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Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE): protocol for a multisite prospective observational study of the causes of fever in Africa and Asia

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ABSTRACT

Introduction Fever commonly leads to healthcare seeking and hospital admission in sub-Saharan Africa and Asia. There is only limited guidance for clinicians managing non-malarial fevers, which often results in inappropriate treatment for patients. Furthermore, there is little evidence for estimates of disease burden, or to guide empirical therapy, control measures, resource allocation, prioritisation of clinical diagnostics or antimicrobial stewardship. The Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE) study seeks to address these information gaps.

Methods and analysis FIEBRE investigates febrile illness in paediatric and adult outpatients and inpatients using standardised clinical, laboratory and social science protocols over a minimum 12-month period at five sites in sub-Saharan Africa and Southeastern and Southern Asia. Patients presenting with fever are enrolled and provide clinical data, pharyngeal swabs and a venous blood sample; selected participants also provide a urine sample. Laboratory assessments target infections that are treatable and/or preventable. Selected point-of-care tests, as well as blood and urine cultures and antimicrobial susceptibility testing, are performed on site. On day 28, patients provide a second venous blood sample for serology and information on clinical outcome. Further diagnostic assays are performed at international reference laboratories. Blood and pharyngeal samples from matched community controls enable calculation of AFs, and surveys of treatment seeking allow estimation of the incidence of common infections. Additional assays detect markers that may differentiate bacterial from non-bacterial causes of illness and/or prognosticate illness severity. Social science research on antimicrobial use will inform future recommendations for fever case management. Residual samples from participants are stored for future use.

Ethics and dissemination Ethics approval was obtained from all relevant institutional and national committees; written informed consent is obtained from all participants or parents/guardians. Final results will be shared with participating communities, and in open-access journals and other scientific fora. Study documents are available online (https://doi.org/10.17037/PUBS.04652739).

Strengths and limitations of this study

- Harmonised protocol at multiple sites to allow comparison of results across diverse epidemiological, geographic and cultural settings.
- Collection of data from inpatients, outpatients and community controls of all ages ≥2 months, at multiple sites across Africa and Asia, over the course of >12 months at each site to capture seasonal variation.
- Inclusion of a control group at each site to aid attribution and to allow estimation of disease incidence.
- Standardised diagnostic testing at pathogen-specific reference laboratories according to internationally accepted clinical case definitions.
- Current study limited to only five sites; protocol and supporting documents are freely available to other researchers who may wish to undertake similar work.

INTRODUCTION

Fever is one of the most common symptoms leading to healthcare seeking and hospital admission in sub-Saharan Africa and Asia.[1 2] Current age-specific WHO algorithms for the primary care level provide only limited guidance to clinicians for the management of non-malarial fevers. If the malaria test is negative, the patient is classified as 'fever: no malaria' in the Integrated Management of Childhood Illness guidelines[3] or in the Integrated Management of Adolescent and Adult Illness guidelines,[4] and advice is given to 'treat according to the apparent cause of fever.' Many febrile illnesses present with

non-specific symptoms and signs, and the current recommendations often result in treatable diseases being left untreated or treated with inappropriate antimicrobials on the one hand and overtreatment of self-limiting conditions with antimicrobials on the other, with important implications for the development of antimicrobial resistance.[5 6]

Little is currently known about the causes of fever in many low-income and middle-income countries (LMICs),[7–9] so there is sparse evidence on which to base empirical treatment guidelines for febrile patients, especially in more remote areas. Some studies provide an indication of the clinical spectrum of febrile illness,[10 11] but these studies were often disease specific, for example, focussing on urinary tract infections in Nigeria[12] or arboviruses in Asia.[13] A few studies designed to look at aetiologies of fever in given locations have been published recently.[2 14–17] While the results are useful within the specific study areas, the epidemiology of infections varies in place and time, so the generalisability of single-site studies is uncertain. Furthermore, the study approaches were heterogeneous—with differences in patient age, type of health facility, seasons covered, inclusion criteria, study design, sampling techniques and pathology tests employed—making it difficult to compare findings across sites and to produce a clear picture of the most common causes of fever in each geographical setting, age group and at each level of care. In addition, there is disabling heterogeneity in eligibility criteria, case definitions, use of diagnostic tests that are not sufficiently validated or standardised and lack of control groups, preventing calculation of attributable fractions (AFs). Recently, two multisite, prospective, case–control studies demonstrated the potential of using harmonised research protocols with standardised diagnostic methods to investigate the causes of clinical syndromes with high morbidity and mortality in resource-limited settings: the Global Enteric Multicenter Study[18] and the Pneumonia Aetiology Research for Child Health study[19] determined the predominant infectious causes of diarrhoea and pneumonia, respectively, among children in multiple African and Asian countries.

Improved diagnosis and treatment of febrile illness matter both for the care of individual patients and for public health goals. Besides data gaps on prevalence of specific infections in febrile patients, there is very little information on incidence for many of the infections thought to be clinically important in Africa and Asia. Consequently, there is little information on which to base estimates of burden of disease or to guide empirical therapy, control measures and resource allocation.[20] In addition, the ability to differentiate between bacterial and viral infections and between broad groups of bacterial pathogens based on antimicrobial susceptibility, could have a major global impact on antimicrobial resistance by limiting the unnecessary use of antimicrobials. However, there are limited data on antimicrobial usage and how and why the frequency and appropriateness of usage vary across LMICs. There is increasing emphasis on identification and incorporation into point-of-care diagnostic tests of markers of immune and endothelial activation (hereafter 'biomarkers') that can distinguish between bacterial causes of fever requiring antimicrobial treatment and viral or self-limiting infections,[21] or that can identify current or incipient severe illness.[22 23]

The Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE) study has been designed to help address these information gaps. FIEBRE is a multisite investigation in paediatric and adult outpatients and inpatients, using standardised clinical, reference laboratory and social science protocols, in low-resource regions from which few or no data are available. FIEBRE is being conducted at five sites in sub-Saharan Africa and Southeastern and Southern Asia, and the full protocol, data collection forms, standard operating procedures and other supplementary information are freely available to researchers who may wish to conduct harmonised work at other sites (accessible on the FIEBRE study website (https://doi.org/10.17037/PUBS.04652739) or from coinvestigators). This paper describes the clinical, epidemiological and laboratory activities of FIEBRE, which seek to identify infections that are treatable (eg, with specific antimicrobials) and/or preventable (eg, with vaccination or vector-control approaches), to document antimicrobial susceptibility in isolated micro-organisms and to evaluate biomarkers that may be useful in distinguishing bacterial from other causes of fever and/or in prognosis. An overview of the social science work and its relationship to the broader study is also provided, with country-specific protocols available on the FIEBRE study website (https://doi.org/10.17037/PUBS.04652739).

METHODS AND ANALYSIS

Study design

FIEBRE is a study of febrile illness in people aged 2 months and older residing at one of five sites (three sites in sub-Saharan Africa, one in Southeastern and one in Southern Asia). The study's specific objectives are listed in box 1. Patients who present with fever at the selected facilities are recruited (day 0) if they or their guardians/caregivers (in the case of minors or unconscious patients) provide written informed consent. Study staff take a targeted illness and exposure history and perform a physical examination. Nasopharyngeal and/or oropharyngeal swabs and a venous blood sample are collected from all participants; a urine sample is collected from selected participants. Tests for malaria and for HIV (at sites where HIV prevalence exceeds 1% in the general adult population, for patients not already known to be infected), serum cryptococcal antigen (CrAg) and urinary lipoarabinomannan (uLAM) detection, and blood and urine cultures are performed on site; bacteria and fungi isolated from clinical specimens are identified and tested for antimicrobial susceptibility. At day 28 after enrolment, study patients are asked to provide a further venous blood sample for serology, and clinical outcome is evaluated.

Box 1 Specific objectives of Febrile Illness Evaluation in a Broad Range of Endemicities

Primary objectives

1. To determine the treatable and/or preventable causes of fever in children aged ≥2 months and in adults presenting as outpatients, and among those admitted to hospitals, in areas represented by the study sites.
2. To determine how fever aetiology varies according to patient age, geographical area, local malaria and HIV prevalence, and other risk factors.
3. To determine the prevalence and spectrum of antimicrobial resistance among bacterial pathogens identified in clinical specimens from febrile patients.

Secondary objectives

1. To generate data on incidence of specific infections in study site catchment areas and therefore contribute key data on disease burden for some infections that are not counted in current global burden of disease estimates.
2. To build an archive of well-characterised and geographically diverse biological samples from patients with well-characterised clinical phenotypes, and from community controls, for use in evaluation of new diagnostic and prognostic tests and in identification of human-related and pathogen-related biomarkers that may improve case management strategies.
3. To evaluate available biomarker assays to assess their performance and potential utility in fever case management in the study areas.
4. To collect social science data on the roles of antimicrobials in fever case management for prescribers, local residents and a range of stakeholders.
5. To generate data to inform the development of new evidence-based fever case management algorithms which may be evaluated in future studies.

Study patients are managed by the clinical staff responsible for usual patient care at each study site, according to local standard of care. Results of diagnostic tests performed at or near the study site are provided to the clinical staff as soon as available. Other diagnostic tests are performed at internationally recognised reference laboratories (see the Specific laboratory assessments section).

Recruitment to the study is over a minimum continuous 12-month period at each site to ensure that seasonal variations in causes of fever are captured. Blood and pharyngeal samples from matched community controls enable the calculation of AFs. In addition, control participants are surveyed to obtain representative data about treatment seeking and medicine use. By combining data on causes of fever at study sites with the estimate of the proportion of patients with fever seeking care at those facilities, the incidence of common infections in the study area can be estimated, in order to contribute to efforts to define the burden and impact of infectious diseases.[24–27] Social science research is carried out to capture people's responses to febrile illness and the nature of antimicrobial use in different settings, the findings from which will be used to inform changes in fever case management.

Study sites, population and participant selection

The study is being conducted at five sites, all of which have little or no published data on causes of fever, and where suitably qualified research teams and capacity are available. Current study sites include outpatient and inpatient facilities in Bangladesh, Lao People's Democratic Republic (Laos), Malawi, Mozambique and Zimbabwe. These sites have been selected because they fulfil the aforementioned criteria and because, based on available data, there is substantial between-site variation in the prevalence of HIV and malaria (table 1).

The study recruits both febrile patients (cases) and community controls. Patients are drawn from those who present for healthcare at the selected healthcare facilities. All patients aged 2 months and older are eligible for enrolment. Patients are recruited if they fulfil all of the following criteria:

1. Tympanic or axillary temperature of ≥37.5°C at presentation.
2. *Not* having been hospitalised or having undergone surgery in the previous month.
3. Age ≥2 months (2 months or older).
4. For *out*patients, residence (at the time of enrolment) within the defined catchment area around the health facility.
5. For outpatients aged ≥15 years, *absence* of symptoms of lower respiratory infection and of diarrhoeal diseases as defined by
   a. Cough and ≥1 of the following: cough productive of green/yellow sputum or haemoptysis.
   b. Loose stools (≥3) within the previous 24 hours.
6. For outpatients aged ≥2 months to <15 years, absence of symptoms of diarrhoeal diseases as defined by ≥3 loose stools within the previous 24 hours.
7. Willingness and ability to provide demographic and clinical information, and clinical samples, at the time of enrolment and 28 days later.
8. Provision of written informed consent for adult participants; or for children, provision of written consent from a parent/guardian and assent from the child (according to local regulations and practices at each study site).

Social science research is conducted with purposive samples of prescribers, medicine sellers and residents in the study catchment areas in two countries, as well as with stakeholders in the wider public health community.

Participant recruitment began in Zimbabwe in June 2018, in Malawi in July 2018, in Laos in October 2018 and in Mozambique in December 2018; following confirmation of funding, a fifth site is expected to begin in Bangladesh in mid-2020.

Data and sample collection at the time of patient enrolment (day 0)

At patient enrolment, study staff collect basic demographic data and information on the history of the present illness. A study staff clinician performs a physical examination, including signs that may be used to calculate a

Table 1 Characteristics of the study sites for the Febrile Illness Evaluation in a Broad Range of Endemicities

	Bangladesh	Lao People's Democratic Republic	Malawi	Mozambique	Zimbabwe
Site-specific ethics committees*	Bangladesh Medical Research Council National Research Ethics Committee, Chittagong Medical College Ethical Review Committee, Oxford Tropical Research Committee†	National Ethics Committee for Health Research, Oxford Tropical Research Ethics Committee†	University of Malawi College of Medicine Research and Ethics Committee, Liverpool School of Tropical Medicine Research Ethics Committee†	Comité Institucional de Bioética para a Saúde do Centro de Investigação em Saúde de Manhiça, Comité Nacional de Bioética em Saúde de Moçambique	Medical Research Council of Zimbabwe
Name of health facilities where patients are recruited	CMCH and Bangladesh Institute of Tropical and Infectious Diseases	Phonhong Vientiane Provincial Hospital	Chikwawa District Hospital	Manhiça District Hospital	Harare Central Hospital, Chitungwiza General Hospital and three primary care clinics in Harare City
Region of country	Southeast	Northwest	South	South	North central
Demographic classification	Urban, periurban and rural	Periurban and rural	Rural	Rural	Urban
HIV epidemiology (2018 national seroprevalence among adults aged 15–49 years‡ unless otherwise indicated)	<0.1%	0.3%, no site-specific estimates available	9.2%, no site-specific estimates available	12.6%, 39.7% among adults aged 18–47 years in Manhiça in 2012§	12.7%, 11.5% in Harare‡
Malaria epidemiology	Low transmission of *Plasmodium falciparum* and *P. vivax*, peaking from June to September; 2013–2016 average annual incidence of 4.53 per 1000 population¶; in 2019, 1.7% of CMCH febrile inpatients screened had positive malaria test**	Low transmission of *P. falciparum* and *P. vivax*;<1% of symptomatic patients in 2008–10 had laboratory-confirmed malaria††	Perennial transmission of *P. falciparum*, peaking from December to May; over 12 months in 2016–2017, 12.5% of surveyed children aged <5 years had symptomatic malaria‡‡	Perennial transmission of *P. falciparum*, with marked seasonality peaking from November to April; approximately 7% malaria prevalence in children <5 years of age§§	No local malaria transmission; Harare health facilities may receive malaria-infected patients referred or visiting from endemic areas of Zimbabwe¶¶

Continued

Table 1 Continued

	Bangladesh	Lao People's Democratic Republic	Malawi	Mozambique	Zimbabwe

*All implemented versions of the protocol are approved by the site-specific ethics committee/s for each site and by the research and ethics committee of the London School of Hygiene & Tropical Medicine.
†Oxford Tropical Research Ethics Committee and Liverpool School for Tropical Medicine Research Ethics Committee have reciprocal agreements for protocol review and approval with the research and ethics committee of the London School of Hygiene & Tropical Medicine.
‡UNAIDS AIDSinfo Data Sheet, 2018 national data (and subnational data for Zimbabwe) (http://aidsinfo.unaids.org/).
§González R, *et al* 'HIV incidence and spatial clustering in a rural area of southern Mozambique,' *PLoS One*, 2015 Jul 6;10(7):e0132053.
¶Reported in Mayxay *et al*.[2]
**Kabaghe AN, *et al*., 'Short-term changes in anaemia and malaria parasite prevalence in children under 5 years during 1 year of repeated cross-sectional surveys in rural Malawi.' *Am J Trop Med Hyg*, 97(5), 2017, pp. 1568–1575, doi:10.4269/ajtmh.17–0335.
††Personal communication, Quique Bassat.
‡‡US President's Malaria Initiative Malaria Operational Plan for Zimbabwe, fiscal year 2017.
§§Noé A, *et al*'Mapping the stability of malaria hotspots in Bangladesh from 2013 to 2016,' *Mal J*, 2018; 17:259–79
¶¶Personal communication, Chittagong Medical College Hospital, Malaria Research Group, Chattogram, Bangladesh.
CMCH, Chittagong Medical College Hospital.

severity score (eg, FEAST Paediatric Emergency Triage[28] and Lambaréné Organ Dysfunction Score[29 30] for children aged <15 years, and quick Sequential Organ Failure Assessment[31–33] and the 'universal vital assessment'[34] score for older patients).

Study staff collect pharyngeal swabs and a venous blood sample from each participant using standard age-based and weight-based thresholds for blood volumes obtained.[35] In addition, a urine sample is collected from patients aged <2 years (using clean-catch methods where possible, although this is recognised to be challenging) and from older patients who have dysuria, frequent micturition, suprapubic tenderness or costovertebral angle tenderness. Study staff prepare the samples and conduct the diagnostic tests described. All other care is provided by health facility staff according to local standards.

The FIEBRE study collects clinical samples for two purposes: for assays that are of immediate clinical benefit to patient care (malaria testing, HIV testing, serum CrAg, uLAM, and blood and urine cultures, performed at or near the clinical site) and for research purposes (serological and nucleic acid assays for pathogen-specific diagnoses, assays of immune and endothelial activation markers, and RNA analysis in a subset of participants, all of which will be done in the future at specialised laboratories).

Data and sample collection at the time of patient follow-up (day 28)

All patients are asked to return to the study site for one follow-up visit 28 days after enrolment (acceptable range: 26–48 days, inclusive, after enrolment). At each patient's day 28 follow-up visit, study staff record the clinical outcome of the illness (complete recovery, improvement but incomplete recovery, same as on day 0, worse than on day 0, death and loss to follow-up) and obtain a convalescent venous blood sample for paired serology and biomarker testing. In the event that a patient is lost to follow-up or deceased, information is collected from other household members where possible.

Recruitment, data and sample collection for control participants

Interpreting the results of some serological, molecular and pharyngeal swab assays requires knowledge of background prevalence of infection or colonisation in the study population. To address this need, ≥600 control participants are enrolled at each study site. Control participants are community members in the study site health facilities' catchment areas, frequency matched 1:2 (or >1:2, where logistically feasible) to participating outpatients by month of enrolment, age, gender and geographical location of residence to the outpatients. No controls are specifically recruited for the inpatient population, as inpatients may be referred to the participating health facilities from a wider geographical area and therefore may be less representative of the epidemiology in the study area. Potential control participants are approached at their place of residence by study staff, with assistance from established

community health workers, where locally appropriate. Controls are recruited two times per month at each site and enrolled if they or their parents/guardians provide informed consent. The informed consent document and process for controls include an explanation that control participants are not likely to benefit directly from study participation, but that their participation may lead to better understanding of febrile illnesses in their community and others like it.

Study staff collect basic demographic data from control participants. Sample collection and diagnostic testing are identical for controls and patients, with three exceptions: blood for culture, convalescent sera and urine are not collected from controls. Venous blood is drawn from control participants using standard age-based and weight-based volume guidelines.

Healthcare utilisation survey for estimation of incidence of infections

In addition, questionnaires are administered to the community control participants in order to capture representative data about treatment seeking and antimicrobial and other medicine use. The control (or healthcare decision-maker if the participant is a child) is asked about treatment-seeking practices for each household member.[24 36] This healthcare utilisation survey provides an estimate of the period prevalence of fever, as well as the proportion of individuals with fever in the community who present to the study enrolment sites for care. The fraction of people with fever presenting to a study site will be used to estimate the population-based incidence of fever overall and the incidence of specific causes of fever in the catchment area of study healthcare facilities.[1 24 25 37 38]

Social science methods

To capture responses to febrile illness and the nature of antimicrobial use among prescribers, medicine retailers and residents, social scientists use qualitative and quantitative methods derived from medical anthropology. The first research phase involves household medicine surveys in the study catchment areas, a central feature of which is the use of 'drug bags' (a collection of physical examples of locally available antibiotics) that enable the production of qualitative and quantitative data about antibiotic recognition, use and access. Details of this method have been described elsewhere.[39] The second phase is longitudinal ethnographic fieldwork, including participant observation and key informant interviews with residents, medicine retailers (pharmacists, drug shop workers and market vendors) and healthcare workers in clinics and hospitals. With prescribers and retailers, qualitative methods are complemented and contextualised by the collection of quantitative data about antimicrobial prescription, stocks and sales. Alongside ethnography, in-depth interviews with stakeholders in the wider public health community are conducted to situate local fever management and antimicrobial use within broader public and global health discourses.

Specific laboratory assessments

Laboratory assessments for detection and diagnosis of infectious causes of fever focus on those that are treatable and/or preventable (table 2). With a few exceptions, the same pathogens are sought in samples from all participants at all sites, including: blood parasites; bacterial, mycobacterial and fungal bloodstream infections; typhus group and spotted fever group *Rickettsia* spp; *Orientia tsutsugamushi*; *Coxiella burnetii*; *Leptospira* spp; *Brucella* spp; *Borrelia* spp that cause relapsing fever; *Leishmania* spp; and arboviruses.

Table 2 describes pathogen-based diagnostic tests that are performed at or near the point of care at each study site; these results are made available in real time to treating clinicians for use in patient care decisions. In order to standardise diagnostic testing for study results, external quality assessment of site results and further diagnostic assays for which capacity does not exist currently near the research sites are performed at internationally recognised reference laboratories (table 2). Cryopreserved samples of all microorganisms isolated in culture from blood and urine are shipped on dry ice to a reference laboratory for confirmation of identification and of antimicrobial susceptibility testing to international standards. Participants' pharyngeal swabs and blood samples (EDTA whole blood, serum, plasma and buffy coat) are aliquoted, stored at −80°C and shipped to the collaborating reference laboratories. For each pathogen of interest, all samples from all study sites are tested at the same reference laboratory, and diagnostic strategies meet internationally accepted laboratory-based case definitions.

In addition to diagnostic testing for specific infectious agents, a set of assays is carried out to detect host (patient) biomarkers that have been identified in previous studies as potentially useful in differentiating between bacterial and non-bacterial causes of illness and/or as prognosticators of illness severity. These include C reactive protein,[40 41] a triggering receptor expressed on myeloid cells (sTREM-1),[40 42 43] angiopoietin 2,[43 44] heparin-inding protein[40 44 45] and others. The biomarkers sought prioritise assays that are most likely to lead to public health benefit in fever case management for patient populations typified by FIEBRE participants. The diagnostic and prognostic value of these biomarkers will be assessed to determine their utility alone and in combination for predicting severe outcomes, using mortality and severity scores as endpoints.

Sample archive

Informed consent is sought from study participants or parents/guardians at recruitment for the future use of their biological samples and anonymised data, including for the development and evaluation of new diagnostic tests, for example, new point-of-care diagnostic tests intended to guide the management of febrile patients and

Table 2 Pathogen-based diagnostic testing for the Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE)

Infection or pathogen sought	Sample type	Diagnostic test	Notes
Pathogen-based diagnostic tests to be performed at or near the point of care			
Malaria (*Plasmodium* species)	EDTA whole blood	Antigen-detecting lateral flow malaria rapid diagnostic test (mRDT)	Combination test detects histidine-rich protein 2 and *Plasmodium* lactate dehydrogenase
	Thick and thin blood smear	Expert light microscopy for the presence versus the absence of asexual parasites, species and density	For all mRDT-positive samples and 10% of mRDT-negative samples
HIV*	EDTA whole blood	Antibody-detecting rapid tests according to national guidelines	Confirmatory molecular testing according to national guidelines for infants who test antibody-positive
Bacteraemia and/or fungaemia	Whole blood	Aerobic culture, identification and antimicrobial susceptibility testing performed for isolated microorganisms	Single culture bottle; blood volume of ≤10 mL, weight-based volumes for small children
Mycobacteraemia*	Whole blood	Mycobacterial culture	For patients aged ≥15 years who are HIV-infected and/or admitted as inpatients
Mycobacterium tuberculosis	Urine	Urinary lipoarabinomannan rapid test	For patients who are HIV-infected and/or admitted as inpatients
Cryptococcus species	Serum	Antigen-detecting lateral flow rapid diagnostic test	For patients who are HIV-infected and/or admitted as inpatients
Nitrites and leucocyte esterase (evidence of urinary tract infection)	Urine	Urine dipstick	Urine culture performed on samples positive for nitrites and/or leucocyte esterase
Bacteriuria	Urine	Culture, identification and antimicrobial susceptibility testing performed for isolated microorganisms	For samples dipstick-positive for nitrites and/or leucocyte esterase
External quality assessment of diagnostic results obtained at or near the point of care, to be performed at internationally recognised reference laboratories			
Malaria (*Plasmodium* species)	Thick and thin blood smear	Expert light microscopy for the presence versus the absence of asexual parasites, species and density	Randomly selected sample of 10% of microscopy-positive and 10% of microscopy-negative smears from each site
Bacteria and fungi isolated from blood and urine at sites	Cryopreserved isolates	MALDI-TOF MS for identification and drug susceptibility testing to EUCAST standards	–
Mycobacteria isolated from blood at sites	Cryopreserved isolates	Identification using subculture and molecular testing, drug susceptibility testing depending on organisms identified	–
Pathogen-based diagnostic tests to be performed at internationally recognised reference laboratories			
Borrelia species (louse-borne and tick-borne relapsing fevers)	Thick and thin blood smear	Expert light microscopy	Random 10% sample of all smears from each site; if positives are identified, a larger proportion are to be read

Continued

Table 2 Continued

Infection or pathogen sought	Sample type	Diagnostic test	Notes
Arboviruses: chikungunya, dengue, Japanese encephalitis, o'nyong 'nyong, Zika	Serum	Africa-specific or Asia-specific IgG ELISA and qPCR, microneutralisation for samples positive by ELISA	A proportion of African samples to be tested for Japanese encephalitis virus, and a proportion of Asian samples to be tested for o'nyong 'nyong virus; if positives are identified, a larger proportion of samples are to be tested
Brucella species (brucellosis)	Serum	Brucella IgM EIA, *Brucella* microagglutination test for samples positive by EIA	Convalescent sera screened for exposure using EIA; positives tested by IgM EIA and microagglutination on acute and convalescent sera
Leptospira species (leptospirosis)	Serum	Microagglutination test	–
Rickettsiae: *Orientia* species, *Rickettsia* (typhus group and spotted fever group), *Coxiella burnetii*	Serum and buffy coat	IgG and IgM IFA; qPCRfor samples positive by serological screen	Buffy coat is preferred sample for *Orientia* and rickettsial species detection, serum to be used for *C. burnetii*
Visceral leishmaniasis	Serum	Direct agglutination test	–
Histoplasma species (histoplasmosis)	Serum	Histoplasma EIA††	–
Respiratory pathogens: influenza A and B, respiratory syncytial virus†	Nasopharyngeal±oropharyngeal swab	Luminex respiratory panel	–
Paediatric viraemia and/or bacteraemia‡	EDTA whole blood	PCR	(Details to be determined)

*At sites where HIV prevalence is >1% in the general adult population.
†The Luminex respiratory panel also detects adenovirus, parainfluenza viruses 1–4, enterovirus, rhinovirus, B virus, coronaviruses (229E, OC43, HKu1 and NL63), metapneumovirus, bocavirus, *Legionella pneumoniae*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.
‡To be performed on samples from children aged <5 years, from whom blood volumes will not be adequate for all serology tests listed.
EIA, enzyme immunoassay; EUCAST, European Committee of Antimicrobial Susceptibility Testing; IFA, immunofluorescence assay; MALDI-TOF MS, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry; mRDT, malaria rapid diagnostic test; qPCR, quantitative PCR.

assays detecting host transcriptomic signatures of specific infections. Residual blood and pharyngeal samples from participants are stored and monitored in a central laboratory facility. Access to the samples follows a formal process of application and requires approval from both the FIEBRE consortium and an independent committee including senior scientists as well as lay members.

Data sharing

Anonymised data outputs are shared on institutional data repositories. All data releases are assigned persistent interoperable digital object identifier (DOI) numbers (ISO 26324). Nucleic acid sequences and associated datasets will be released on relevant data archives (eg, EMBL-ENA and GENBANK). Data outputs which reasonably, ethically and legally can be shared will be released on open-attribution ShareAlike licenses, such as the Creative Commons Attribution-ShareAlike V.2.0 Generic (CC BY-SA V.2.0).

Sample size considerations

Any single pathogen or fever aetiology is likely to be rare in the study populations.[2 15 16] The prevalence of respiratory viruses (eg, influenza and respiratory syncytial virus) detected by PCR, and of baseline seropositivity to other pathogens, will be compared between cases and controls. The prevalence of seropositivity to common causes of fever is assumed to be approximately 5% in the general population. To identify causes of fever, a sample size of 600 patients per group will enable estimation of the prevalence of an infection whose true prevalence is 5%, with a precision of ±1.7% with 95% confidence, and to estimate the prevalence of an infection whose true prevalence is 1%, with a precision of±0.8%. A sample of 600 outpatients and 300 controls will provide >90% power to show a significant difference between a prevalence of 12% in cases and 5% in controls. Therefore, 600 febrile patients are to be enrolled in each of four analysis groups (children aged ≥2 months to <15 years, and patients aged ≥15 years, with stratified enrolment so that within each of the two age groups, approximately half are inpatients and half are outpatients), for a total of 2400 patients per site, plus 300 controls in each of the two age groups at each site (total 600). Site-specific recruitment strategies allow enrolment of a representative sample of patients presenting over the course of the study at each site. The social science research will involve 100–150 participants per site, with the sample size determined by data saturation.

Data analysis plan for primary outcomes

To determine the treatable and/or preventable causes of fever in the study population, the AF will be calculated for each pathogen or group of pathogens. This will be done separately for each site and age stratum. For each group of patients, the OR for the association between each pathogen and fever will be calculated, using logistic regression, by comparing cases and controls. Strata based on geographical location and season will be defined at each site, and the analysis will be adjusted for age, sex and stratum. A weighted analysis will be performed to reflect the relative frequency of fevers that present in each season. For pathogen A, the AF (AF_A) will then be calculated as $AF_A=p_A(1\text{-}OR_A)$, where p_A is the proportion of cases with pathogen A, and OR_A is the OR for the association between presence of pathogen A and being a case. CIs will be calculated using bootstrap methods.

To determine the prevalence and spectrum of antimicrobial resistance among bacterial pathogens identified in the study, at each site, the proportion of bacterial pathogens with antimicrobial resistance defined by standardised criteria will be calculated. The proportion of common organisms demonstrating resistance to a standard panel of antimicrobials will be reported.

To generate data on incidence of specific infections among study participants and contribute to estimates of disease burden, for an area with known or estimated population size, responses to the healthcare utilisation survey questions will be used to estimate r, the proportion of fevers for which treatment was sought at the study facility. The total number of cases seen at the study site from the defined population will be multiplied by $1/r$ to obtain the total cases in a year from the defined population. This will be divided by the size of the population to estimate incidence. Incidence of fever caused by specific pathogens will be calculated by multiplying the incidence by the AF for that pathogen.

To assess the performance and potential utility of biomarker assays to guide fever case management in the study areas, each biomarker and biomarker combinations will be compared with mortality and severity scores calculated using clinical data and with pathogen-specific diagnoses.

To generate data to support the development of new fever case management algorithms which may be evaluated in future studies, the association between the presence of pathogens with predefined clinical and other variables will be examined, and social science research findings will shape recommendations. Social science data will be analysed iteratively as themes emerge and are followed up during ethnographic fieldwork. Patterns in the data will be interrogated in consultation with wider social theory, building on our reviews and analyses of 'the social' in fever case management[46] and antimicrobial resistance.[47]

Ethics and dissemination

Ethics approval of the study protocol was obtained from all relevant institutional and national committees (table 1). Written informed consent was obtained from all participants, or their parents/guardians, for study participation and for future use of biological samples. No individual participant identities will be used in any reports or publications resulting from the study.

Before beginning study activities at each site, meetings are held with community leaders and representatives of the public, and with staff at participating health facilities,

to provide information about the aims of the study and the methods to be used. When final results are available, feedback and dissemination meetings will be held at each site both for healthcare staff and for the communities who participated in the study.

Investigators and study staff engage with national and international networks to ensure that researchers, public health advocates and policy makers at various levels are aware of the study. The study protocol, standard operating procedures, data collection tools and other study documents are freely available on request from coinvestigators and at the FIEBRE study website (DOI: https://doi.org/10.17037/PUBS.04652739). Press releases and website updates publicise study progress. Study results will be prepared for publication in open-access peer-reviewed journals, and presented at national and international scientific conferences as soon as possible after study completion.

Patient and public involvement

Patients were not directly involved in the development of the research questions, the design of this study or the conduct of the study. The FIEBRE study does include substantial interaction with communities in the study areas to promote awareness and acceptance of patient recruitment at health facilities, and to encourage participation as community controls and in social science activities. Study results will be disseminated in participating communities at each site through the collaborating research group's public engagement teams and community advisory groups, using locally appropriate strategies. Community feedback, as well as findings from social science research, will be incorporated into future recommendations for improved fever case management.

DISCUSSION

The FIEBRE study is designed to investigate causes of febrile illness and antimicrobial resistance at multiple sites in Africa and Asia, where currently there is little evidence and very limited diagnostic capacity to guide fever case management. FIEBRE focuses on detecting infections that are treatable (eg, with specific antimicrobials) and/or preventable (eg, with vaccination or vector-control approaches). Across all sites, the study uses a common design, selection criteria, case definitions, laboratory procedures and analysis plan. This harmonised approach will generate reliable and comparable data that can contribute to updated recommendations on the clinical management and prevention of febrile illnesses, adapted to local contexts. In addition, due to the inclusion of community control participants, the study will provide data to support reliable estimates of the incidence and, in turn, burden of disease.

This study provides a unique opportunity to collect and store biomedical samples with data from a large and well-characterised group of febrile patients and controls from representative settings in Africa and Asia. The samples will be useful for identification of novel diagnostic targets and to guide prioritisation for the development and evaluation of new point-of-care diagnostic tests intended to guide the management of febrile patients. New tests could include those that predict severity of illness, detect specific infections, and/or differentiate between bacterial and viral infections to help guide antimicrobial therapy, identified as high priority at a WHO meeting of experts convened in 2015.[21]

It is anticipated that data generated by FIEBRE on causes of febrile illness and antimicrobial susceptibility, alongside the social science work on the role of antimicrobials in fever case management, will be incorporated into new diagnostic strategies and case management guidelines which can then be evaluated and optimised in various contexts.

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