for data processing and interpretation still hamper the availability of genomic data from sub-Saharan Africa (see literature review, Chapter 4). The COVID-19 pandemic has changed the availability and use of whole genome sequencing in sub-Saharan Africa.[9] The increased sequencing capacity will hopefully be used for other (non-SARS-CoV-2) pathogens in the future.

This PhD also found an association between HIV infection and AMR. The systematic review undertaken as part of the background section showed that while there is evidence for an association between AMR and HIV for Gram-positive organisms, data for Gram-negatives are very limited. The ARGUS study showed that HIV infection is associated with third-generation cephalosporin resistance for *E. coli*. Given the high burden of HIV and infections in general in sub-Saharan Africa, clinicians need to consider the higher risk of resistance in patients with HIV and prioritize them for diagnostic testing. However, access to diagnostic testing including culture, bacterial identification and AST is limited in LMICs. Diagnostic tests are unavailable, unaffordable and of suboptimal quality. In addition slow turnaround times limit their impact on clinical decision making and patient care.[10]

As part of this PhD two studies evaluated novel culture media which may be used to overcome some of these challenges, expand laboratory testing and potentially be used for surveillance. The studies show that InTray COLOREX Screen/ESBL and Compact Dry have a good performance in comparison to conventional microbiology tests for bacterial identification and detection of resistance to third-generation cephalosporins (InTray ESBL). A study from Lao PDR also found that InTrays are easy to use and have a good performance in a low-resource setting.[11]

This PhD also explored antibiotic use and perceptions with regards to AMR and antibiotic prescriptions. The studies show that exposure to antibiotics in the community is frequent. According to the study findings, one in five patients may have been exposed to antibiotics prior to seeking primary care. This may be explained by the socio-economic situation in Zimbabwe and high costs associated with accessing formal healthcare. Nurses and midwives, who are the

main antibiotic prescribers in the outpatient setting in Zimbabwe acknowledged that antibiotics are overprescribed and overused and highlighted the need for training on AMR and prescribing. Point-of-care tests would constitute an attractive strategy for expanding microbiology testing and reducing diagnostic uncertainties while providing rapid results. However, point-of-care tests for detecting AMR are still to be developed. A promising strategy for reducing antibiotic prescriptions and thus influence AMR would be the use of C-reactive protein at the point of care which has been trialled mainly in South-East Asia.[12,13]

Strengths of this study

The strengths of this PhD lie in its multi-faceted approach to understanding AMR in Zimbabwe. I performed a systematic review to identify previously published studies. There was limited data investigating the association between HIV and Gram-negative resistance and hence the ARGUS study investigating this association and recruiting more than 1000 participants from ten PHCs in Harare filled an important gap. I sought to determine other factors, and in particular prior antibiotic exposure, that may contribute to the development of AMR in this setting. I also investigated the underlying mechanisms of resistance by performing whole genome sequencing. Findings from this study and other research work in Zimbabwe[14] highlighted the challenges in accessing diagnostic testing which in turn impacts on the availability of AMR data. For this reason, I evaluated two novel culture techniques that have some advantages over conventional culture methods and may be useful in LMIC laboratories. Finally, I explored the attitudes and beliefs of healthcare workers in relation to AMR and antibiotic prescriptions. This approach provides a comprehensive picture of the factors driving AMR in this setting which were outlined in Chapter 1 (Figure 1).

A further strength was the recruitment of participants accessing ten PHCs in Harare. These PHCs serve more than 70% of the low-income population residing in the city[15] and participants are thus likely to be representative of the urban population of Harare and of patient populations from similar settings. The study had a very high participation rate with only two patients refusing to participate.

AST and the evaluation of novel culture media was done in a research laboratory with quality assurance methods in place to ensure high quality results.

Limitations

The study was unable to recruit the targeted sample size of 1500 due to the COVID-19 pandemic which led to country-level lockdowns and clinic closures. Specifically, in Zimbabwe a national lockdown was declared at the end of March 2020 and recruitment into the study had to be suspended for 8 weeks. Furthermore, lack of personal protective equipment and limited SARS-CoV2 testing capacity, meant that clinics were frequently closed when a SARS-CoV-2 infection was identified among the healthcare staff. This was worsened by the economic situation in Zimbabwe leading to healthcare staff not being able to afford transport to their workplace which in turn led to staff shortages and additional clinic closures. In August 2020, only a third of the city's clinics were operational.[16] Because of clinic closures and slow recruitment, the study stopped recruitment early in July 2020. Although, we could show an association between HIV-infection and third-generation cephalosporin resistance, which was the main outcome of the ARGUS study, the association between HIV status and other antibiotics did not reach statistical significance.

Loss to follow-up at the first follow-up visit for those with a positive urine culture was high (19%). Most participants were followed through home visits, but were often not at home at the time of the scheduled visit. Additionally, during the COVID-19 national lockdown research activities ceased resulting in missed follow-up appointments. The analysis investigating whether participants who were appropriately treated were more likely to report symptom resolution and have a negative urine culture at follow-up assumed that follow-up cultures were missing at random. This is likely to be true especially for the follow-up data missing due to the national lockdown. Therefore, the results of this study are generalizable to patients presenting to primary care with symptoms of UTI in Zimbabwe.

Implications for policy

The findings from this PhD highlight the need for high-quality, representative AMR data from sub-Saharan Africa. Improved laboratory capacity, decentralisation and/or efficient transport systems and laboratory networks, and novel diagnostics are required. WHO-GLASS is currently designing a strategy on how to conduct surveys for obtaining representative AMR data from low-resource settings. AMR prevalence surveys from selected countries and/or regions will be initiated in the next year. Because of the work I conducted as part of my PhD, I am acting as an advisor to the WHO for this initiative. The high prevalence of resistance to antibiotics used for first-line treatment of UTIs and neonatal sepsis emphasizes the need for updated guidelines informed by local epidemiology. Following discussions of the ARGUS

study results with national policymakers and programmers, nitrofurantoin was added to the national guidelines as first-line treatment of uncomplicated UTIs.

Excessive antibiotic use is a well-recognized driver of AMR. While healthcare workers acknowledge that antibiotics are overprescribed, the decisions to prescribe antibiotics are influenced by social and economic circumstances and diagnostic uncertainties. Furthermore, there is a need for formalised training initiatives of healthcare workers which may lead to optimisation of antibiotic prescribing and reduce use.

Further research

The work in this PhD showed that the performance of novel culture media is comparable to that of routine microbiology methods for bacterial identification and detection of resistance. These media however were evaluated within a research laboratory setting. It remains to be seen if these or similar methods can be implemented in routine laboratories and whether their use is cost-effective. Currently these media are costly. Scale up of AMR surveillance in LMICs may help negotiating prices and create demand. Strategies for reducing turnaround times for critical specimens should be evaluated within specifically designed studies with a focus on the effect of these strategies on patient morbidity and mortality.

Countries in sub-Saharan Africa have a high burden of HIV and the highest mortality associated with AMR.[6] Little data are available on the association between HIV and Gram-negative resistance and this should be further explored in other countries and patient populations.

Although relevant to understanding AMR in Zimbabwe, the work done in this PhD did not sufficiently investigate the community-related factors associated with excessive and inappropriate antibiotic use described in Figure 1 (Chapter 1). A different approach would be needed to evaluate non-prescription antibiotic use, the challenges in accessing healthcare and how these can be addressed, as well as the views of individuals in the community on the role of antibiotics and on AMR. More in-depth analyses of antibiotic exposure in the community should be performed to investigate their sources and potential environmental exposures ultimately leading to the development of strategies for reducing antibiotic use in the community.

Concluding remarks

The studies comprised in this PhD report on the prevalence of AMR in Zimbabwe, on the association between HIV and AMR, on methods that can improve and scale up microbiology testing, and on the extensive use of antibiotics and insufficient prescriber training. Zimbabwe has put forward a National Action Plan for AMR[17], enrolled into GLASS,[18] and has received funding from several international organizations to support AMR surveillance.[19,20] However, sustained efforts are needed to acquire representative AMR data using high-quality testing and use these data to inform treatment decision aiming to improve patient outcomes. Reducing antibiotic use is challenging in this setting due to the economic situation, high burden of infections, diagnostic uncertainties and the need to provide care.

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APPENDIX 1

Supplementary materials for Chapter 1 (published paper)

The association between antimicrobial resistance and HIV infection: a systematic review and meta-analysis

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SUPPLEMENTARY MATERIALS

TABLE OF CONTENTS

Methods: Search strategy, data extraction and quality assessment

Antiretroviral therapy (ART) was introduced towards the late 90s' in the United States and Europe while the ART rollout in sub-Saharan Africa occurred in the mid-2000s'.[1, 2] Region and country-specific cut-offs for ART roll-out were used: 1996 and 2004 for studies conducted in the United States/Europe and in sub-Saharan Africa/Asia/South America respectively.

The search strategy is presented in Table S1. Variables abstracted from the full-text publications are described in Table S2.

In order to determine if findings are consistent across different domains, we performed sensitivity meta-analyses with the studies grouped according to: year of publication, time when the studies were conducted in relation to widespread ART use, country income, HIV prevalence among adults in the country at the time when the study was conducted; CD4 cell count level and ART use among HIV-infected study participants. Results are shown in Table S6 and Figures S7 and S8).

A quality assessment was performed using a modified Newcastle Ottawa Scale. The studies were evaluated on the following domains: selection of the exposed and unexposed groups, ascertainment of exposure, comparability between groups, assessment of outcome and handling of missing data (non-participation, and missing/untested samples).

Table S1. Search strategy for EMBASE, Medline and Web of Science

SEARCH STRATEGY: EMBASE AND MEDLINE
Antimicrobial resistance
('anti-bacterial resistan*' OR 'anti$bacterial resistan*' OR 'antibiotic resistan*' OR 'antimicrobial resistan*' OR 'drug resistan*' OR 'multi-drug resistan*' OR 'multidrug resistan*' OR 'multiple drug resistan*' OR 'antibiotic susceptib*' OR 'antimicrobial susceptib*' OR 'drug* susceptib*' OR 'multi-drug susceptib*' OR 'multidrug susceptib*' OR 'multiple-drug susceptib*' OR 'multiple drug susceptib*' OR 'AMR' OR 'anti microbial resistan*' OR 'ESBL' OR 'extended spectrum beta lactamase*' OR 'resistan*') AND
MeSH terms: exp Drug resistance, beta-lactamases
HIV
'HIV' OR 'HIV-1' OR 'HIV-2' OR 'ART' OR 'ARV' OR 'HAART' OR 'antiretroviral*' OR 'human immunodeficiency virus' OR 'AIDS' OR 'acquired immunodeficiency syndrome' OR 'acquired immune-deficiency syndrome'
MeSH: exp HIV, HIV infections, Antiretroviral Therapy, Highly Active; Anti-Retroviral Agents
Bacteria and infection
('E$ coli' OR 'Escherichia coli' OR 'Klebsiella' OR 'Enterobacteriaceae' OR 'enterobacteria' OR 'Salmonella' OR 'Shigella' OR 'Proteus' OR 'Enterobacter' OR 'Serratia' OR 'Citrobacter' OR 'gram negative*' OR 'gram positive' OR 'Staphylococc*' OR 'staphylococcal' OR 'MRSA' OR 'methicillin resistan*' OR 'Streptococc*' OR 'streptococcal' OR 'penumocc*' OR 'Enterococc*' OR 'enterococcal' OR 'VRE' OR 'urinary

tract infection*' OR 'UTI' OR 'cystitis' OR 'pyelonephritis' OR 'bloodstream infection*' OR 'blood-stream infection*' OR 'BSI' OR 'bacteremia*' OR 'bacteraemia*' OR 'diarrhea*' OR 'diarrhoea*' OR 'typhoid' OR 'typhi' OR 'cholecystitis' OR 'appendicitis' OR 'coloni?ation' OR 'pneumonia' OR 'meningitis' OR 'abscess' OR 'skin infection*' OR 'otitis' OR 'carriage' OR 'coloni?e* OR 'carry*' OR 'microbiome' OR 'sepsis' OR 'septic*' OR 'gonorrh*' OR 'Neisseria')

MeSH: Enterobacteriaceae, Staphylococcus, Enterococcus, Streptococcus, Urinary Tract Infections, Cholecystitis, Intraabdominal Infections; Diarrhea; Gram-Negative Bacteria, Gram-positive Bacteria; Sepsis; Bacterial infections; Neisseria; Gonorrhea

SEARCH STRATEGY: WEB OF SCIENCE

TS=((antibiotic resistan* OR antimicrobial resistan*) AND (HIV) AND (bacteria*))

SEARCH STRATEGY: LILACS

TW: (antibiotic resistan$ OR antimicrobial resistan$) AND HIV AND bacteria$

TW: (resistencia antibiótica OR resistencia antimicrob$ OR resistência a antibióticos OR resistência antimicrob$) AND (VIH OR HIV OR SIDA OR AIDS)

Table S2. Variables abstracted from the full-text publications

CATEGORY			
General	Author Year of publication Years of study Country	Study design Data collection Facility type Number of facilities	Age category Population recruited
HIV-specific	HIV status ascertainment Number HIV+ ART coverage	Co-trimoxazole prophylaxis	CD4 cell count
Laboratory testing	Sample type Infection or carriage Organism(s) isolated Description of methods	Methods for bacterial identification Methods for antimicrobial susceptibility testing	Standards for interpretation Laboratory quality control Antibiotics tested
Quality assessment	Representativeness of exposed	Selection of non-exposed Exposure ascertainment	Comparability STROBE checklist

Quality assessment using a modified Newcastle Ottawa Scale [3]

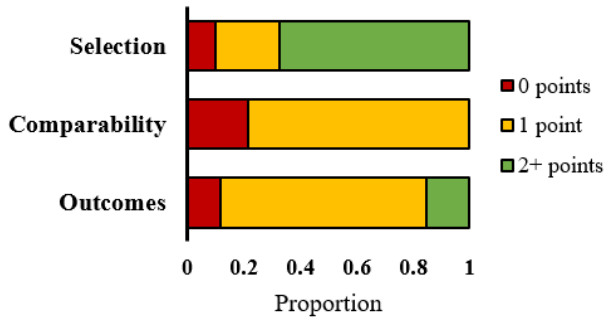
Items were grouped according to the original scale into (1) Selection; (2) Comparability; and (3) Outcome. HIV infection was considered to be the exposure and AMR was the outcome. The items used were:

- Selection: representativeness of HIV-infected patients (max 2 points); selection of non-exposed participants (max 2 points); and ascertainment of HIV infection (max 2 points);
- Comparability of participants with and without HIV based on study design and data analysis (max 1 point);
- Outcome: ascertainment of outcome (max 1 point); and handling of missing data from patients/ samples (max 1 point).

The most common factors impacting on the quality of the included studies were: the lack of representability of the exposed participants, ascertainment of the exposure (HIV infection) and a lack of information on missing participants or samples.

Studies did not consistently report on how HIV status was ascertained. Selection and enrolment of study participants was generally reported. However, study participants were not always representative of the underlying population and comparability between PLWH and HIV-negative people was sometimes uncertain.

Figure S1. Quality assessment of studies using the Newcastle Ottawa Scale



Laboratory methods

Of the 46 studies presenting data on bacteraemias, 11/46 (24%) reported using an automated blood culture system, 2/46 (4%) used manual methods and 33/46 (72%) did not describe how the blood culture was processed. In the majority of studies, organisms were identified using only manual methods and conventional biochemical tests (n=40, 43%). Eighteen studies used addition automated systems (Vitek: n=7, MALDI-TOF: n=4, Phoenix: n=4, Xpert: n=1, Microscan: n=2). Thirty-four studies did not describe the methods used for bacterial identification. Most studies performed antimicrobial susceptibility testing (AST) using manual methods (n=58, 63%): disc diffusion (n=24), minimum inhibitory concentration (MIC) determination (n=10) or a combination of methods (n=24). Automated methods for AST were used in 18 studies (Vitek: n=8, Phoenix: n=4, Microscan: n=4; Sensititre: n=2); 16 studies did not detail the method used. The standards used for AST interpretation were reported in 60 (65%) studies. These were NCCLS/CLSI (n=54), EUCAST (n=2) and a combination of other standards (n=4). Only 14 (15%) studies reported performing quality control procedures for any of the laboratory tests with 13/14 of these being from low- and middle-income countries in sub-Saharan Africa and Asia.

For *S. aureus,* methicillin resistance was determined using cefoxitin (n=8), oxacillin (n=9) or methicillin (n=2) discs or media. Chromogenic media was used in six studies, and PCR-based methods in seven; among the latter two used a combination of PCR and other methods. Seventeen studies did not describe the methods used for MRSA testing.

Of 22 studies reporting on penicillin susceptibility in *S. pneumoniae*, 16 performed MICs for confirmation, while six did not describe how penicillin testing was performed.

Antimicrobial resistance in *Salmonella spp.*

Six studies including 422 PLWH and 2040 individuals without HIV infection reported on infections with *Salmonella spp.* Three studies evaluated resistance in non-typhoid salmonellae only, two in both non-typhoid *Salmonella* and *S.* Typhi, and one did not identify beyond species level. Isolates were from blood (n=3), stool (n=1) or mixed samples (n=2). Only two studies reported on use of co-trimoxazole prophylaxis in the study populations at 8% and 31% respectively. The prevalence of ceftriaxone resistance in non-typhoid salmonellae was described in three studies and ranged from 7% to 13% among PLWH and from 10% to 14% among individuals without HIV infection. Multidrug resistance was described by two studies in non-typhoid salmonellae and ranged from 28% to 72% among HIV-infected and from 17% to 56% among HIV-uninfected individuals. Fluoroquinolone resistance was not consistently described. The pooled OR for co-trimoxazole resistance in non-typhoid salmonellae was 2.76 (95%CI 1.25-6.09).

Figure S2. Pooled prevalence for antimicrobial resistance in HIV-infected individuals. *S. pneumoniae* penicillin non-susceptibility (a) and co-trimoxazole resistance (b); *S. aureus* methicillin resistance (c) and co-trimoxazole resistance (d): *E. coli* (infection only) third-generation cephalosporin resistance (e) and co-trimoxazole resistance (f). ES: estimate

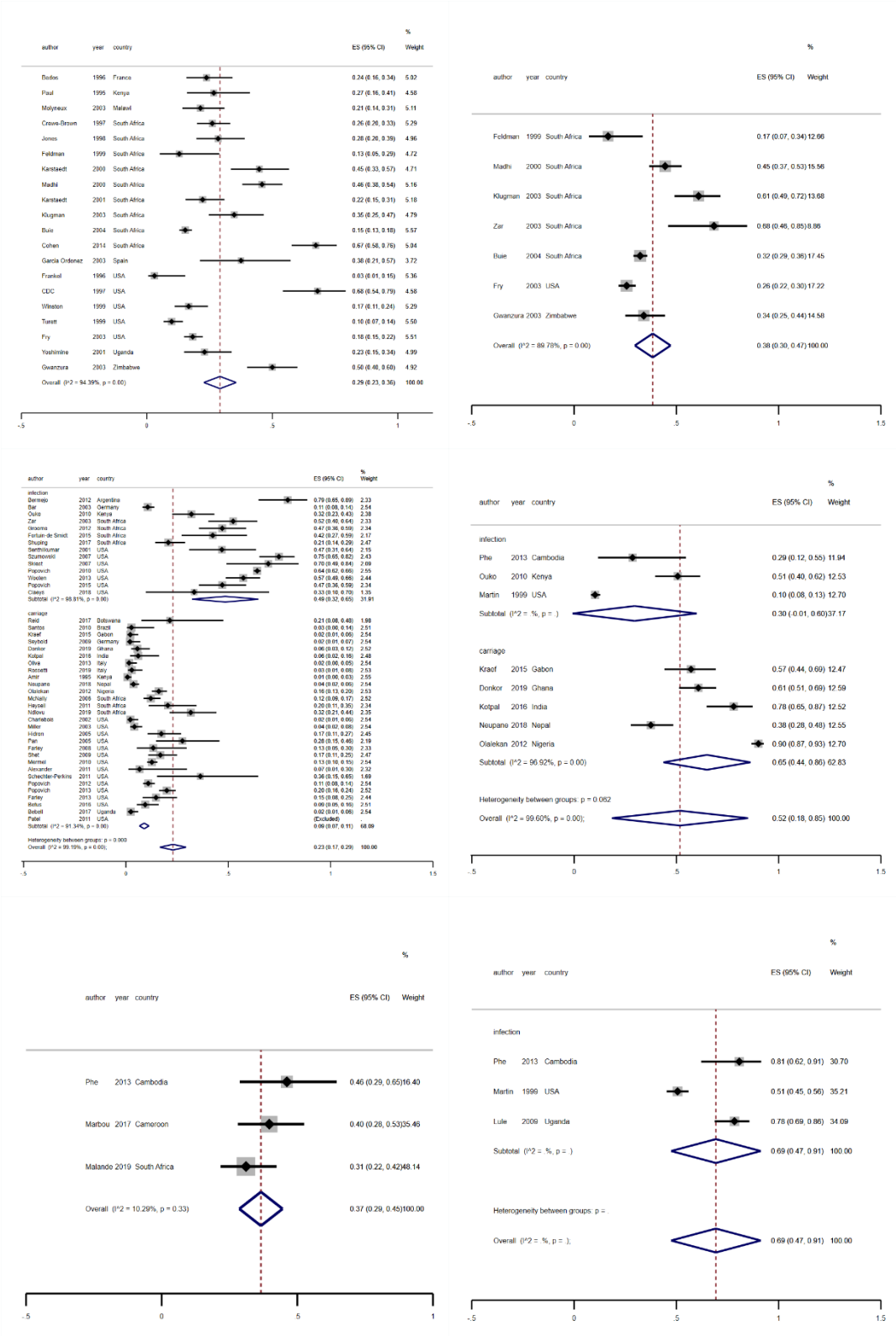


Figure S3. Pooled prevalence for antimicrobial resistance in HIV non-infected individuals. *S. pneumoniae* penicillin non-susceptibility (A) and co-trimoxazole resistance (B); *S. aureus* methicillin resistance (C) and co-trimoxazole resistance (D): *E. coli* third-generation cephalosporin resistance (E) and co-trimoxazole resistance (F). ES: estimate

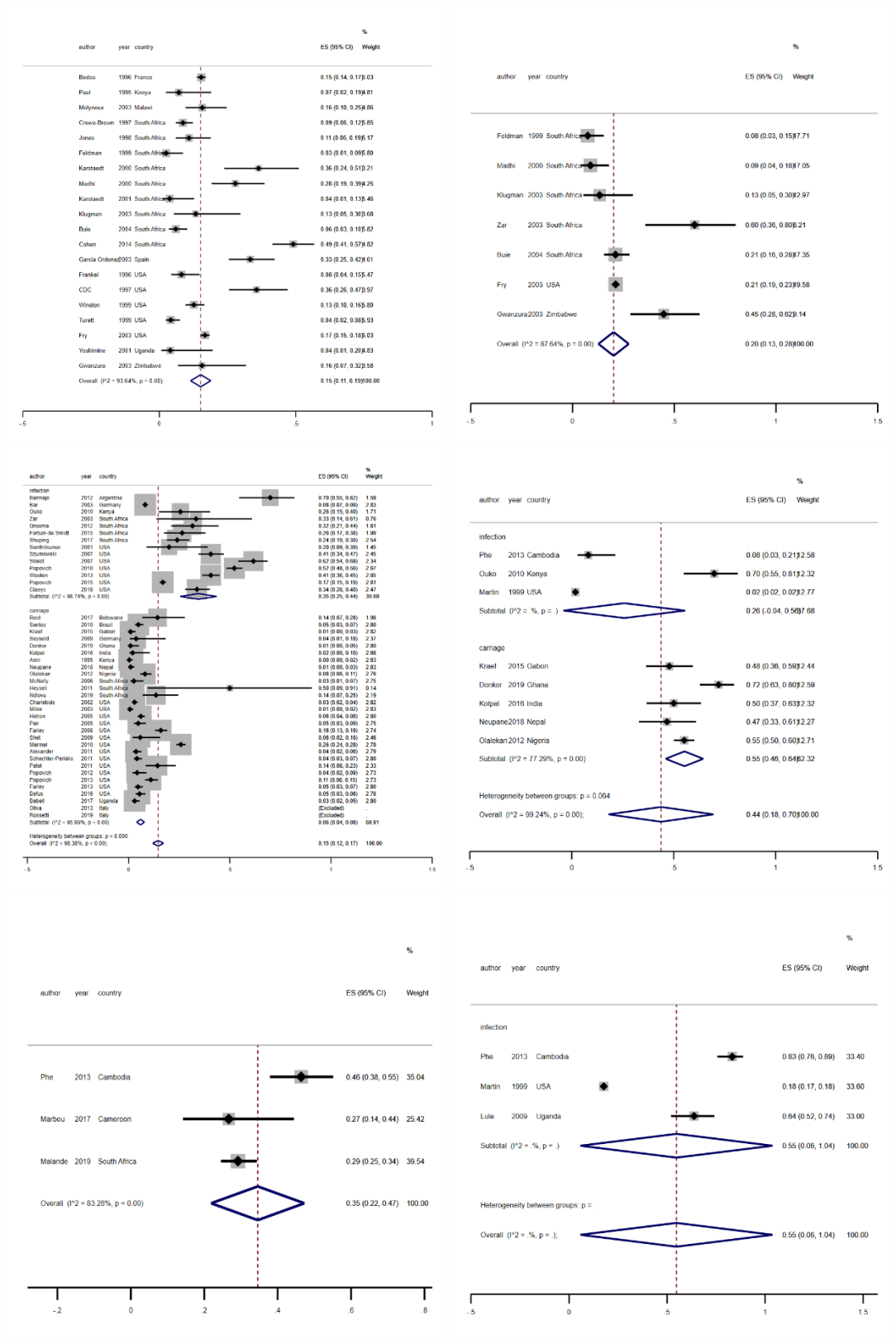
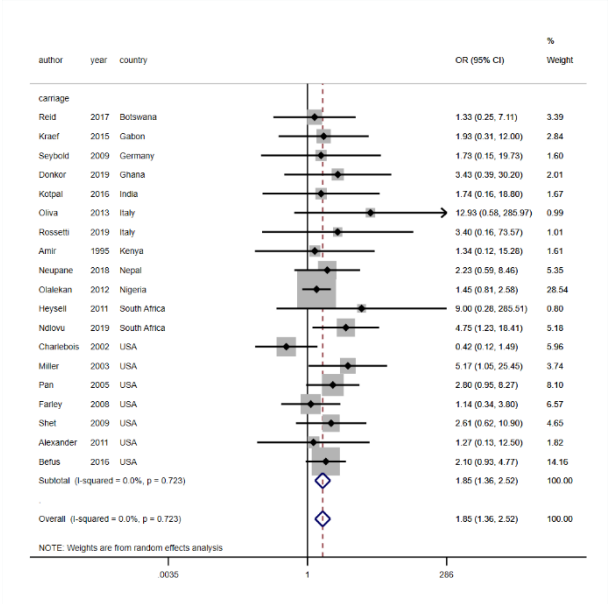
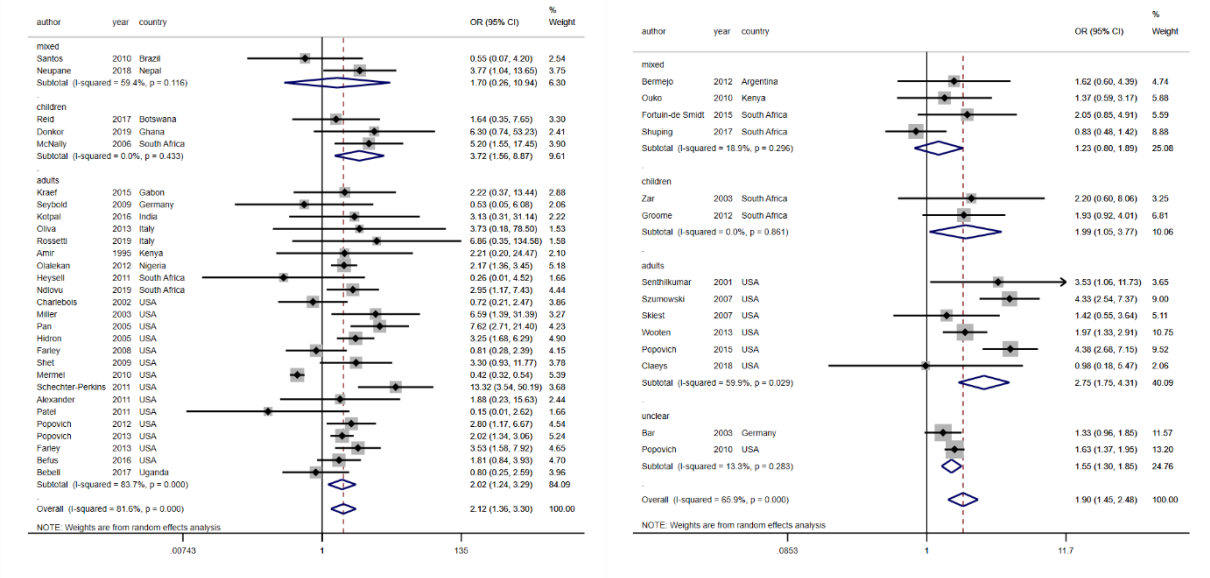


Figure S4 Carriage of MRSA among individuals with *S. aureus* colonization



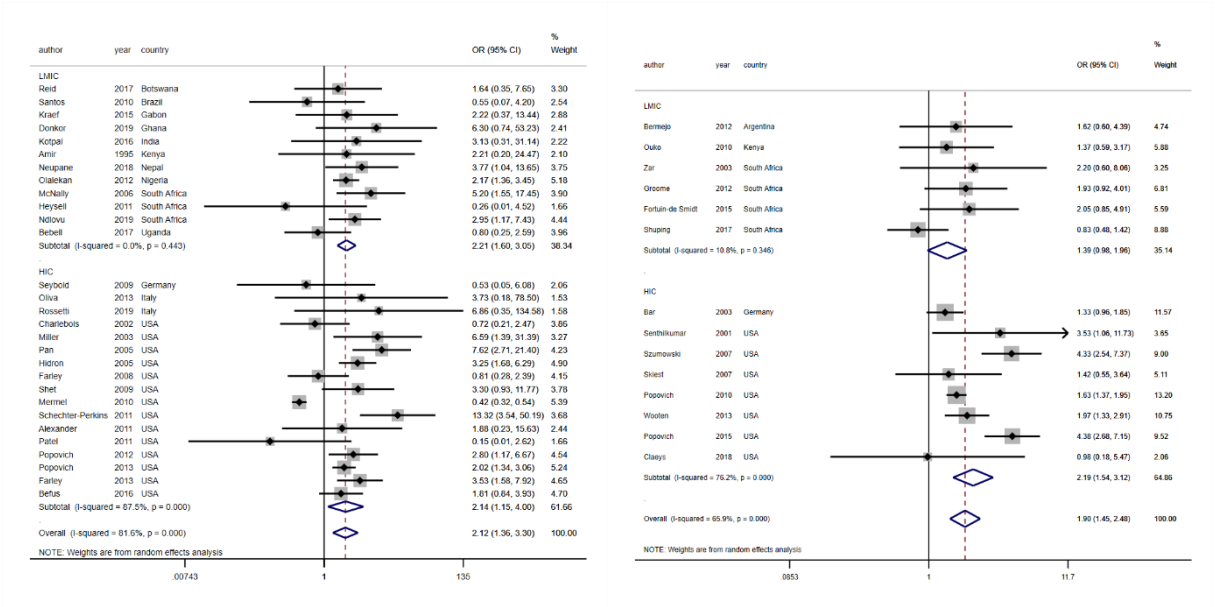
Because of the use of selective methods for resistance testing (e.g. chromogenic media or molecular methods), colonisation with MRSA was evaluated using also the number with S. aureus colonisation as a denominator. OR: odds ratio

Figure S5. MRSA carriage (left) and infection (right) according to age



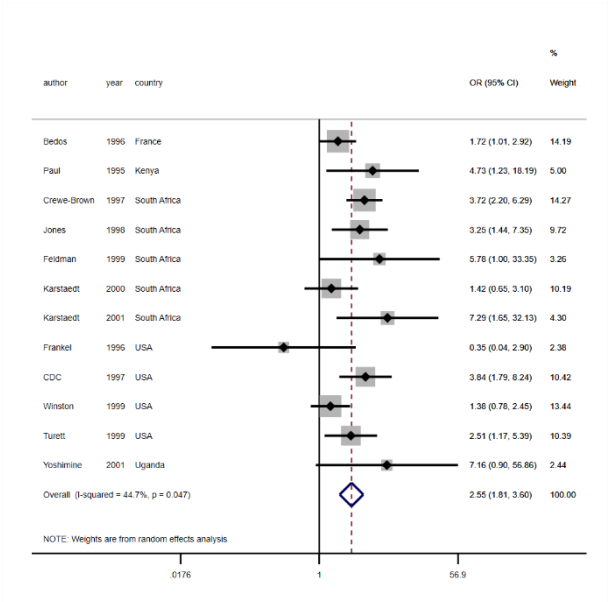
OR: odds ratio

Figure S6. MRSA carriage (left) and infection (right) according to country income



OR: odds ratio

Figure S7. Penicillin non-susceptibility of *S. pneumoniae* in studies that describe performing MICs for penicillin



OR: odds ratio

Table S4. Effect of different variables on the association between HIV and MRSA for explaining heterogeneity of studies.

Variable potentially explaining heterogeneity	**Between study variability τ^2; relative reduction**	**Residual I^2**
None	0.4187	96.57%
Year of publication	0.4305, -2.81%	95.52%
Continent of study	0.4313, -2.99%	96.64%
Country income (LMIC vs. HIC)	0.4159, 0.67%	96.37%
Study design	0.4898, 1.85%	94.66%
Data collection (prospective vs. retrospective)	0.4276, 0.54%	95.39%
Patient type (inpatients vs. outpatients)	0.1327, 7.95%	60.04%
Patient age (children vs. adults)	0.4027, -3.77%	82.36%
Isolate type (infection vs. carriage)	0.4294, -2.54%	95.37%

Only patient type can explain some of the heterogeneity between studies with most of the heterogeneity remaining unexplained. LMIC: low- and middle-income countries; HIC: high-income countries.

Table S5. Effect of different variables on the association between HIV and penicillin-nonsusceptible *S. pneumoniae* for explaining heterogeneity of studies.

Variable potentially explaining heterogeneity	**Between study variability τ^2; relative reduction**	**Residual I^2**
None	0.1355	59.47%
Year of publication	0.1461, -7.81%	60.18%
Continent of study	0.1102, 18.66%	52.64%
Country income (LMIC vs. HIC)	0.0679, 49.88%	35.30%
Study design	0.1985, -12.29%	67.57%
Data collection (prospective vs. retrospective)	0.1494, -10.21	60.72%
Patient age (children vs. adults)	0.1566, -22.47%	54.70%

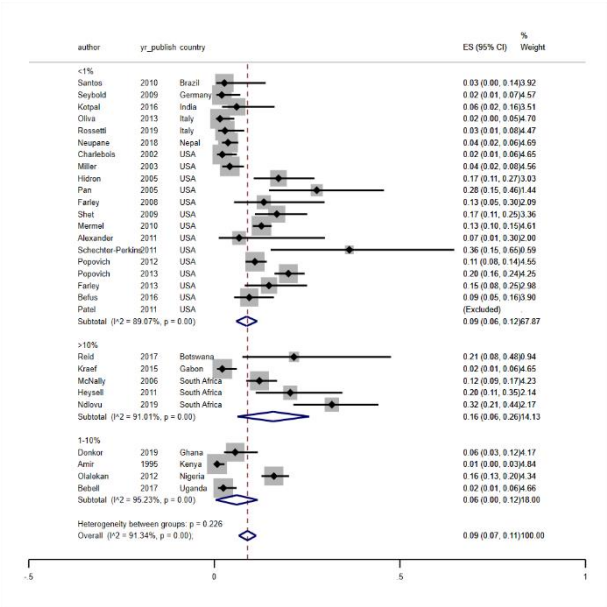
Country income and continent of study can explain some of the heterogeneity between studies. LMIC: low- and middle-income countries; HIC: high-income countries.

Table S6. Subgroup meta-analyses for *S. aureus* and *S. pneumoniae*

Resistance/ organism	Category	Type	Studies	OR (95% CI)
Methicillin resistant *S. aureus*	All studies	Carriage	29	2.12 (1.36-3.30)
		Infection	14	1.90 (1.45-2.48)
	Pre-ART	Carriage	2	4.37 (1.48-12.90)
		Infection	2	2.84 (1.18-6.85)
	ART	Carriage	27	2.04 (1.29-3.22)
		Infection	11	1.88 (1.40-2.53)
	Published since 2015	Carriage	9	2.17 (1.41-3.33)
		Infection	4	1.75 (0.65-4.69)
	LMIC	Carriage	12	2.21 (1.60-3.05)
		Infection	6	1.39 (0.98-1.96)
	HIC	Carriage	17	2.14 (1.15-4.00)
		Infection	8	2.19 (1.54-3.12)
	CD4 <200	Carriage	2	1.02 (0.13-7.76)
		Infection	3	1.67 (1.41-1.99)
	CD4 ≥200	Carriage	11	2.42 (1.78-3.28)
		Infection	3	3.67 (2.31-5.85)
	ART <50%	Carriage	4	3.33 (1.67-6.67)
		Infection	1	3.53 (1.06-11.73)
	ART ≥50%	Carriage	6	2.99 (1.65-5.43)
		Infection	2	3.24 (1.58-6.62)
Penicillin non-susceptible *S. pneumoniae*	All studies	Infection	20	2.28 (1.75-2.97)
	Pre-ART	Infection	18	2.39 (1.78-3.22)
	ART	Infection	2	1.82 (1.09-3.03)
	Published after 2010	Infection	1	2.14 (1.27-3.59)
	LMIC	Infection	13	2.74 (2.14-3.49)
	HIC	Infection	7	1.60 (1.10-2.32)
	CD4 <200	Infection	1	2.51 (1.17-5.39)
	CD4 ≥200	Infection	4	3.64 (1.21-10.90)

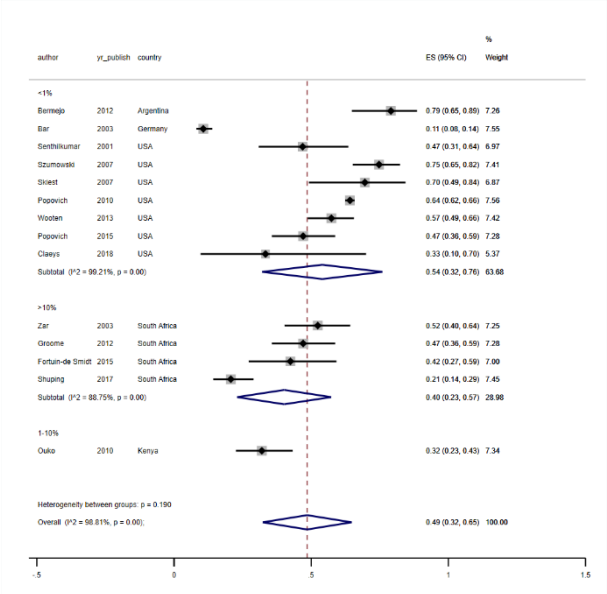
For one study it was unclear if it was conducted pre-ART or when ART was widely used; ART: antiretroviral therapy, HIC: high-income countries; LMIC: low- and middle-income countries; OR: odds ratio.

Figure S8. Carriage of methicillin-resistant *S. aureus* according to HIV prevalence in the country where the study was conducted. HIV prevalence (headings) was categorised in <1%, between 1% and 10% and >10%



HIV prevalence is for the age group 15-49 and for the years when the study was conducted.

Figure S9. Infection with methicillin-resistant *S. aureus* according to HIV prevalence in the country where the study was conducted. HIV prevalence (headings) was categorised in <1%, between 1% and 10% and >10%



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[3] Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available from: http://www.ohri.ca/programs/clinicalepidemiology/oxford.asp.

Table S3. Main characteristics of studies reporting on antimicrobial resistance in bacterial pathogens isolated from HIV-positive and HIV negative individuals

Author (year) [Ref]	Country	Years of data collection	Data collection	Study design	Study population	Setting of infection	Age category	Organism	Isolate type	Isolate site	Number of participants	% HIV+ on SXT prophylaxis	% HIV+ on ART
Alexander (2011) [1]	USA	2006-2007	Prospective	Cohort	Haemodialysis patients and controls	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 15 HIV- 301	ND	ND
Amir (1995) [2]	Kenya	1992	Prospective	Cross-sectional	Inpatients+ outpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 264 HIV- 290	ND	ND
Bebell (2017) [3]	Uganda	2015	Prospective	Cross-sectional	Inpatients+ outpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 166 HIV- 334	ND	ND
Befus (2016) [4]	USA	2009-2011	Prospective	Cross-sectional	Incarcerated	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 117 HIV- 351	ND	ND
Charlebois (2002) [5]	USA	1999-2000	Prospective	Cross-sectional	Community (urban poor)	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 141 HIV- 686	ND	ND
Donkor (2019) [6]	Ghana	2017	Prospective	Case-control	Outpatients and community controls	NA	Children	*S. aureus*	Carriage	Nasal	HIV+ 107 HIV- 107	89%	90%
Farley (2008) [7]	USA	2006	Prospective	Cross-sectional	Newly arrested men	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 30 HIV- 572	ND	ND
Farley (2013) [8]	USA	2009-2010	Prospective	Cohort	Psychiatric inpatients	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 68 HIV- 430	ND	ND
Heysell (2011) [9]	South Africa	2008	Prospective	Cohort	Inpatients with TB	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 44 HIV- 2	60%	ND
Hidron (2005) [10]	USA	2003	Unclear	Case-control	Inpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 81 HIV- 645	ND	ND
Kotpal (2016) [11]	India	2010	Prospective	Case-control	Outpatients and controls	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 50 HIV- 50	0%	0%
Kraef (2015) [12]	Gabon	2013	Prospective	Case-control	Outpatients+ healthy community controls	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 141 HIV- 206	34%	35%
McNally (2006) [13]	South Africa	2001-2002	Prospective	Cross-sectional	Inpatients	NA	Children	*S. aureus*	Carriage	Nasal	HIV+ 239 HIV- 116	ND	ND
Mermel (2010) [14]	USA	2006-2007	Prospective	Cross-sectional	Inpatients+ outpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 655 HIV- 1400	ND	ND
Miller (2003) [15]	USA	1999-2000	Prospective	Cross-sectional	Outpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 193 HIV- 307	ND	24%
Ndlovu (2019) [16]	South Africa	2012-2015	Prospective	Cohort	Inpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 60 HIV- 59	ND	ND
Neupane (2018)	Nepal	2016	Prospective	Cross-	Unclear	NA	All ages	*S. aureus*	Carriage	Nasal	HIV+ 300	ND	ND

[17]				sectional							HIV- 300		
Olalekan (2012) [18]	Nigeria	2008-2010	Prospective	Case-control	Outpatients and healthy controls	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 374 HIV- 370	ND	ND
Oliva (2013) [19]	Italy	2008-2012	Retrospective	Cross-sectional	Outpatient	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 131 HIV- 96	16%	98%
Pan (2005) [20]	USA	2002	Prospective	Cross-sectional	Centres and clinics	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 29 HIV- 210	ND	ND
Patel (2011) [21]	USA	2007-2008	Prospective	Cohort	Outpatients on dialysis	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 19 HIV- 84	ND	ND
Popovich (2012) [22]	USA	2010-2011	Prospective	Cross-sectional	Outpatients	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 458 HIV- 143	86%	20%
Popovich (2013) [23]	USA	2011-2012	Prospective	Cross-sectional	Inpatients	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 374 HIV- 371	ND	ND
Reid (2017) [24]	Botswana	2013	Prospective	Cross-sectional	Outpatients	NA	Children	*S. aureus*	Carriage	Nasal	HIV+ 14 HIV- 42	ND	100%
Rossetti (2019) [25]	Italy	2011-2012	Prospective	Cohort	unclear	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 105 HIV- 100	ND	99%
Santos (2010) [26]	Brazil	2006-2007	Prospective	Cohort	Inpatients	NA	All ages	*S. aureus*	Carriage	Nasal	HIV+ 37 HIV- 436	ND	ND
Schechter-Perkins (2011) [27]	USA	2009-2010	Prospective	Cross-sectional	Outpatient	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 11 HIV- 389	ND	ND
Seybold (2009) [28]	Germany	2006-2007	Unclear	Cross-sectional	Outpatient	NA	Adults	*S. aureus*	Carriage	Nasal	HIV+ 100 HIV- 27	ND	ND
Shet (2009) [29]	USA	2005-2006	Prospective	Case-control	Outpatients and healthy controls	NA	Adults	*S. aureus*	Carriage	Multiple	HIV+ 107 HIV- 52	ND	69%
Bär (2003) [30]	Germany	1995-2000	Unclear	Cross-sectional	Unclear	Unclear	Unclear	*S. aureus*	Infection	SSTI	HIV+ 432 HIV- 3668	ND	ND
Bermejo (2012) [31]	Argentina	2009-2011	Prospective	Cross-sectional	Outpatients	CA	All ages	*S. aureus*	Infection	SSTI	HIV+ 43 HIV- 40	16%	ND
Claeys (2018) [32]	USA	2012-2015	Retrospective	Cross-sectional	Unclear	Unclear	Adults	*S. aureus*	Infection	SSTI	HIV+ 6 HIV- 225	ND	ND
Delorenze (2013) [33]	USA	1995-2010	Retrospective	Cohort	Inpatients+ outpatients	55% CA	All ages	*S. aureus*	Infection	Mixed	HIV+ 14,060 HIV- 6,597,396[#]	ND	78%
Farr (2012) [34] [#]	USA	1997-2006	Retrospective	Cross-sectional	Inpatients	CA	All ages	*S. aureus*	Infection	Mixed	HIV+ 4074 HIV- 10,538,033[#]	ND	ND
Fortuin-de Smidt (2015) [35]	South Africa	2012-2013	Retrospective	Cross-sectional	Inpatients	Most HA	All ages	*S. aureus*	Infection	Blood	HIV+ 33 HIV- 68	ND	59%
Groome (2012) [36]	South Africa	2005-2006	Retrospective	Cross-sectional	Inpatients	CA	Children	*S. aureus*	Infection	Blood	HIV+ 68 HIV- 57	ND	ND
Ouko (2010) [37]	Kenya	NA	Prospective	Cross-sectional	Inpatients	HA	All ages	*S. aureus*	Infection	Mixed	HIV+ 75 HIV- 43	ND	ND
Popovich (2010) [38]	USA	2000-2007	Retrospective	Cohort	Inpatients+ outpatients	CA	Unclear	*S. aureus*	Infection	SSTI	HIV+ 2939 HIV- 601	34%	ND
Popovich (2015) [39]	USA	2006-2011	Retrospective	Case-control	Incarcerated	Unclear	Adults	*S. aureus*	Infection	Mixed	HIV+ 68 HIV- 2122	ND	ND

Senthilkumar (2001) [40]	USA	1994-1997	Retrospective	Cross-sectional	Inpatients	80% CA	Adults	*S. aureus*	Infection	Blood	HIV+ 32 HIV- 25	40%	ND
Shuping (2017) [41]	South Africa	2014	Retrospective	Cross-sectional	Inpatients	HA	All ages	*S. aureus*	Infection	Blood	HIV+ 116 HIV- 229	ND	ND
Skiest (2007) [42]	USA	2003	Prospective	Cross-sectional	Inpatients	CA and HCA	Adults	*S. aureus*	Infection	Mixed	HIV+ 23 HIV- 167	ND	ND
Szumowski (2007) [43]	USA	1998-2005	Retrospective	Cross-sectional	Outpatient	CA	Adults	*S. aureus*	Infection	SSTI	HIV+ 95 HIV- 229	ND	61%
Wooten (2013) [44]	USA	2005-2010	Retrospective	Case-control	Inpatients	63% hospital onset	Adults	*S. aureus*	Infection	Respiratory samples	HIV+ 129 HIV- 490	ND	ND
Rodriguez-Barradas (1997) [45]	USA	1994	Prospective	Case-control	Unclear	NA	Adults	*S. pneumoniae*	Carriage	Nasal and pharyngeal	HIV+ 39 HIV- 103	ND	ND
Aspa (2008) [46]	Spain	1999-2000	Retrospective	Cross-sectional	Inpatients	CA	Adults	*S. pneumoniae*	Infection	Mixed	HIV+ 61 HIV- 577	ND	ND
Bedos (1996) [47]	France	1991-1992	Retrospective	Cross-sectional	Inpatients	Not specified (lab survey)	All ages	*S. pneumoniae*	Infection	Mixed	HIV+ 80 HIV- 1846	ND	ND
Buie (2004) [48]	South Africa	1996-2002	Retrospective	Cross-sectional	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 785 HIV-199	ND	ND
CDC (1997) [49]	USA	1995	Retrospective	Cross-sectional	Not specified	ND	All ages	*S. pneumoniae*	Infection	Mixed	HIV+ 50 HIV- 73	42%	ND
Cohen (2014) [50]	South Africa	2010-2012	Prospective	Case-control	Inpatients	ND	Children	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 101 HIV- 161	47%	26%
Cornick (2011) [51]	Malawi	2004-2006	Retrospective	Cross-sectional	Inpatients	ND	Children	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 100 HIV- 71	ND	ND
Crewe-Brown (1997) [52]	South Africa	1993-1995	Retrospective	Cross-sectional	Inpatients	Predominant CA	All ages	*S. pneumoniae*	Infection	Blood	HIV+ 165 HIV- 312	ND	ND
Crowther-Gibson (2012) [53]	South Africa	2003-2008	Retrospective	Cross-sectional	Not specified	ND	All ages	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 4636 HIV- 1028	ND	ND
Feldman (1999) [54]	South Africa	not specified	Retrospective	Cross-sectional	inpatients	ND	Adults	*S. pneumoniae*	Infection	Blood	HIV+ 31 HIV- 80	ND	ND
Frankel (1996) [55]	USA	1992-1993	Retrospective	Cross-sectional	Inpatients	ND	All ages	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 33 HIV- 111	27%	ND
Fry (2003) [56]	USA	1998-1999	Retrospective	Cross-sectional	Not specified	ND	Adults	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 416 HIV- 1930	ND	ND
Garcia Ordonez (2003) [57]	Spain	1995-2000	Retrospective	Cross-sectional	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Blood	HIV+ 24 HIV- 117	ND	ND
Gwanzura (2003) [58]	Zimbabwe	1994-2000	Retrospective	Cross-sectional	Inpatients	ND	All ages	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 92 HIV- 32	ND	ND
Isea-Pena (2013) [59]	Spain	2007-2011	Retrospective	Cross-sectional	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 24 HIV- 98	ND	ND
Jones (1998) [60]	South Africa	1996	Retrospective	Cross-sectional	Inpatients	ND	All ages	*S. pneumoniae*	Infection	Blood	HIV+ 81 HIV- 92	ND	ND
Karstaedt (2000) [61]	South Africa	1986/1996	Retrospective	Cohort	Inpatients	ND	Children	*S. pneumoniae*	Infection	Blood	HIV+ 67 HIV- 44	19%	ND
Karstaedt	South	1986/1996	Retrospective	Cohort	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Blood	HIV+ 108	0%	ND

(2001) [62]	Africa										HIV- 53		
Klugman (2003) [63]	South Africa	1998-2000	Prospective	RCT	Inpatients	ND	Children	*S. pneumoniae*	Infection	Mixed	HIV+ 69 HIV- 30	ND	ND
Madhi (2000) [64]	South Africa	1997-1999	Retrospective	Cross-sectional	Inpatients	ND	Children	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 146 HIV- 79	ND	ND
Mollendorf (2014) [65]	South Africa	2003-2010	Retrospective	Cross-sectional	Not specified	4% nosocomial	All ages	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 1493 HIV- 590	ND	ND
Paul (1995) [66]	Kenya	1992	Prospective	Cross-sectional	Inpatients	CA	Adults	*S. pneumoniae*	Infection	Mixed, invasive	HIV+ 45 HIV- 42	ND	ND
Stephen (2008) [67]	Germany	2001-2005	Retrospective	Cross-sectional	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Mixed	HIV+ 64 HIV- 71	ND	ND
Turett (1999) [68]	USA	1992-1996	Retrospective	Cohort	Inpatients	ND	All ages	*S. pneumoniae*	Infection	Blood	HIV+ 233 HIV- 239	24%	2%
Winston (1999) [69]	USA	1994-1996	Retrospective	Case-control	Inpatients	ND	Adults	*S. pneumoniae*	Infection	Mixed	HIV+ 120 HIV- 356	ND	ND
Yoshimine (2001) [70]	Uganda	1996-1998	Prospective	Cohort	Inpatients	CA	Adults	*S. pneumoniae*	Infection	Mixed	HIV+ 74 HIV- 25	ND	ND
Hosuru Subramanya (2019) [71]	Nepal	2016-2017	Prospective	Case-control	Outpatients and healthy controls	NA	All ages	ESBL-enterobacteria	Carriage	Stool	HIV+ 119 HIV- 357	ND	ND
Tellevik (2016) [72]	Tanzania	2010-2011	Prospective	Case-control	Inpatients+ outpatients	NA	Children	Enterobacteria	Carriage	Stool	HIV+ 29 HIV- 319	ND	ND
Nelson (2014) [73]	Tanzania	2013	Prospective	Cross-sectional	Inpatients	NA	Adults	Enterobacteria	Carriage	Stool	HIV+ 13 HIV- 100	ND	ND
Reinheimer (2017) [74]	Germany	2014-2016	Retrospective	Case-control	Inpatients	NA	Adults	*E. coli*	carriage	Stool	HIV+ 109 HIV- 109	ND	ND
Abebe (2014) [75]	Ethiopia	2013	Prospective	Cross-sectional	Outpatients	NA	Adults	Enterococci	Carriage	Stool	HIV+ 103 HIV- 98	ND	99%
Ali (2018) [76]	Ethiopia	2017	Prospective	Cross-sectional	Outpatients	NA	Adults	Enterococci	Carriage	Stool	HIV+ 150 HIV- 150	ND	ND
Buys (2016) [77]	South Africa	2006-2011	Retrospective	Cross-sectional	Inpatients	86% HA, HCA 9%, 5% CA	Children	*K. pneumoniae*	Infection	Blood	HIV+ 82 HIV- 206	46%	ND
Malande (2019) [78]	South Africa	2005-2014	Retrospective	Cross-sectional	Inpatients	45% CA	Children	*E. coli*	Infection	Blood	HIV+ 74 HIV- 329	ND	ND
Marbou (2017) [79]	Cameroon	not specified	Prospective	Cross-sectional	Unclear	ND	Adults	*E. coli*	Infection	Stool	HIV+ 58 HIV- 30	ND	ND
Akullian (2018) [80]	Kenya	2007-2014	Prospective	Cohort	Inpatients+ outpatients	CA	All ages	*NTS*	Infection	Blood and stool	HIV+ 50 HIV- 253	ND	ND
Keddy (2016) [81]	South Africa	2003-2013	Unclear	Cohort	Unclear	14% nosocomial, rest CA	All ages	*NTS*	Infection	Sterile specimens	HIV+ 1900 HIV- 258	31%	20%
Luvsansharav (2020) [82]	Kenya	2009-2014	Mixed	Cross-sectional	Inpatients + outpatients	unclear	Children	*NTS*	Infection	Blood	HIV+16 HIV- 70	ND	ND
Phu (2016) [83]	Vietnam	2008-2013	Retrospective	Cohort	Inpatients	ND	Adults	*NTS*	Infection	Blood	HIV+ 62 HIV- 28	8%	22%
Bhavnani	USA	1995-1997	Retrospective	Case-	Inpatients	15% CA	Adults	*Enterococci*	Infection	Blood	HIV+ 5	ND	ND

(2000) [84]				control							HIV- 295		
Murray (2017) [85]	USA	2013-2015	Retrospective	Cross-sectional	Unclear	ND	All ages	*Shigella*	Infection	Not specified	HIV+ 108 HIV- 451	ND	ND
Kownhar (2007) [86]	India	2001-2004	Prospective	Case-control	Unclear	ND	Adults	*Campylobacter spp., Shigella spp.*	Infection	Stool	HIV+ 37 HIV- 13	ND	ND
Lule (2009) [87]	Uganda	2001-2002	Prospective	Cohort	Community	ND	Adults	*E. coli, Aeromonas, Shigella, Salmonella, Campylobacter*	Infection	Stool	HIV+ 185 HIV- 133	ND	ND
Martin (1999) [88]	USA	1988-1995	Retrospective	Cross-sectional	Inpatients+ outpatients	ND	All ages	*E. coli, K. pneumoniae, S. aureus, Enterobacter, Morganella, Proteus, Serratia.*	Infection	Not specified	HIV+ 1399 HIV- 23251	ND	ND
Molyneux (2003) [89]	Malawi	1997-2001	Prospective	RCT	Inpatients	ND	Children	*S. pneumoniae, H. influenzae*	Infection	CSF	HIV+ 121 HIV- 188	ND	ND
Phe (2013) [90]	Cambodia	2009-2011	Retrospective	Cross-sectional	Unclear	CA	Adults	*E. coli, S. aureus, S.* Typhi, *NTS*	Infection	Blood	HIV+ 57 HIV- 190	26%	60%
Zar (2003) [91]	South Africa	1998	Prospective	Cross-sectional	Inpatients	CA	Children	*K. pneumoniae, S. aureus, S. pneumoniae, H. influenzae*	Infection	Respiratory samples	HIV+ 145 HIV- 76	67%	ND
Wolday (1998) [92]	Ethiopia	1991-1995	Retrospective	Cross-sectional	Unclear	ND	Adults	*S.* Typhi, NTS	Infection	Mostly blood, but also urine and stool	HIV+ 27 HIV- 22	ND	ND

Bar et al. excluded organisms which are not common causes of SSTI and were likely wound colonizers (e.g. E. coli, P aeruginosa, CNS), the streptococcal species was not specified for most isolates and only a small percentage was S. pyogenes; #Delorenze et al. and Farr et al. did not report on the numbers of S. aureus infections but the total number of individuals who were health plan members or hospitalized. There studies were not included in the meta-analysis. CA: community acquired, CSF: cerebrospinal fluid; ESBL: extended-spectrum beta-lactamases; HA: hospital associated, HCA: healthcare associated; NA: not applicable; ND: not described; NTS: non-typhoid salmonellae, SSTI: skin and soft tissue infection; SXT: co-trimoxazole; TB: tuberculosis.

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APPENDIX 2

Supplementary materials for Chapter 2 (published paper)

Prevalence of extended-spectrum beta-lactamase producing *Escherichia coli* in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe

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Supplementary material

TABLE OF CONTENTS

Laboratory methods and sample processing

Urine samples underwent dipstick, microscopy and culture on the day of collection. Urine dipstick was considered positive if either nitrites or leucocytes were present. Leukocyturia was considered to be present if the sample had ≥10 white blood cells/µl on microscopy.

A volume of 1µL of the sample was inoculated on chromogenic agar (Brilliance UTI agar, Oxoid, UK) and incubated at 37°C for 24 hours. Urine cultures were considered positive if there was growth of $\geq 10^3$ colony forming units (cfu)/mL. This threshold for positivity was used given that lower bacterial counts can be significant in patients with UTI symptoms.[13] Cultures were considered contaminated if growth of a non-uropathogen was present or if ≥2 organisms were isolated in the absence of a clear predominance of one organism. To minimise contamination, samples were refrigerated if a prolonged transportation time was anticipated.

E. coli was identified by colony appearance on chromogenic media while other *Enterobacterales* were identified using APIs (Analytical Profile Index, bioMérieux, France). This strategy may have led to the misidentification of a small number of *Citrobacter spp.*, as *E. coli.* However, it is anticipated that misidentification was infrequent and it would not have impacted the overall study results. The identity of presumptive *E. coli* isolates showing discoloration on chromogenic media was confirmed using APIs.

Drugs used for AST are shown in Table S1. Fosfomycin AST was done for *E. coli* using disc diffusion testing. Fosfomycin AST for other *Enterobacterales* requires determination of minimum inhibitory concentrations which was not available.

Table S1 Antimicrobial susceptibility testing according to pathogen

Organism	**Drugs tests for AST[#]**
E. coli	Ampicillin, Amoxicillin/ clavulanic acid, cefpodoxime, ceftazidime, ceftriaxone, cefoxitin, imipenem, ciprofloxacin, amikacin, gentamicin, co-trimoxazole, chloramphenicol, nitrofurantoin, fosfomycin
Other *Enterobacterales*	Ampicillin, Amoxicillin/ clavulanic acid, cefpodoxime, ceftazidime, ceftriaxone, cefoxitin, imipenem, ciprofloxacin, gentamicin, co-trimoxazole, chloramphenicol, nitrofurantoin
Enterococcus spp.	Ampicillin, ciprofloxacin, nitrofurantoin
Staphylococcus saprophyticus	Cefoxitin, ciprofloxacin, nitrofurantoin, co-trimoxazole

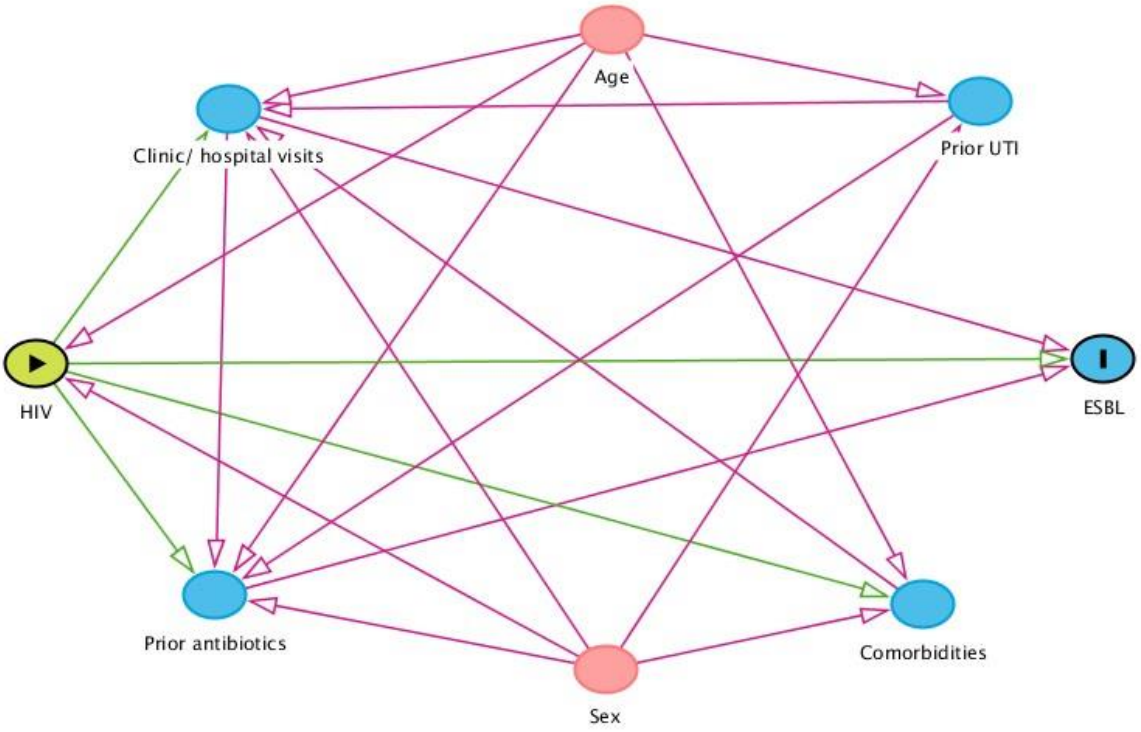
[#]Internal quality control for AST was conducted and interpreted in accordance to EUCAST recommendations (Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST Version 9.0, valid from 2019-01-01, www.eucast.org)

Testing for ESBL and AmpC production was performed according to EUCAST recommendations.[15] Briefly, screening for the presence of ESBLs was done using cefpodoxime. Isolates positive on the screening test underwent confirmation by synergy testing with amoxicillin/clavulanic acid and ceftazidime. Screening for AmpC was performed using cefoxitin and ceftazidime and was confirmed by cloxacillin synergy testing.[15]
Quality of laboratory testing was ensured by using standard operating procedures, training and regular re-training on laboratory procedures and supervision by a senior clinical microbiologist.

Sample size and statistical analysis

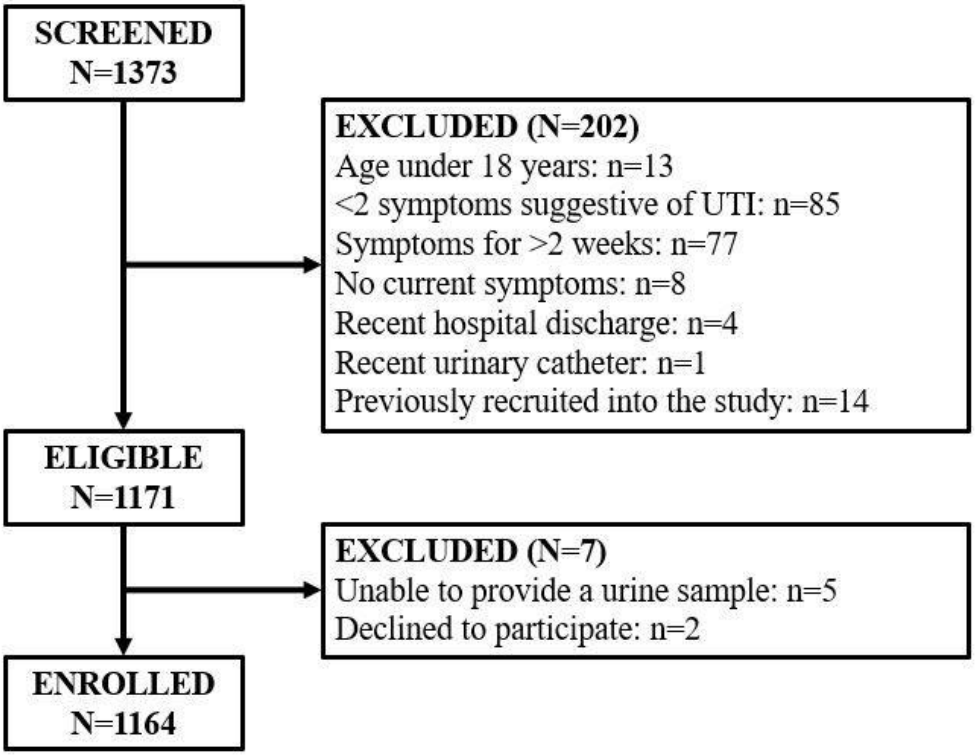
Sample size calculations were conducted for the association between HIV status and infection with ESBL-*E. coli*. The following was assumed: i) 30% of urine cultures positive, ii) 90% of organism *E. coli*, iii) 25% of participants infected with HIV, 15% of ESBL-*E. coli* among HIV uninfected and 30% among HIV-infected participants. A sample size of 1404 would detect a difference with 80% power and a level of significance of 5%.
For the association between HIV infection and infection with *ESBL-E. coli*, a multivariate analysis using logistic regression was performed. Directed acyclical graphs were used to establish variables on the causal pathway between the exposure and the outcome and these were not adjusted for in the multivariate model (Figure S1. Variables that were associated with the exposure of interest (HIV infection) and with the outcome (infection with ESBL-*E. coli*) were included in the multivariate model.

Supplementary Figure 1 Directed acyclic graph for the relationship between HIV infection (exposure) and infection with ESBL-*E. coli* (outcome). Variables in red are potential confounders while the green arrows show a causal path. Variables in blue are ancestors of the outcome.



Clinic/ hospital visits, prior antibiotics (including co-trimoxazole prophylaxis) and comorbidities are on the causal pathway between the exposure of interest (HIV infection) and the outcome (infection with ESBL-producing organisms). These variables cannot be considered confounders of the association. The diagram was created using www.dagitty.net.

Supplementary Figure 2. Flow diagram of patients included in the study



The study was unable to recruit to the planned sample size because of suspension of research activities during the national COVID-19 lockdown (April-May 2020) and because recruitment had to be stopped earlier than anticipated (July 2020) due to COVID-19-related clinic closures.

Urine dipstick results

A positive urine dipstick was recorded in 411 (35.4%) and 321 (27.6%) had positive urine microscopy for leucocytes. Overall, the urinary dipstick had a sensitivity of 71% (95% CI 66-76) and a specificity of 80% (95%CI 77-83) for predicting a positive urine culture (Table S2). There was a good concordance between microscopy and dipstick leucocyte esterase results of 88.9% (1033/1162).

Supplementary Table 2. Bacterial growth in urine cultures and urine dipstick results according to HIV status.

Patient category	Leucocyte esterase positive N (%)	Nitrite positive N (%)	Combined leucocyte esterase or nitrite positive	
			Sensitivity	**Specificity**
HIV negative (n=678)			69% (62-76)	81% (77-84)
Culture results				
Enterobacterales (n=169)	123 (73)	72 (43)		
Gram-positive bacteria (n=23)	8 (35)	0 (0)		
Contamination (n=42)	14 (32)	1 (2)		
Culture negative (n=444)	84 (19)	6 (1)		
HIV positive (n=387)			68% (59-77)	81% (76-86)
Culture results				
Enterobacterales (n=91)	69 (76)	34 (37)		
Gram-positive bacteria (n=19)	5 (26)	0 (0)		
Contamination (n=18)	5 (28)	2 (11)		
Culture negative (n=259)	50 (19)	4 (2)		

Dipstick could not be performed in 2 patients because of sample spillage during transportation. For the sensitivity and specificity calculations, contaminated cultures were excluded.

Prevalence of resistance

The prevalence of resistance among the isolated bacterial species are shown in Table S3. For *E. coli* prevalence with 95% CIs are shown in Table S4.

Supplementary Table 3. Antimicrobial resistance according to bacterial species isolated from urine samples from individuals presenting with symptoms of urinary tract infection to public health clinics in Harare, Zimbabwe (percentages are shown in brackets)

	AMP	AMC	CRO	IMP	CIP	GENT	NIT	FOS	SXT	CHL
E. coli (n=254)	209 (82)	104 (41)	46 (18)	0 (0)	57 (22)	42 (17)	14 (6)	5 (2)	223 (88)	28 (11)
Other *Enterobacterales (n=39)*	-	13 (34)	6 (16)	0 (0)	2 (5)	4 (11)	14 (37)	-	23 (61)	4 (11)
Enterococcus spp. (n=40)	5 (28)	-	-	-	7 (18)	-	4 (10)	-	-	-

AMP: ampicillin; AMC: amoxicillin/ clavulanic acid; CRO: ceftriaxone; IMP: imipenem; CIP: ciprofloxacin; GENT: gentamicin; NIT: nitrofurantoin; FOS: fosfomycin; SXT: co-trimoxazole; CHL: chloramphenicol. Other Enterobacterales were: Klebsiella pneumoniae (n=20); Enterobacter spp. (n=6); Proteus mirabilis (n=6); Citrobacter spp. (n=2); Klebsiella oxytoca (n=1); not identified (n=4 – these showed growth of blue colonies on the Brilliance UTI agar). Missing: AST not done (n=2, K. pneumoniae and Enterococcus spp.); AST for ciprofloxacin missing (n=1, E. aerogenes); AST for ampicillin missing (n=21, Enterococcus spp.); AST for Fosfomycin missing (n=30, E. coli). S. saprophyticus (n=3) and S. aureus (n=2) AST not included.

Supplementary Table 4. Prevalence of resistance in *E. coli* with 95% confidence intervals.

Antibiotic	Percentage resistant (95%CI)
Amoxicillin	82% (77-87)
Amoxicillin/clavulanic acid	41% (35-47)
Ceftriaxone	18% (14-23)
Imipenem	0% (0-1)
Ciprofloxacin	22% (17-28)
Gentamicin	17% (12-22)
Amikacin	0% (0-2)
Co-trimoxazole	88% (83-92)
Chloramphenicol	11% (7-16)
Nitrofurantoin	6% (3-9)
Fosfomycin	2% (1-5)

AST is shown for all E. coli isolates (N=254) except for amikacin and fosfomycin where n=228 were tested.

Risk factor analysis and the association between HIV and infections with ESBL-*E. coli*

Characteristics of study participants with and without ESBL-*E. coli* infections are shown in Table S5. The univariate and multivariate analyses of the association between HIV and infection with ESBL-*E. coli* are shown in Table S6.

Supplementary Table 5. Characteristics of individuals presenting to public health clinics in Harare, Zimbabwe, with *E. coli* UTIs, stratified by presence of ESBL

Risk factors for resistance	ESBL-*E. coli* N=49	Non-ESBL *E. coli* N=205	p-value
Age (years), median (IQR)	37.3 (30.6-60.4)	31.8 (24.8-46.7)	0.016
Female sex	36 (73.5)	171 (83.4)	0.107
Pregnancy	3 (8.6)	17 (10.9)	0.685
HIV infection	22 (53.6)	60 (32.3)	0.010
Prior UTI	14 (30.4)	35 (17.4)	0.046
Prior antibiotic use (last 12 months)			
Any antibiotic	21 (44.7)	65 (32.3)	0.109
Amoxicillin	11 (22.5)	34 (16.6)	0.334
Fluoroquinolone	10 (20.4)	9 (4.4)	<0.001
Co-trimoxazole (incl. prophylaxis)	11 (22.5)	38 (18.5)	0.533
Prior hospital admission (last 12 months)	3 (6.4)	15 (7.5)	0.797

UTI: urinary tract infection. Missing: unknown HIV status (n=27); prior UTI (n=7); pregnancy status (n=16); age (n=1); previous hospital admission (n=6).

Supplementary Table 6. Univariate and multivariate analysis of the association between HIV and urinary tract infection with ESBL-*E. coli*

Risk factors for resistance	Unadjusted OR (95%CI)	p-value	Adjusted OR** (95% CI)	p-value
Age*	1.02 (1.00-1.04)	0.030	1.02 (0.99-1.04)	0.128
Female sex	0.80 (0.34-1.91)	0.621	1.16 (0.45-3.02)	0.755
HIV infection	2.43 (1.22-4.83)	0.011	2.13 (1.05-4.32)	0.036

**Age was included as a linear variable. There was no departure from a linear trend for the age variable. **The OR was adjusted for age and sex.*

APPENDIX 3

Supplementary materials for Chapter 6 (paper under review)

Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe

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Knowledge, attitudes and practices relating to antibiotic use and resistance among prescribers from public primary healthcare facilities in Harare, Zimbabwe

GENERAL INFORMATION	
Age	☐ <25 years ☐ 25-34 years ☐ 35-44 years ☐ 45-54 years ☐ ≥55 years
Gender	☐ Male ☐ Female
What type of healthcare facility do you work in?	☐ Public health facility only ☐ Private health facility only ☐ Both public and private ☐ Other (please specify): _____
What is your place of work? (tick **ALL** that apply)	☐ Primary care facility (polyclinic) ☐ Private practice (for outpatients) ☐ Hospital ☐ Other (please specify): _____
Level of training	☐ Student nurse ☐ Junior nurse ☐ Senior nurse ☐ Midwife ☐ Other (please specify): _____
Years of professional experience (after graduation)	☐ <5 years ☐ 5-10 years ☐ >10 years

DIAGNOSTICS

Approximately, how many times in the last month did you

Order/ perform a urine culture:	☐ Never	☐ 1-2	☐ 3-5	☐ 5-10	☐ >10
Order/ perform a stool culture:	☐ Never	☐ 1-2	☐ 3-5	☐ 5-10	☐ >10
Order/ perform a sputum test for TB:	☐ Never	☐ 1-2	☐ 3-5	☐ 5-10	☐ >10
☐ I was not working in a clinic during the previous month					

In your practice, what is the importance of the following problems in affecting <u>the availability of microbiology results</u> in your health facility?

	Very important	Important	Neutral	Un-important	Very un-important	Do not know
Microbiology lab is not available	☐	☐	☐	☐	☐	☐
Microbiology lab is often not taking samples	☐	☐	☐	☐	☐	☐
Most patients cannot afford the tests	☐	☐	☐	☐	☐	☐
Tests that I order are not being done	☐	☐	☐	☐	☐	☐
I do not receive the lab results back	☐	☐	☐	☐	☐	☐
Other (please specify)_____	☐	☐	☐	☐	☐	☐

In your practice, what is the importance of the following problems in affecting <u>the use of microbiology results</u> in your health facility?

	Very important	Important	Neutral	Un-important	Very un-important	Do not know
Nurses are confident with empirical treatment and do not need the microbiology results for guidance	☐	☐	☐	☐	☐	☐
Microbiology results are received late	☐	☐	☐	☐	☐	☐
Microbiology results are often unreliable	☐	☐	☐	☐	☐	☐
High patient workload	☐	☐	☐	☐	☐	☐
Microbiology samples are collected after antibiotics have been given	☐	☐	☐	☐	☐	☐
Other (please specify)_____	☐	☐	☐	☐	☐	☐

What antibiotic resistant organisms do you encounter in your practice (<u>select ALL that apply</u>)?

☐ MRSA
☐ ESBL
☐ Resistant *Salmonella* Typhi
☐ Drug-resistant TB
☐ Other (please specify): _____
☐ Do not know

ANTIBIOTIC PRESCRIBING

How many times do you prescribe antibiotics in a regular week

☐ 0 (did not prescribe) ☐ 1-2 ☐ 3-5 ☐ >5

What influences/ guides your prescribing decision in your routine practice (<u>select ALL that apply</u>)

☐ My own previous experience/ training
☐ Seeking advice from a senior colleague
☐ Use of national prescribing guidelines
☐ Unsure
☐ Other (please specify): _____

The following scenarios are causes of antibiotic resistance. Which in your opinion are more, and less, important?

	Very important	Important	Neutral	Un-important	Very un-important	Do not know
Too many antibiotics are prescribed	☐	☐	☐	☐	☐	☐
Too many broad-spectrum antibiotics used	☐	☐	☐	☐	☐	☐
Too long durations of antibiotic treatment	☐	☐	☐	☐	☐	☐
Too low a dose of antibiotics	☐	☐	☐	☐	☐	☐
Poor hand hygiene practices	☐	☐	☐	☐	☐	☐
Insufficient infection control practices	☐	☐	☐	☐	☐	☐
Too much access to antibiotics from the informal market	☐	☐	☐	☐	☐	☐
Too much access to antibiotics from pharmacies without a prescription from a doctor/nurse	☐	☐	☐	☐	☐	☐
Patient poor adherence to antibiotics	☐	☐	☐	☐	☐	☐
Sub-standard quality of antibiotics	☐	☐	☐	☐	☐	☐

What measures do you think would be helpful in improving antibiotic prescribing?

	Very helpful	Helpful	Neutral	Unhelpful	Very unhelpful	Do not know
Educational sessions on prescribing	☐	☐	☐	☐	☐	☐
Availability of local/national guidelines/ protocols	☐	☐	☐	☐	☐	☐
Availability of national resistance data	☐	☐	☐	☐	☐	☐
Availability of local resistance data	☐	☐	☐	☐	☐	☐
Availability of antibiotics in my clinic	☐	☐	☐	☐	☐	☐
Improved prevention of infections in the community	☐	☐	☐	☐	☐	☐
Advice from senior colleagues	☐	☐	☐	☐	☐	☐
Restriction of prescription of certain antibiotics	☐	☐	☐	☐	☐	☐
Regular audit and feedback on antibiotic prescribing on your ward/ clinic	☐	☐	☐	☐	☐	☐

In the last year, have you received any training in antibiotic prescribing?

☐ Yes
☐ No

If yes, how was the training delivered (select all that apply)

☐ Lecture at the hospital/ clinic
☐ Workshop
☐ Informal education during routine work (e.g. ward rounds)
☐ Self-directed learning

IMPORTANCE OF ANTIMICROBIAL RESISTANCE

Do you think antibiotic resistance is a global problem?	☐ Yes	☐ No	☐ Unsure
Do you think antibiotic resistance is a national problem?	☐ Yes	☐ No	☐ Unsure
Do you think antibiotic resistance is problem in your health facility?	☐ Yes	☐ No	☐ Unsure

DECISION MAKING

My decision on whether to start or not to start antibiotic treatment is guided by

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree	Do not know
How sick is the patient	☐	☐	☐	☐	☐	☐
If the patient is immunodeficient	☐	☐	☐	☐	☐	☐
The patient's expectations	☐	☐	☐	☐	☐	☐
I tend to follow local policy/ guidelines	☐	☐	☐	☐	☐	☐
My senior's expectations	☐	☐	☐	☐	☐	☐
Clinical picture and laboratory results	☐	☐	☐	☐	☐	☐
I have in mind the potential emergence of resistance to antibiotics	☐	☐	☐	☐	☐	☐
I take into consideration the potential side effects of the antibiotic treatment	☐	☐	☐	☐	☐	☐

Antibiotic prescriptions	Strongly agree	Agree	Neutral	Disagree	Strongly disagree	Do not know
I feel pressure from the patients to prescribe antibiotics	☐	☐	☐	☐	☐	☐
I feel pressure from colleagues/ seniors to prescribe antibiotics	☐	☐	☐	☐	☐	☐
I have to prescribe antibiotics because laboratory tests are unavailable/ unreliable	☐	☐	☐	☐	☐	☐
Antibiotic prescriptions should be reduced in hospitalized patients	☐	☐	☐	☐	☐	☐
Antibiotic prescriptions should be reduced in the outpatient setting (polyclinics)	☐	☐	☐	☐	☐	☐

In your setting, how often are antibiotics prescribed when they are not necessary?

☐ Very often (>90%) ☐ Often (>70%) ☐ About half the time (50%)
☐ Sometimes (<30%) ☐ Almost never (<10%)

What resources do you use to increase your knowledge on antibiotic prescribing? (select ALL that apply)

☐ Textbooks ☐ Professional meetings
☐ Medical journals ☐ Tablet or smartphone apps
☐ National guidelines (e.g. EDLIZ) ☐ Internet
☐ International guidelines ☐ Consultation with colleagues

☐ Lectures
☐ Other (please specify): _____

KNOWLEDGE

A 30-year old man presents to the clinic for a 3-day history of fever and feeling unwell. His brother says that he has travelled back from Beitbridge a week before. What diagnostic tests would you order? (select ALL that apply)

☐ SARS-CoV2 PCR
☐ SARS-CoV2 RDT (antibody test)
☐ Blood culture
☐ Other (please specify): _____

In the clinic, you see a 35-year old man who has well-controlled HIV (CD4+ count 345/mmc) has been coughing for 2 weeks. He was given a course of amoxycillin which has not helped. He has been feeling a little feverish but examination is unremarkable. What is your preferred strategy?

☐ Reassure him that he probably has a viral infection and it will resolve by itself
☐ Refer him to hospital for a course of ceftriaxone
☐ Prescribe doxycycline to cover atypical/resistant respiratory pathogens
☐ Order a sputum test for tuberculosis

A 19- year old woman from Budiriro comes to the clinic with a 6-day history of fever, headache and abdominal pain. On examination she appears to be very unwell and slightly confused. You suspect typhoid fever.

What antibiotic(s) would you prescribe? Please list: _____

A 20-year old women who is 12-weeks pregnant presents to the clinic complaining of vaginal discharge. She suspects that her partner has been unfaithful.

What treatment will you prescribe? _____

A 12-year old child is brought to the clinic by his mother for a 3-day history of fever (39˚C), runny nose and a dry cough. What would you do? (select **ALL** that apply)

☐ Prescribe a course of amoxicillin
☐ Prescribe paracetamol
☐ Reassure the mother that the child does not need antibiotics

END OF QUESTIONS

THANK YOU FOR PARTICIPATING IN THE SURVEY

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	6-7
Outcome data	15*	Report numbers of outcome events or summary measures	6-7, Figure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

APPENDIX 4

Supplementary materials for Chapter 8 (published paper)

Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe

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Supplementary materials

Additional Table S1. Characteristics of participants in a study of patients presenting with symptoms of urinary tract infection to primary care clinics in Harare, Zimbabwe

Characteristic	N=414
Age, median (IQR)	36 (26-46)
Female sex, n (%)	263 (63.5)
Pregnant, n (%)	32 (12.5)
HIV+, n (%)	169 (42.7)
On co-trimoxazole prophylaxis, n (%)	77 (45.6)
Urine dipstick positive, n (%)	125 (30.3)
Leucocyturia on microscopy, n (%)	81 (19.6)
Reported prior antimicrobials, n (%)	14 (3.4)

This analysis excludes participants with contaminated urine cultures on Brilliance UTI agar (n=17). Missing information: women who did not know if they were pregnant (n=6); participants who did not know their HIV status (n=18); dipstick, urine microscopy and antibiotic assay could not be performed because of sample spillage during transport (n=1). Urine dipstick was considered positive if it was positive for nitrites and/or leucocytes.

Fig. S1 Appearance of different colony types on InTray Screen culture plates (from left to right 1. *E. coli;* 2. Coliforms; 3. *Enterococcus spp.*; 4. *Staphylococcus aureus*; 5. *Proteus mirabilis*; 6. Staphylococci/ streptococci (contaminants))

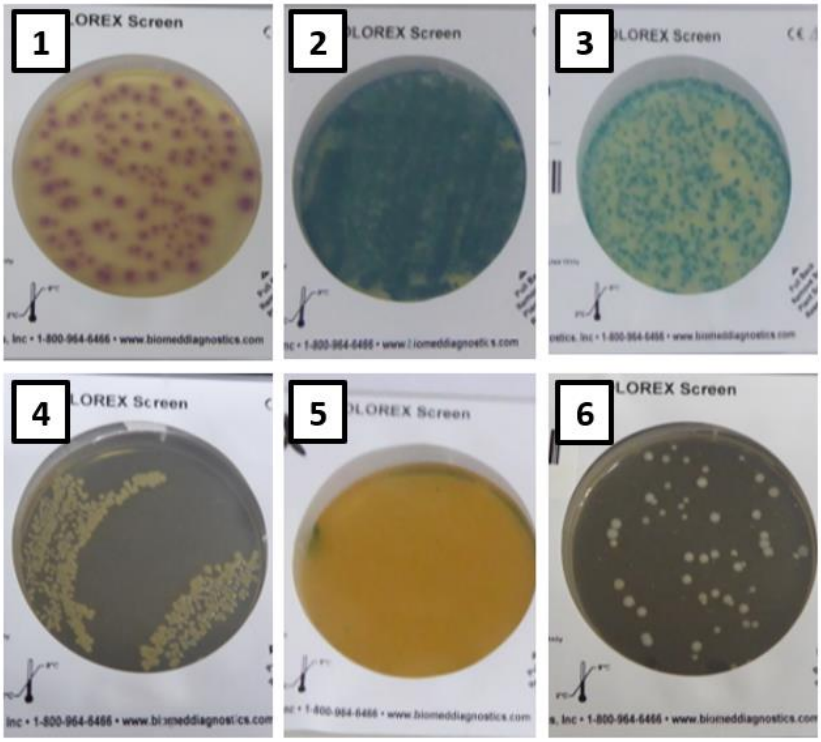
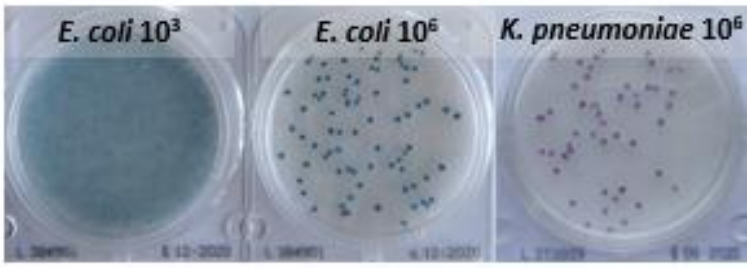


Fig. S2 Appearance of E. coli and K. pneumoniae at different dilutions using Compact Dry EC



APPENDIX 5

Supplementary materials for Chapter 9 (published paper)

Evaluation of a novel culture system for rapid pathogen identification and detection of cephalosporin resistance in neonatal Gram-negative sepsis at a tertiary referral unit in Harare, Zimbabwe

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SUPPLEMENTARY MATERIALS

Table of contents

Supplementary table 1. Criteria used for the diagnosis of neonatal sepsis (adapted from the NICE guidelines)[1]

MAJOR CRITERIA (start antibiotics if any of these are present)	
Confirmed sepsis or chorioamnionitis in mother Confirmed or suspected sepsis in twin Seizures Severe respiratory distress in a term infant Respiratory distress starting more than 4h after birth Signs of shock	
MINOR CRITERIA (start antibiotics if any two available)	
Antenatal	Rupture of membranes >18h Spontaneous preterm birth <37/40 weeks GBS sepsis in previous baby or documented GBS carriage in this pregnancy (urine or vaginal swab)
Natal	Born before arrival Meconium stained liquor
Postnatal	Respiratory distress that is not obviously related to: • Environmental hypothermia • "Delayed transition to extra-uterine life" i.e. mild to moderate respiratory distress apparent soon after birth that is improving with time. Hypoxia Apnoea Hypoglycaemia/ hyperglycaemia not otherwise explained Temperature instability not explained by environmental factors Acidosis not obviously related to HIE Unexplained bleeding or thrombocytopenia Mild encephalopathy/ altered responsiveness Altered tone not otherwise explained Feed intolerance/ feeding difficulty Abnormal heart rate (<90 or >160/min) Jaundice in first 24h

GBS: group B streptococcus; HIE: hypoxic-ischemic encephalopathy.

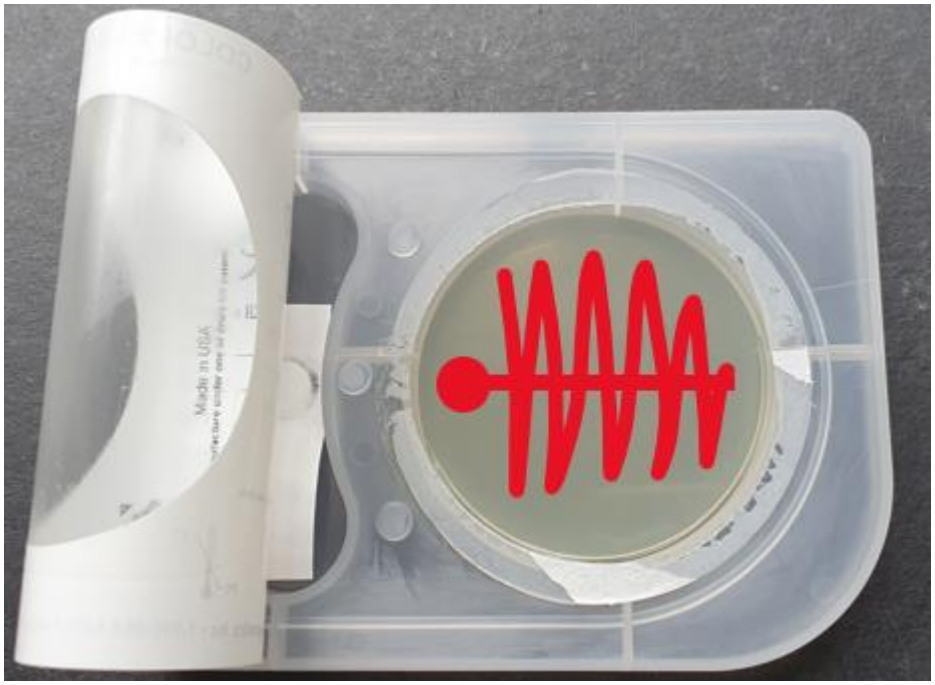
1. National Institute for Healthcare Excellence (NICE). Neonatal infection (early onset): antibiotics for prevention and treatment. Clinical guideline (August 2012). NICE, UK. Available from https://www.nice.org.uk/guidance/cg149. .
2. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.

Supplementary table 2. Bacterial identification and antimicrobial susceptibility testing according to pathogen

Gram-stain result	Drugs tests for AST[#]	Other tests for identification
Gram positive cocci (clusters)	Cefoxitin, vancomycin	Catalase, coagulase
Gram positive cocci (chains)	Ampicillin, vancomycin	Catalase, bile-aesculin
Gram negative bacilli	Ceftriaxone, imipenem, ciprofloxacin, chloramphenicol, gentamicin, amikacin	API 20E*

All positive blood cultures were plated on blood, chocolate and MacConkey agar.
**API 20E results were interpreted using the API 20E version 5.0 database.*
[#]Internal quality control for AST was conducted and interpreted in accordance to EUCAST recommendations (Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST Version 10.0, valid from 2020-01-01, www.eucast.org)
AST: antimicrobial susceptibility testing

Supplementary figure 1. Inoculation technique for the InTrays. (one drop of blood from the positive blood culture is inoculated and streaked on the plate).



Risk factor analysis for isolation of *K. pneumoniae* from blood cultures

Characteristics between neonates with *K. pneumoniae* sepsis and those where the pathogen was not isolated were compared. Differences between groups were assessed using the $\chi 2$ test for categorical and the Mann-Whitney U test for continuous variables. The level of significance was considered at $p \leq 0.05$. For the risk factor analysis, the outcome was isolation of *K. pneumoniae* from the blood culture. Multivariable analysis using logistic regression was performed for variables which showed an association in the bivariate analysis at a p-value of <0.20.

Infections with *K. pneumoniae* were more frequent in neonates where the blood culture was collected within 72 hours of birth 38/75 (50.7%) compared with those who had blood cultures done more than 72 hours after birth 16/124 (12.9%, p<0.001). Among the baseline characteristics, low or very low birth weight and age of sepsis onset, were associated with *K. pneumoniae* infection (Supplementary Table S4) in the bivariate analysis and after adjustment. Neonates with a low or very low birth weight had an adjusted OR for *K. pneumoniae* infection of 4.5 (95% CI 1.95-10.21, p<0.001).

Supplementary table 4. Risk factors for *Klebsiella pneumoniae* infections

Characteristic	Odds ratio	95% CI	p-value	Adjusted Odds Ratio	95% CI	p-value
Maternal age	1.003	0.96-1.05	0.895			
Number of ANC visits	0.933	0.77-1.13	0.480			
Mother is HIV+	1.776	0.69-4.57	0.233			
Primigravida	1.188	0.64-2.21	0.587			
Birth outside a healthcare facility	2.143	0.87-5.28	0.097			
Outborn*	2.198	1.15-4.18	0.016	2.086	1.02-4.25	0.043
Prolonged rupture of membranes	0.780	0.24-2.54	0.680			
Caesarean delivery	1.053	0.50-2.21	0.891			
Female sex	0.548	0.29-1.02	0.059	0.669	0.33-1.35	0.261
Low or very low birth weight	5.397	2.47-11.78	<0.001	4.459	1.95-10.21	<0.001
1-minute Apgar score	1.033	0.89-1.20	0.676			
5-minute Apgar score	1.023	0.86-1.22	0.803			
Required oxygen or respiratory support	1.319	0.71-2.46	0.384			
Difficulties feeding	4.765	1.79-12.66	0.002			
Required surgery	1.871	0.43-8.10	0.402			
Age at blood culture collection	1.093	1.03-1.16	0.005	1.081	1.00-1.17	0.055
Length of hospital stay in days	1.03	1.00-106	0.025	1.008	0.97-1.05	0.696

*Respiratory support = need for supplemental oxygen, CPAP or invasive ventilation. *Outborn: born outside Harare Hospital. Difficulties feeding was not included in the multivariable analysis as it is strongly correlated to birth weight. Birth outside a healthcare facility was not included in the multivariable analysis.*

Outcomes of neonates with ESBL-*K. pneumoniae* sepsis

Supplementary figure 4. Survival analysis for neonates with and without *K. pneumoniae* sepsis