



STUDY PROTOCOL

REVISED **Estimating the burden of antimicrobial resistance in Malawi: protocol for a prospective observational study of the morbidity, mortality and economic cost of third-generation cephalosporin resistant bloodstream infection [version 2; peer review: 2 approved]**

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Abstract

Introduction: Antimicrobial resistance (AMR) is a global public health concern, but the problems are context specific, with each country or setting facing differing challenges. In sub-Saharan Africa, third-generation cephalosporin resistant Enterobacterales (3GCR-E) are of particular concern, given the widespread reliance on ceftriaxone for treatment of severe infection in this setting. In Malawi, despite rising prevalence of 3GCR-E, the health-impact of these infections has not been described. This study is designed to estimate attributable mortality, morbidity and economic cost of 3GCR-E bloodstream infection (BSI) in a large, urban hospital.

Methods: This study will investigate the burden of AMR by recruiting a a prospective longitudinal cohort of patients who have bloodstream infection with 3GCR-E, at Queen Elizabeth Central Hospital, Blantyre, Malawi. Patients whose blood culture is positive for either third-generation cephalosporin susceptible (3GC-S) or third-generation resistant (3GC-R) Enterobacterales will be enrolled and provide clinical and healthcare economic data. Patients will be followed throughout their hospital stay and to 6-months post discharge. The primary outcomes for the study are mortality and morbidity from 3GCR-E. Healthcare economic outcomes will be assessed by comparing healthcare provider costs, indirect patient costs and health-related quality of life outcomes in patients with 3GC-S and 3GC-R BSI. Based on our observation that some patients with clinical suspicion of sepsis and 3GC-R BSI are surviving without an effective antibiotic, we review each patient prospectively and classify what role the isolated bacteria is playing in the patient's clinical presentation. Each BSI episode will be classified into the following categories: definite Gram-negative sepsis, probable Gram-negative sepsis, transient or occult

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

bacteraemia, or contaminated blood culture. These classifications will be incorporated into our analysis.

Ethics and dissemination: The study protocol has been approved by the Malawi College of Medicine Research Ethics Committee and by the Liverpool School of Tropical Medicine Research Ethics committee.

Keywords

Enterobacterales, Extended-spectrum beta-lactamase, Third-generation cephalosporin, Africa south of the Sahara, Antimicrobial resistance



This article is included in the [Malawi-Liverpool Wellcome Trust Clinical Research Programme gateway](#).

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REVISED Amendments from Version 1

We have expanded specific sections of Methods to incorporate reviewers' responses, in particular clarifying the bloodstream infection classification process and the counterfactual approach to analysis. We additionally highlight the difficulties with sepsis severity grading systems in sub-Saharan Africa. The abstract has been expanded to include further details of the bloodstream infection classification process and health-economic methods. We include a new table (Table 2), which describes the antimicrobial discs we will use in antimicrobial sensitivity testing.

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

Introduction**Antimicrobial resistance in sub-Saharan Africa**

Antimicrobial resistance (AMR) is a property of micro-organisms which have evolved to survive exposure to the antimicrobials previously successfully used to treat them. Drug-resistant infections (DRIs) occur when AMR bacteria cause infection and have become a global public health problem¹. In high-income countries, DRIs frequently remain amenable to therapy, albeit with more expensive antibiotics, thus incurring increase in healthcare costs. The greatest burden of DRIs, however, is expected to occur in low and middle-income countries, where alternative antibiotics are frequently unavailable or prohibitively expensive, and the morbidity and mortality from these infections is predicted to be high².

Third-generation cephalosporin resistant Enterobacterales (3GCR-E), have been identified by the World Health Organization (WHO) as critical priority pathogens on which national AMR programmes should focus their surveillance and reporting². These pathogens are of particular importance in sub-Saharan African hospitals, where the third-generation cephalosporin, ceftriaxone, is frequently relied upon in the empirical treatment of sepsis³⁻⁵.

Median proportions of third-generation cephalosporin resistance amongst bloodstream Enterobacterales in sub-Saharan Africa (sSA) are high, approaching 15% in *Escherichia coli* and 50% in *Klebsiella*⁶. The most comprehensive published information on AMR trends in sSA, comes from Malawi, where blood culture surveillance data from patients presenting to Queen Elizabeth Central Hospital (QECH), has shown a recent, rapid rise in third-generation cephalosporin resistance amongst Enterobacterales⁷. Between 2003 and 2016, 3GC resistance rose from 0.7% to 30.3% in *E.coli* and from 11.8% to 90.5% in *Klebsiella*, contemporaneous to the widespread roll-out of ceftriaxone in the hospital since 2005⁷. The lack of availability and prohibitive expense of alternatives to ceftriaxone, means that unlike in high income countries, these infections are locally untreatable.

Knowledge gaps

Despite the rising prevalence of third-generation cephalosporin resistance amongst key pathogens and a reliance on ceftriaxone for management of infection, the health impact

of 3GC-R BSI in sSA has not been described⁶. Findings from large-scale cohorts in high-income settings, suggest that these infections are associated with adverse patient outcomes, including high mortality, length of hospital stay and total healthcare costs⁸⁻¹¹, but only one published study from sSA has investigated health burden from AMR, finding a significant impact of third-generation cephalosporin resistance on mortality¹². Malawi is one of the few countries in sSA with a long-term blood-culture service, but the Malawian dataset, though comprehensive in its AMR prevalence and incidence estimates, does not link drug-resistant infections to clinical metadata such as patient outcomes⁷.

Study approach

This study is designed to help address these knowledge gaps and aims to estimate the attributable mortality, morbidity and economic cost of 3GC-R BSI infection in Malawi by recruiting a prospective longitudinal cohort of patients who have bloodstream infection (BSI) with Enterobacterales. The burden and presentation of clinical infectious disease in sSA may not be the same as in resource rich settings, therefore a key strength of this study is its prospective nature, which enables investigators to collect high quality data by reviewing every patient alive by the time their culture is positive and to determine the role the isolated pathogen is playing in the clinical presentation.

The methodological challenges involved in designing studies that aim to accurately estimate the burden of AMR on patients and health systems, have recently been debated¹³. This consortium reflected upon the need to use the counterfactual approach to assessing burden of AMR, assuming that the likelihood of death would have been different if the pathogen had been susceptible. The counterfactual assumption is that death would not have occurred if the organism causing the infection had been drug-susceptible (or if there had been no infection)^{14,15}. This assumption allows for comparison between patients with resistant and susceptible bacterial infection¹³. This counterfactual approach is the one taken by this study, in which attributable mortality and other health outcomes will be estimated by making comparisons between patients with 3GC-R and comparable 3GC-S bloodstream infections, recruited in a prospective observational cohort.

Typically, a blood culture yielding a member of the family Enterobacterales would be considered to be of high clinical significance and thus trigger antimicrobial therapy to be commenced or refined. It would be unusual to classify such an organism as a contaminant¹⁶. Consequently, Enterobacterales are routinely included in AMR surveillance studies without further consideration¹¹. In the Malawian context, however, the limited availability of carbapenems and aminoglycosides means that patients whose blood culture is positive for 3GCR-E frequently remain untreated with an agent to which the isolate is susceptible. Despite this, patients often recover, posing the question, what role is the bacterial isolate playing in the patient's presentation?

If Enterobacterales are genuinely present in blood cultures as contaminants or cryptic organisms in significant numbers, it

would have profound implications for burden of AMR studies. Before the disease burden attributable to resistant bloodstream infections can be estimated, the role a given blood culture isolate is playing in the clinical episode must be characterised. This protocol has therefore been designed to leverage the prospective nature of this study to propose a method for classifying the impact of each positive blood culture on a patient, and the subsequent incorporation of these classifications into our analysis of morbidity, mortality and cost. We further describe the clinical, laboratory, economic, data-management and ethical components of the study.

Methods

Study design

The study is a prospective longitudinal observational cohort of patients whose blood culture is positive for Gram-negative pathogens, excluding *Salmonellae*, regardless of sensitivity pattern. Patients whose blood culture isolate is susceptible to ceftriaxone will be recruited following the same procedures as those whose blood culture isolate is resistant, so that mortality, morbidity and economic comparisons can be made between the two groups. Detailed inclusion and exclusion criteria are shown in [Table 1](#). *Salmonellae* will be excluded as 3GC-R in non-Typhoidal *Salmonella* remains sporadic and has not yet been reported in *Salmonella* Typhi in Malawi.

Study site

Malawi has a population of 17.5 million people and is classified as low income by the World Bank (2018 GDP of US\$ 7.1 Billion, ranking 149th out of 205 economies)¹⁷. Blantyre is the second city of Malawi, with a population of 800,264 and is located in Blantyre district, population 995,000 (2018 census) Life expectancy at birth is estimated at 64.3 years¹⁸. Malawi was one of 10 low-income countries to reduce its under-five mortality by at least two-thirds between 1990–2018, but infant and neonatal mortality remain high, at 38 and 22 per 1000 live births¹⁹. The study will be being conducted at Queen Elizabeth Central Hospital (QECH), the largest government hospital in the country. QECH provides free healthcare to Blantyre and the surrounding districts, plus tertiary care to Malawi's Southern region. It receives approximately 10,000 adult and

30,000 paediatric admissions per year⁸ and has 1,300 beds, frequently operating above capacity. In July 2017, the Mercy James Centre (MJC) for Paediatric Surgery and Intensive care was opened as a separate 50-bedded building, operating as part of QECH. MJC receives approximately 1,600 admissions per year and houses the country's only Paediatric Intensive Care Unit (PICU).

Blood culture service

A diagnostic blood-culture service, provided through the Malawi-Liverpool Wellcome Trust Clinical Research Programme (MLW) was established in 1998. MLW is affiliated with the Malawi College of Medicine and operates this service, 7 days/week, providing free aerobic blood cultures and cerebral spinal fluid (CSF) analysis to adult medical and paediatric patients. From March 2018, this service was extended to the Department of Obstetrics and Gynaecology, with a limited number of blood cultures offered per month.

Clinical blood culture protocols at QECH state that in adults, 7–10mls of blood should be taken in patients presenting to the emergency department with a fever (axillary temperature > 37.5C) or clinical suspicion of sepsis, severe sepsis or septic shock. In children, 1–2 mls of blood is taken in patients with non-focal febrile illness and a negative malaria test or in children with malaria whose fever persists despite treatment. A blood culture is also recommended in all premature or febrile neonates who are admitted to the neonatal unit. In a busy hospital with constrained resources and limited alternative diagnostics, blood cultures are often done on patients who do not fulfil these criteria, but at the discretion of the attending clinician. These patients will not be excluded from our analysis. The volume of blood taken for cultures in children in this setting is often low, potentially introducing bias to the study and selecting for patients with high bacterial load. This will be noted as a potential limitation to the study since resources for routinely weighing culture bottles are not available.

In the MLW laboratory, blood is inoculated into a single aerobic bottle using the automated BacT/ALERT system (bioMerieux, France). Enterobacterales and *Acinetobacter* are identified to species level using Analytical Profile Index testing (API)

Table 1. Study inclusion and exclusion criteria.

Inclusion criteria
Blood culture is positive for non- <i>Salmonella</i> Enterobacterales or <i>Acinetobacter</i> *
Patient is an inpatient at QECH or can be contacted for admission or assessment
Exclusion criteria
Blood culture is positive for <i>Salmonella enterica</i> (any serovariant)
Patient is unable to provide informed consent and there is no representative to provide informed consent
Patient speaks neither English or Chichewa

*During the set-up period for the study, it became clear that 3GC-R *Acinetobacter* spp. were an emerging problem at QECH, particularly amongst neonates. *Acinetobacter* are closely related to Enterobacterales, often sharing similar AMR profiles, and given their importance as a nosocomial pathogen, these patients will be included. However for analysis purposes we may do the primary analysis on the whole cohort and then Enterobacterales alone

(bioMérieux, France). Before March 2019, antimicrobial sensitivity testing (AST) was carried out as per British Society of Antimicrobial Chemotherapy (BSAC) guidelines²⁰, and from March 2019, as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines²¹ (Table 2). All blood culture isolates for patients recruited to the study will be regrown by a study laboratory technician and AST carried out as per EUCAST. The direct colony suspension method will be used to make a suspension of pure colony in 1ml 0.9% sterile saline solution, to the density of 0.5 McFarland turbidity standard. The resulting suspension will be streaked evenly onto Muller-Hinton agar (MHA), aiming for confluent bacteria growth. Antimicrobial discs will be applied and the resulting AST plates incubated at 35°C for 18 (+/-2 hours). Zones of inhibition for each antimicrobial will be measured to the nearest millimeter and susceptibility categories interpreted according to EUCAST breakpoint tables²². The laboratory adheres to UK National External Quality Assessment Service (NEQAS)²³.

For any Enterobacterales resistant to one or both of cefpodoxime or ceftriaxone on AST, extended spectrum beta-lactamase (ESBL) production will be confirmed using the species dependent combination disc method. The isolate will be cultured overnight on MHA with discs of cefotaxime and ceftazidime (30 micrograms) with and without clavulanic acid (10 micrograms). For all organisms capable of carrying chromosomal AmpC (*Enterobacter spp.*, *Serratia marcescens.*, *Citrobacter freundii*, *Providencia stuartii*, *Morganella morganii*, *Hafnia alvei*), an AmpC-stable cephalosporin, cefipime (30 micrograms), will be used with and without clavulanic acid (10 micrograms). ESBL production is confirmed if there was a difference of at least 5mm between discs with and without clavulanic acid.

The absolute number of blood cultures collected fluctuates on an annual basis, but has approached 21000 per year since 2013. Of these, approximately 13,000 are from paediatrics and 8,000 from adults. The most commonly isolated pathogens are

non-typhoidal Salmonellae, *Salmonella Typhi* and *Streptococcus pneumoniae* (estimated minimum incidence ≥ 300 /year) followed by the other Enterobacterales, in particular *E.coli* and *Klebsiella* spp. (50–299/year)⁷.

Participant selection and enrolment procedures

Daily reviews of the blood culture bench in the MLW microbiology laboratory will be conducted on Monday to Friday, to identify consecutive blood cultures which are positive for pathogens of interest. Blood cultures which become positive over a weekend will be identified by the study team for recruitment on a Monday morning. Once the blood culture result is final, patients will be identified in the hospital and enrolled following informed consent, aiming for recruitment as soon as possible after the final blood culture result is known. If a patient has been discharged by the time the final blood culture result is available, they will be contacted for review and potential recruitment if contact details are available. If a patient has died by the time the final result is available, they will still be included in the study and their medical records will be collected for review and CRF completion.

At enrolment, a baseline questionnaire will be conducted, collecting demographic and clinical information including admission vital signs, pre-hospital healthcare attendance, any treatments including antibiotics administered, health-related quality of life (HRQoL) and a health-care utilisation survey. Pre-hospital healthcare information will be used to classify infections into community acquired or healthcare associated. Questionnaires will be completed by a combination of patient interview, medical note review, and guardian interview if the patient is a child or is obtunded. In patients who have died by the time the blood culture is identified, the same questionnaires will be completed via medical note review, with data recorded as missing if it is not available. Vital signs will be performed by the study nurse at enrolment and patients assessed by a study clinician who will review the admission history and carry out clinical history and examination where indicated.

All participants will be reviewed as soon as possible after recruitment, by the study PI (a specialist trainee in infectious diseases) or a clinical member of the study team and discussed with the PI. If required recommendations on treatment and diagnostics will be made but as this is an observational study, the care of the patient will be directed by the clinical team responsible for the patient.

Sample collection

Blood samples will be collected from participants at enrolment and used to provide a set of baseline parameters that will aid in the clinical assessment of the participants illness. Blood will be tested for Full Blood Count (FBC) and creatinine and for CD4 count if HIV infected. Point of care tests will be carried out on capillary blood for capillary lactate (Lactate Pro 2, Arkray, Japan) and quantitative C-Reactive Protein (CRP) (CRP single test kit, used with the NycoCard II Reader, Abbott, UK, 1116078 and SBUK0028). HIV testing will be done as part of routine patient care, following Malawi national guidelines²⁴. If a patient's HIV status is unknown at the time of recruitment,

Table 2. Antimicrobial discs used in AST for blood culture isolates.

Enterobacterales	<i>Acinetobacter</i>
Co-trimoxazole 25 µg (SXT25)	Co-trimoxazole 25 µg (SXT25)
Gentamicin 10 µg (CN10)	Gentamicin 10 µg (CN10)
Ciprofloxacin 5 µg (CIP5)	Ciprofloxacin 5 µg (CIP5)
Meropenem 10 µg (MEM10)	Meropenem 10 µg (MEM10)
Amikacin 30 µg (AK30)	Amikacin 30 µg (AK30)
Chloramphenicol 30 µg (C30)	
Piperacilin-tazobactam (PTZ 36)	
Co-amoxiclav (AUG 30)	
Cefpodoxime (CPD10)	
Cefoxitin (FOX 30)	
Ceftriaxone (CRO30)	

they will be referred for HIV testing and counselling via standard QECH pathways. Urine will be collected at enrolment for screening dipstick and cultured if the dipstick is positive for leucocytes or nitrites. One stool sample will be taken at enrolment, or a rectal swab if it is not possible for a patient to provide stool. All other sample collection including urinary lipoarabinomannan (uLAM), and Sputum Xpert are done at the discretion of the clinical team providing routine care for the patient.

Follow up procedures

Patients will be followed up throughout their admission until discharge or death, allowing for measurement of in-hospital mortality. Information on treatments administered and vital signs will be recorded daily throughout the admission. To allow for survival analysis and calculation of 28-day and 6-month mortality, patients or their families will be telephoned at 28-days, three and six months post discharge. At follow-up, patients will be questioned to establish details of any antimicrobials received or healthcare facility usage since the last phone contact. If a patient dies, family members are asked the date of death.

Classification of Gram-negative blood culture

A preliminary set of classifications will be developed to describe the impact of each positive blood culture on each participant, following the anecdotal observation that patients were surviving 3GC-R BSI episodes without receiving an antibiotic to which the organism is susceptible. These categories will initially be developed by the study PI (a specialist trainee in infectious diseases), and will be used to broadly classify each BSI episode into the following categories: definite Gram-negative sepsis, probable Gram-negative sepsis, transient or occult bacteraemia, or contaminated blood culture.

An expert panel, consisting of locally experienced physicians, adult and paediatric infectious disease specialists and a consultant microbiologist will then be assembled to pilot and finalise the classifications. This group will be presented with clinical vignettes for six patients and asked to anonymously classify each patient into the set of preliminary categories. An example of these vignettes, as they will be presented to the panel, are shown in [Table 3](#). Any discrepancies in classifications will be discussed between the group and a final set of classifications and definitions decided. These classifications are shown in [Table 4](#).

Table 3. Participant vignettes: three example participants discussed at the consensus meeting, shown with final decisions on patient classification.

<p>Participant-1</p> <p><i>E.coli</i></p> <p>S: ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, meropenem</p> <p>R: ampicillin, cotrimoxazole</p> <p>Classification: Definite Gram-negative sepsis</p>	<p>26 year old female</p> <p>HIV negative, normally fit and well. 2 weeks postpartum. Caesarian section done at Queen Elizabeth Central Hospital. Unwell for 10 days post-operatively: abdominal pain, and fevers.</p> <p>Admission: Temperature 40.0°C. SIRS = Yes</p> <p>Recruitment Day 7: Temperature 37.0°C. SIRS= Yes Wound clean. No urinary catheters. Urine dipstick negative.</p> <p>Antibiotics: 9 days ceftriaxone, 5 days ciprofloxacin</p> <p>Bloods Day 7: WCC 8.3 x10⁹ L⁻¹, CRP 91 mg/L, Lactate 2.6,</p> <p>Outcome: Discharged alive</p>
<p>Participant-2</p> <p><i>E.coli</i></p> <p>S: ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, meropenem</p> <p>R: ampicillin, cotrimoxazole</p> <p>Classification: Definite Gram-negative sepsis</p>	<p>72 year old male</p> <p>HIV positive, on ART 10 years. Benign prostatic hyperplasia. Unwell for 3–4 weeks: confusion, cough, weight loss, lethargy.</p> <p>Pre-hospital: Co-amoxiclav and azithromycin within 1 month of admission. Week 2 TB Treatment</p> <p>Admission: Temperature 35.7°C, GCS 10. SIRS = Yes</p> <p>Recruitment Day 5: Temperature 37.5°C. SIRS = No</p> <p>Bloods Day 5: CD4 158 µL⁻¹, WCC 26.0 x10⁹ L⁻¹, CRP >120mg L⁻¹, lactate 3.4mmol L⁻¹, creatinine 862mmol L⁻¹</p> <p>Antibiotics: Ceftriaxone 24 hours</p> <p>Outcome: Died in hospital</p>

<p>Participant 3</p> <p><i>E.coli</i></p> <p>S: chloramphenicol, gentamicin, amikacin, meropenem, co-amoxiclav</p> <p>R: ceftriaxone, ampicillin, cotrimoxazole, ciprofloxacin</p> <p>Classification: Possible Gram-negative sepsis</p>	<p>32 year old woman,</p> <p>HIV positive, ART 2 years. Mechanical fall into drain, pain in hip and hx of fevers 24 hours later. Associated headache and diarrhoea. Presented to Emergency Department after 5 days, had blood culture and outpatient follow-up arranged. No vital signs available on admission. Discharged on no antibiotics.</p> <p>Bloods Day 6: WCC $6.3 \times 10^9 \text{ L}^{-1}$, creatinine 39 mmol L^{-1}, CD4 $847 \mu\text{L}^{-1}$, lactate 2.6 mmol L^{-1}, CRP 36 mg L^{-1}, urine dip negative</p> <p>Repeat blood culture on Day 6 = negative, no antibiotics in between</p> <p>Antibiotics: one dose of gentamicin on day 6, then discharged on nothing.</p> <p>Outcome: Discharged alive</p>
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Abbreviations: SIRS, systemic inflammatory response syndrome; ART, antiretroviral therapy; WCC, white cell count; CRP, C-reactive protein

Table 4. Classification scheme for Gram-negative blood cultures.

Category	Definition
Definite Gram-negative sepsis consequent upon cultured isolate	The blood culture isolate is contributing to the patient's clinical state and treatment was considered to be required.
Probable Gram-negative sepsis consequent upon cultured isolate	The blood culture isolate is probably contributing to the patient's clinical state, but there is insufficient evidence to confirm or refute this. Treatment was considered to be required
Possible Gram-negative sepsis consequent upon cultured isolate	The blood culture isolate may be contributing to the patient's clinical condition, but the patient improved without antibiotics predicted to be active based on antimicrobial susceptibility testing, and it is not possible to confirm or refute definite/probably Gram-negative (GN) sepsis. i.e. Treatment was considered to be required but the patient improved without antibiotics likely to be active against the isolate.
Occult or transient bacteraemia	The blood culture isolate may have contributed to the patient's clinical condition, but by the time they are assessed with the culture, they have improved. Unlike the definition for 'possible GN sepsis', treatment was not considered to be required, but instead a repeat blood culture was desirable.
Definite contaminant	The isolate has never contributed to patient's condition and was very likely not present in the bloodstream
Probable contaminant	The isolate probably never contributed to patient's condition and was probably not present in the bloodstream/or there is insufficient evidence to say for sure.

On completion of recruitment, the first 50 study participants will be presented to the expert panel and classified into definite/probable or possible Gram-negative sepsis, transient or occult bacteraemia, or definite/probably contaminant (Table 4). For neonatal patients, a consultant neonatologist will be included. The panel will first be asked to anonymously categorise the participants and individual responses will be recorded. Any discrepancies will be discussed and resolved by consensus. Following review and classification of the first 50 participants, the panel will re-assess the classification process to decide if sufficient clinical

and laboratory data are available to confidently classify patients in this manner. At a minimum, patients will be objectively defined on the basis of:

1. Having a severe inflammatory response syndrome (SIRS) or not;
2. Their treatment response (clinical improvement based on factors such as resolution of symptoms and signs including fever, with or without active antibiotics or died with or without active antibiotics).

The lack of robust and universally validated sepsis severity score applicable to a cohort of both adults and children in a low-income setting²⁵, means that SIRS criteria will be used as a minimum to help classify patients, alongside other clinical and biochemical parameters.

In addition, the panel will be asked for consensus on likely focus of clinical infection in patients who are considered to have a definite/probable or possible Gram-negative sepsis (Table 5). The panel will be asked to classify into likely rather than definite focus, because of a desire not to overclassify non-focal sepsis in a setting where lack of diagnostic resources frequently limit the ability to definitively confirm focus of infection.

Data analysis plan for primary outcomes

The primary outcomes for the study are mortality and morbidity from 3GCR-E. The effect of 3GCR -E on mortality and discharge alive will be estimated using a logistic regression model for 28-day mortality and a Cox proportional hazards model for time to death, adjusting for confounders such as patient co-morbidities. In the first instance, these models will be fitted to mortality data from the complete cohort. Uncertainty in true bloodstream infection status will then be explored using a Bayesian latent class model which incorporates the output of the classifications as a latent variable. Taken together with BSI incidence estimates from Blantyre⁷, this will allow estimation of mortality from BSI in the Blantyre, assuming that cases admitted to QECH are representative of the general population. We

will use hospital length of stay as a proxy outcome measure for morbidity⁸. To estimate length of hospital stay associated with 3GC-R-E, we will use multistate modelling, with time from hospital admission as the time scale²⁶. Statistical analyses will be conducted using R (R Foundation for Statistical Computing, Vienna, Austria).

Health economic components

The health economic data collection will allow for three types of comparisons between patients with 3GC-R and 3GC-S BSI: healthcare provider costs, costs incurred by patients and their families as a result of hospitalisation, and health-related quality of life (HRQoL). Primary costing studies and data capture tools described below have been developed and validated for adult inpatients only²⁷, therefore children under 18 are excluded from this component of the study.

Healthcare provider costs. Upon discharge or death, information from the patient's medical record will be extracted by a study clinician, to establish the medications and dosages given, duration of hospital admission, types and numbers of investigations and procedures performed and the participant's outcome. Costs of these healthcare resources will be derived from a previous primary costing study undertaken at QECH²⁷. The international market price will be used to estimate costs for all medication given²⁸.

Direct non-medical and indirect costs. Questionnaires will be administered to patients and their guardians as soon as possible after recruitment. All questionnaires are provided as extended data²⁹. Data collected will include cost of transportation, food, drinks, toiletries, clothing and other items bought during the hospital admission. For indirect costs, any time off work taken by participants or their guardians is recorded together with self-reported income. The development and language translations of these questionnaires followed previous procedures^{27,30}.

Health-related quality of life. The Chichewa version of the EuroQoL EQ-5D-3L will be used to assess HRQoL of participants at recruitment and discharge from hospital as well as at 28-day follow-up³¹. The EQ-5D has a descriptive component asking participants to rate their health status across a number of domains and a visual analogue scale (VAS) similar to a thermometer, and ranges from 100 (best imaginable health state) to 0 (worst imaginable health state). EQ-5D utility scores will be derived from responses to the descriptive components using the Zimbabwean EQ-5D tariff set³².

Mean differences in total direct health provider cost, total direct non-medical and indirect cost and HRQoL outcomes between participants with 3GC-R and 3GC-S BSI will be estimated. Non-parametric bootstrap methods will be used to account for possible skewness in distribution of economic data. Multivariable analysis will be undertaken to explore the

Table 5. Clinically suspected focus of infection.

Focus
Non-focal
Central-line associated
Clear focus of infection
<ul style="list-style-type: none"> • Urinary tract infection • CNS • Skin and soft tissue • Gastrointestinal (hepatobiliary) • Gastrointestinal (non hepatobiliary) • Cardiovascular system • Respiratory tract infection (other than VAP) • Reproductive tract infection • Surgical site infection • Bone and joint • Post-operative, non-focal • VAP
Unknown
Other

Abbreviations: CNS, central nervous system; VAP, ventilator associated pneumonia

independent effects 3GC-R on these economic costs and HRQoL outcomes. For HRQoL outcomes, EQ-5D utility and VAS scores will also be compared between participants at recruitment, discharge and follow-up where data are available.

Data capture and storage

Data will be collected using [Open Data Kit](#) software (ODK, 1.4.10) and [TeleForm Data Capture Software](#) (10.7). Completed ODK forms are pushed daily to a dedicated secure SQL database. Teleform paper forms will be checked, scanned and validated by the MLW data team, in discussion with the clinical team if required and validated TeleForm data pushed to the SQL database. Completed paper TeleForm records will be stored securely in the MLW data department. All data on the study database will be stored securely with access restricted to the study PI and the database administrators in the MLW data department. Results of laboratory investigations in the MLW laboratory will be stored in the MLW PreLink laboratory information management system (LIMS), anonymised and linked only to the participant unique study ID number.

Sample size considerations

The study is powered to detect a difference in 28-day mortality rates between participants with 3GC-R and 3GC-S BSI. There are no studies from sSA powered to detect mortality from ESBL BSI, on which to guide our sample size estimates, but a large multi-centre European study found that mortality was 14% higher in patients who had an ESBL positive BSI versus those who had ESBL negative BSI³³. Based on this, we aim to recruit 250 patients to the cohort, which would provide 80% power to detect a difference in 28-day mortality rates of 10% vs. 24.1%. If this recruitment target is not achieved, a more modest 200 patients would still provide 80% power to detect mortality of 10% vs 25.8%. These calculations assume a 50:50 split in 3GC-R and 3GC-S infections, based on 2016 figures⁷. An imbalance in this split will have minimal impact on the statistical power of the study. We are aiming to recruit 250 patients who have definite, probable or possible Gram-negative sepsis, therefore have inflated the overall sample size to 350 participants given that some patients will be censored from the study following expert case review.

Ethics

Ethical approval for the study was granted by the Malawi College of Medicine Research Ethics Committee (COMREC), protocol number P.10/17/2299 and by the Liverpool School of Tropical Medicine Research Ethics committee, protocol number 17-063. LSTM acted as the study sponsor. Written informed consent is obtained from study participants, the participant's parent/guardian if they are a child aged 18 years, or from a guardian if the patient lacks capacity to consent. Written informed consent is obtained from study participants, the participant's parent/guardian if they are a child aged 18 years, or from a guardian if the patient lacks capacity to consent. See below for

explanation of consent for patients who have died. In addition to parental consent, assent was sought from children aged eight years and above, in accordance with WHO guidelines and the requirements of the local ethics committee³⁴.

As consent was not possible for patients who had died by the time of enrolment, all medical records were anonymised by the study clinical officer, who was a Malawi Ministry of Health employee, at the request of COMREC. Once recruited, no other member of the study team had access to any identifiable medical records for these patients.

Dissemination

Results from this study will be presented internally within the College of Medicine and QECH, Malawi College of Medicine Research Ethics Committee and disseminated to the Ministry of Health, Malawi. Manuscripts will subsequently be prepared for publication in peer-reviewed journals, which will be made freely available via open-access publication.

Study status

Recruitment to the study is currently ongoing and is expected to be completed in March 2020.

Discussion

This study is designed to investigate the attributable morbidity, mortality and economic cost of third-generation cephalosporin resistant bloodstream infections in Malawi, a country which has the largest bacteraemia and AMR surveillance dataset from sSA, but in which the health burden of AMR infections is currently unknown. We aim to address this knowledge gap by assessing the healthcare burden of resistance to one of the most commonly used and frequently last-line antibiotics in hospitalised inpatients in Malawi.

Our approach of prospective recruitment and detailed characterisation of all BSI episodes will generate reliable data on the impact of bloodstream infection on patients, and in turn, the burden of 3GC-R infection. In line with recently published guidance on quality reporting of AMR data³⁵, we will be able to provide a clear account of microbiological sampling criteria, sampling frame and laboratory methods, as well as clinical metadata including empiric antibiotic regimens, HIV status and healthcare attendance.

This study is limited to one hospital but it is hoped that these data will be used to generate accurate burden estimates for Malawi, and that the methods will be replicated by future investigators wishing to generate robust data on the impact of drug-resistant infections. By estimating attributable mortality, morbidity and economic cost in a prospective cohort, we will generate high quality data that will be amongst the first of their kind from sSA and that will consequently be able to inform global burden of disease estimates

Data availability

Underlying data

No data are associated with this article

Extended data

Zenodo: Case report forms (CRFs) used for the publication: Estimating the burden of antimicrobial resistance in Malawi: protocol for a prospective observational study of the morbidity, mortality and economic cost of third-generation cephalosporin resistant bloodstream infection. <http://doi.org/10.5281/zenodo.3634466>²⁹

This project contains the following extended data:

- CRFs_Wellcome.pdf (Study questionnaires)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

The authors would like to thank the clinical staff and patients at Queen Elizabeth Central Hospital and the Laboratory Staff at MLW.

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Priscilla Rupali

Infectious Diseases Training and Research Center, Christian Medical College, Vellore, India

I am happy with the revisions and explanations provided. I give my approval for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious Diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 09 June 2020

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Bieke Tack 

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Jan Jacobs

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A non-blocking comment from our side is about the qSOFA versus the SIRS score: as the authors have written in their reply to our comments, a sepsis classification system is extremely challenging in a low-resource setting. However, we do not agree that the cited letter to the editor¹ provides enough evidence to state that qSOFA score is not likely to be useful for clinical management or research in a

low-resource setting. The letter questions the superiority of the qSOFA score to the SIRS score, which was assessed by Rudd *et al.* in ten low- and middle income countries². They state that the data from Rudd *et al.* are too biased for comparison of the predictive performance between the qSOFA and SIRS score. However, even so, Rudd *et al.* provide interesting data that support the use of qSOFA as a bedside clinical decision-making tool. In contrast to the SIRS score, the qSOFA score does not need laboratory testing and is a less technically challenging alternative for the low-resource context.

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Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical Bacteriology.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 27 April 2020

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Priscilla Rupali

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The idea is definitely good and will provide meaningful data which will likely help in investment of resources in bringing AMR down.

Overview: This study protocol attempts to in a prospective manner compare mortality, morbidity and economic impact between infections which are caused by 3rd GC resistant vs sensitive GNB *E.coli* and *Klebsiella spp.* Data regarding this is available from high income countries and they hope to derive this information in sub Saharan Africa.

Ethical approval has been granted for the study.

Comments:

Study Approach: In this section, the authors have described the general approach and various strength of their study. I agree with the authors that a prospective study studying this is important and may provide new contextual information relevant to the specific clinical setting in SSA.

- Classifying patients by experts may not be required if the definitions set out for definite, probable and transient BSI are followed by the study team for recruitment. I am not sure, review by experts adds anything to this.
- The primary outcomes are not well defined and I presume are mortality, LOS as predictor of morbidity and costs.
- Counterfactual approach – not validated terminology and is best excluded as it confuses the readers.
- Severity of disease needs to be better defined by a validated score qSOFA or APACHE rather than SIRS as it is likely to have bearing on the primary outcome.
- "If Enterobacteriales are genuinely present in blood cultures as contaminants or cryptic organisms in significant numbers, it would have profound implications for burden of AMR studies." - I am not sure I agree with this. If a positive blood culture is classified as a contaminant – in the absence of corroborating clinical features, antibiotics are unlikely to be given and this contribution to AMR burden is unlikely to be significant.

Data Analysis: "Uncertainty in true bacterial infection will be explored by a latent variable approach and a bayesian paradigm". Generally this approach is used for a diagnostic test where there is no established gold standard. It is not applicable here.

- It is unclear exactly what variables are being collected which could impact the primary outcome of death in the 2 groups.
- Antibiotics - appropriateness, duration, empirical antibiotics given or not are not mentioned.
- Primary outcomes and variables affecting that are not mentioned in coherent detail.
- Appreciate that economic and HRQoL outcomes have been mentioned in a fair bit of detail.
- It has been mentioned "In patients who have died by the time the blood culture is identified, the same questionnaires will be completed via medical note review, with data recorded as missing if it is not available" - will these missing data be included in the analysis?

Participant Selection and Enrollment Procedures:

- Table 1 - "**Patient** speaks neither English or Chichewa" - is an exclusion criteria but it is unclear what happens when recruiting children or if patient passes away before blood culture is obtained.
- "If a patient has died by the time the final result is available, their medical records will be collected for review" - it is unclear how informed consent will be obtained from a family member .

Follow-up Procedures: "If a patient dies, family members are asked the date of death", thus this mean only date of death or details as mentioned earlier regarding antimicrobial therapy or healthcare facility admissions? Kindly clarify.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious Diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 May 2020

Rebecca Lester, Liverpool School of Tropical Medicine, Liverpool, UK

Comments:

Study Approach: In this section, the authors have described the general approach and various strength of their study. I agree with the authors that a prospective study studying this is important and may provide new contextual information relevant to the specific clinical setting in SSA.

· **Classifying patients by experts may not be required if the definitions set out for definite, probable and transient BSI are followed by the study team for recruitment. I am not sure, review by experts adds anything to this.**

Many thanks for this feedback. We believe that the expert panel review is essential to the classifications process as expertise in local infectious disease management is important when making decisions surrounding the complex patients in this setting. Infectious disease clinicians are experienced in making decisions about the need for antibiotics in patients with a positive blood culture whereas the study team are not. Additionally, at the point of recruitment it is often the case that not all the required clinical information (such as blood tests) are available, and these need to be collated for these discussions.

The study team will reflect on whether this was a useful exercise post-hoc in the results manuscript.

The primary outcomes are not well defined and I presume are mortality, LOS as predictor of morbidity and costs.

We have added a sentence to the data analysis section as follows:

The primary outcomes for the study are mortality and morbidity from 3GCR-E.

Counterfactual approach – not validated terminology and is best excluded as it confuses the readers.

Please also see our response to reviewer 1's comments on the counterfactual approach. We have chosen to keep this terminology, which is a standard approach for causal inference in epidemiological studies, but expanded on our explanation of the term and included further references, including to the article written by members of the Surveillance and Epidemiology of

Drug-resistant Infections Consortium (SEDRIC) consortium, which describes the importance of the counterfactual approach in AMR research.

Severity of disease needs to be better defined by a validated score qSOFA or APACHE rather than SIRS as it is likely to have bearing on the primary outcome.

Please also see our response to reviewer 1's comments, with regards to the difficulties with these scores. Notably, qSOFA is not validated in low income settings[1], nor in paediatric populations, and APACHE scores require parameters which are not available in our study setting, such as serum sodium and potassium. SIRS has been chosen because the required parameters are readily available, but will simply be used as a minimum criteria for classifications, which will also take into account other variables such as CRP and lactate.

"If Enterobacteriales are genuinely present in blood cultures as contaminants or cryptic organisms in significant numbers, it would have profound implications for burden of AMR studies." - I am not sure I agree with this. If a positive blood culture is classified as a contaminant – in the absence of corroborating clinical features, antibiotics are unlikely to be given and this contribution to AMR burden is unlikely to be significant.

Many thanks for this feedback and we agree that this is a complex area. However, in the majority of cases, and particularly in Malawi, Enterobacteriales are rarely if ever considered contaminants by clinicians and are invariably treated with active antibiotics where they are available. Further, data from diagnostic microbiology laboratories are frequently reported to ministries of health and thence to GLASS as aggregate data, without any reference to clinical metadata beyond that which is on the request form. The lack of standardisation of how microbiological data are captured is a major problem in assessing the global burden of drug resistant infection, which may well lead to overestimates. It is the prospective nature of this study and expert review of all cases that allows the distinctions between contaminants and true infections to be made and we believe this is a strength of the study.

Data Analysis: "Uncertainty in true bacterial infection will be explored by a latent variable approach and a bayesian paradigm". Generally this approach is used for a diagnostic test where there is no established gold standard. It is not applicable here.

Please also see our response to Reviewer 1's comments. A Bayesian latent class model will be used to explore uncertainty in true BSI status given the clinical classifications decided by the expert panel. This is a standard approach for dealing with uncertainty in models from clinical observational studies in addition to studies evaluating diagnostic tests[3].

It is unclear exactly what variables are being collected which could impact the primary outcome of death in the 2 groups.

Unfortunately, due to space limitations we are unable to describe all of these variables fully in the text. However, the study CRFs are included as extended data and referenced in the main paper. For example, we will collect data on patient co-morbidities, baseline vital signs and previous healthcare exposures – all of which could be acting as confounders in outcome models.

Antibiotics - appropriateness, duration, empirical antibiotics given or not are not mentioned.

As this is an observational study, these will depend on the clinical care provided by the patient's team and will not be prescribed as part of the study. We have clarified this in the text (Methods/Participant selection and enrolment procedures) (please also see response to reviewer 1's comments).

Primary outcomes and variables affecting that are not mentioned in coherent detail

We have now clarified the primary outcomes. As mentioned above, study CRFs with all variables collected are included as extended data.

Appreciate that economic and HRQoL outcomes have been mentioned in a fair bit of detail.

We thank the reviewer for their support

It has been mentioned "In patients who have died by the time the blood culture is identified, the same questionnaires will be completed via medical note review, with data recorded as missing if it is not available" - will these missing data be included in the analysis?

Any data that are available from patients who have died will be included, and missing data will be accounted for in the final analysis through a process of multiple imputations.

Participant Selection and Enrollment Procedures:

Table 1 - "Patient speaks neither English or Chichewa" - is an exclusion criteria but it is unclear what happens when recruiting children or if patient passes away before blood culture is obtained.

Please see the Ethics section for an explanation of consent in children. We have added a sentence about assent, so the paragraph now reads:

Written informed consent is obtained from study participants, the participant's parent/guardian if they are a child aged 18 years, or from a guardian if the patient lacks capacity to consent. See below for explanation of consent for patients who have died. In addition to parental consent, assent was sought from children aged eight years and above, in accordance with WHO guidelines and the requirements of the local ethics committee.

"If a patient has died by the time the final result is available, their medical records will be collected for review" - it is unclear how informed consent will be obtained from a family member .

An ethical waiver for informed consent was obtained from the local Research Ethics Committee. Instead, files of patients who have files are anonymised by a hospital employee and then reviewed by a member of the study team. We have added the following paragraph:

As consent was not possible for patients who had died by the time of enrolment, all medical records were anonymised by the study clinical officer, who was a Malawi Ministry of Health employee, at the request of COMREC. Once recruited, no other member of the study team had access to any identifiable medical records for these patients.

Follow-up Procedures: "If a patient dies, family members are asked the date of death", thus this mean only date of death or details as mentioned earlier regarding antimicrobial therapy or healthcare facility admissions? Kindly clarify.

Family members are asked date of death in addition to the other questions asked to surviving patients at follow-up.

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Competing Interests: No competing interests were disclosed.

Reviewer Report 02 March 2020

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Bieke Tack

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Jan Jacobs

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The paper describes a study protocol used to assess the impact (mortality, direct and indirect costs and other healthcare outcomes) compared between patients infected with ceftriaxone-resistant versus ceftriaxone-susceptible *Enterobacteriales* bloodstream infections. The study protocol has already been granted ethical approval.

Major comments

- The authors correctly observed that patients with clinical suspicion of sepsis and culture-proven bloodstream infection caused by third generation cephalosporin resistant *Enterobacteriales* survived despite treatment with non-effective antibiotics. In addition to "*question the role of the bacterial isolate playing in the patient's presentation*", there is the fact that also in the pre-antibiotic era, a proportion of patients survived bloodstream infections – see Friedman (2016)¹. So the reasoning of "survived = probably contaminant" may not be apply in all situations, e.g. not in settings with less severe symptoms and correctable causes such as urinary catheter-related bacteremia. It would be good to address this in the Discussion session.

- P3: it is not clear how data of patients who died are processed "...by reviewing every patient alive by the time their culture is positive" means that patients who died with 24h – 48h (before expected growth of blood cultures) are not included? This may impact the study results, as – depending on the referral track and type of patients (children) – mortality may be highest in the first 24h upon admission. However, on P4 it is stated that "If a patient has died by the time the final result is available, their medical records will be collected for review".
- P5: the authors may have good reasons to stick to "severe SIRS" but it I suggest to address these reasons (e.g. why not using the qSOFA) or any other gradable disease severity classification applicable to adults, children and neonates– this may be challenging. Is there any use of the lactate or CRP results (one or serial measurements)?
- P5 statistics: I am not an expert in this domain. My main comment is: "*latent variable approach and a Bayesian paradigm*" is a bit vague, do the authors mean they will use both approaches in 1 model (e.g. Bayesian Latent Variable Modeling) or build 2/more models (Bayesian stats, structural equation modelling,...) and compare them? Further, it is not clear how they will build the latent variable, will it be built on the criteria that will used for the classification? Is there any validation to assume the length of hospital stay as a proxy for morbidity? It would be good to assess also how differences in co-morbidity (and their effect on the outcomes) are taken into account.
- P5: suggest to specify how "got better" - Fever resolution, discharge, symptom resolution...
- P4 Representativeness: blood culture sampling indications may not be strictly followed, with possibilities to under- and over-sampling (see P4) – is a "screening log" applied to allow estimation of the non- or incorrectly sampled patients?
- P5 Clinical follow-up: both in the paragraph on recruitment and follow-up procedures, I miss data about treatment guidelines and collection of treatment data (including correct dosage in children). If I understand it correctly, not only the patients treated with ceftriaxone will be enrolled? Is there any classification according to (available and affordable) treatment alternatives in case of ceftriaxone-resistance? Is there a hospital policy about choice of second- and third-line antibiotics?
- P7 Table 3: I suggest to take into account the issue of healthcare-associated infections, for which certain *Enterobacteriales* (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*...) and *Acinetobacter* may be more likely to involved (transient bacteremia; pseudo-bacteremia) than for instance *E. coli* which is more likely to be endogenous.
- P7 Table 4: some of the "*clear focus*" may be difficult to assess – for instance "Urinary Tract Infection" and the "Respiratory tract infection" and "VVAP"
- P6 – P7: Clinical care and follow-up: it is not clear at which moment the patients will be assessed and by who: if the assessment is done by a trained panel or experts, one can expect sensible and relevant therapeutic and diagnostic actions during the hospital course (e.g. extra cultures at surgical debridement, additional x-ray...). They may influence patient outcome. How will this be handled?

- Table 1: Not clear why *Acinetobacter* is included – in terms of habitat and behavior, it is different from *Enterobacteriales* as well as from the other Gram-negative fermentative rods.
- P3: the "counterfactual" approach is not well described for naïve readers – this should be addressed as it is key to the approach.
- "*Blood cultures which are positive over a weekend will be identified on Monday morning*" meaning that a 2-day delay in identification and antibiotic susceptibility testing will occur and that directed treatment will be delayed – this can be important in situations where empiric treatment did not include effective treatment against *Enterobacteriales* – is this an issue or is ceftriaxone a part of the standard empiric treatment?

Minor comments

- Sensitive: suggest to replace by "Susceptible".
- Study Site: for reasons of representativeness, comparison and generalizability, some national demographic and health data could be provided (aside from the GDP).
- Study Site: why are only the admission data for the MJC supplied (and not those for the entire hospital)?
- Study Site: I suggest to give an overview of the (estimated) distribution adult versus children blood culture samples as well as community versus hospital-associated infections.
- Diagnostic bacteriology: blood volumes sampled are (too) low: this is a reality in low-resource settings but its implications should be discussed – it is possible that mainly patients with high bacterial loads will be selected and included.
- Diagnostic bacteriology: not clear which antibiotics will be tested and of ESBL testing is done.
- Clinical presentation P5: not clear what is meant with "*admission physiology*": vital signs, clinical signs and symptoms, illness severity,...?
- The Abstract might be restructured to give more essential and detailed study information such as the (i) concrete classification of the role of the isolated bacteria and (ii) the healthcare economic data.
- Abstract: Some abbreviations not explained previously (3GC-S) and there is some inconsistency (3GCR-E versus 3GC-R) - probably it will be easier if no abbreviations are used.
- In line with the Keywords, "Africa" (L3) might be changed to "sub-Saharan Africa".
- References: Some references are (too) briefly cited (e.g. ref. 15 "World Bank). Suggest to add websites + date of access where appropriate.
- Consistency of abbreviations or unusual abbreviation: 3GCR-E versus 3GC-R and DRI – only used a few time in the text.
- Style: P4 and elsewhere: paragraph of methods mixes past, future and present tense.

References

1. Friedman ND, Temkin E, Carmeli Y: The negative impact of antibiotic resistance. *Clin Microbiol Infect.* 2016; **22** (5): 416-22 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical Bacteriology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 23 May 2020

Rebecca Lester, Liverpool School of Tropical Medicine, Liverpool, UK

The paper describes a study protocol used to assess the impact (mortality, direct and indirect costs and other healthcare outcomes) compared between patients infected with ceftriaxone-resistant versus ceftriaxone-susceptible *Enterobacteriales* bloodstream infections. The study protocol has already been granted ethical approval.

Major comments

The authors correctly observed that patients with clinical suspicion of sepsis and culture-proven bloodstream infection caused by third generation cephalosporin resistant *Enterobacteriales* survived despite treatment with non-effective antibiotics. In addition to "question the role of the bacterial isolate playing in the patient's presentation", there is the fact that also in the pre-antibiotic era, a proportion of patients survived bloodstream infections – see Friedman (2016)¹. So the reasoning of "survived = probably contaminant" may not be apply in all situations, e.g. not in settings with less severe symptoms and correctable causes such as urinary catheter-related bacteremia. It would be good to address this in the Discussion session.

Many thanks for this feedback and reference. Patients will not automatically be classified as probable contaminant if they survive the episode, but classified based on discussion of the case by the panel, taking into account all available information on each patient – including but not limited to their treatment response. For example, if a patient recovers because of source control (e.g. catheter removal) this would be noted by the panel and taken into account. The definition of "probable contaminant" in Table 3, is "*the isolate probably never contributed to patient's condition*

and was probably not present in the bloodstream/or there is insufficient evidence to say for sure." This definition allows for a more nuanced approach which accounts for all available parameters and the clinical judgement from the panel. The criteria are described in Table 4.

P3: it is not clear how data of patients who died are processed "...by reviewing every patient alive by the time their culture is positive" means that patients who died with 24h – 48h (before expected growth of blood cultures) are not included? This may impact the study results, as – depending on the referral track and type of patients (children) – mortality may be highest in the first 24h upon admission. However, on P4 it is stated that "If a patient has died by the time the final result is available, their medical records will be collected for review".

All patients who have died before the final blood culture result is available are screened and included in the study if possible. Information from their medical notes is recorded in the study CRFs via a retrospective note review. We have clarified this by removing the sentence "...by reviewing every patient alive by the time their culture is positive" and will expand the sentence on page 4 to read:

If a patient has died by the time the final result is available, they will still be included in the study and their medical records will be collected for review and CRF completion.

P5: the authors may have good reasons to stick to "severe SIRS" but it I suggest to address these reasons (e.g. why not using the qSOFA) or any other gradable disease severity classification applicable to adults, children and neonates– this may be challenging. Is there any use of the lactate or CRP results (one or serial measurements)?

We agree that a sepsis classification system applicable to this cohort is extremely challenging and a universal grading system which is validated across all age groups in a low-income setting, is not available. qSOFA scores, for example, are not validated in LMICs and are not likely to be useful for clinical management or research in this setting[1]. We have chosen SIRS therefore, as a *minimum* criteria for objective classification. However, when the panel reviews the clinical vignettes, all available information, including both lactate and CRP, will be taken into account when deciding if a patient has sepsis. We now explain this approach further on P5, with the following text:

The lack of robust and universally validated sepsis severity score applicable to a cohort of both adults and children in a low-income setting[1], means that SIRS criteria will be used as a minimum to help classify patients, alongside other clinical and biochemical parameters.

P5 statistics: I am not an expert in this domain. My main comment is: "latent variable approach and a Bayesian paradigm" is a bit vague, do the authors mean they will use both approaches in 1 model (e.g. Bayesian Latent Variable Modeling) or build 2/more models (Bayesian stats, structural equation modelling,...) and compare them? Further, it is not clear how they will build the latent variable, will it be built on the criteria that will used for the classification? Is there any validation to assume the length of hospital stay as a proxy for morbidity? It would be good to assess also how differences in co-morbidity (and their effect on the outcomes) are taken into account.

This will be one model –which takes a Bayesian latent class approach. This approach is simply a method to explore the uncertainty in each patient's true BSI status, acknowledging that it is error-prone. The latent variable will incorporate the output of the classifications. We have altered the text to make this clearer:

Uncertainty in true bloodstream infection status will then be explored using a Bayesian latent class model which incorporates the output of the classifications as a latent variable.

Any confounders such as co-morbidities will be conditioned upon in the mortality models. We have clarified this in the text with an additional sentence in the data analysis section.

Hospital length of stay is a frequently used as a proxy outcome measure of patient morbidity. Other morbidity outcome measures such as ICU admissions, need for surgery, re-hospitalisation and disability status at discharge or follow-up, are more variably used, and not always applicable to low-income settings[2]. We have included an additional reference for this and clarified this in the text.

P5: suggest to specify how "got better" - Fever resolution, discharge, symptom resolution...

We have changed this to "clinical improvement based on factors such as resolution of symptoms and signs including fever". We have not defined specific criteria as these may not be applicable to the entire cohort.

P4 Representativeness: blood culture sampling indications may not be strictly followed, with possibilities to under- and over-sampling (see P4) – is a "screening log" applied to allow estimation of the non- or incorrectly sampled patients?

We are reliant on blood cultures obtained through the routine clinical service at QECH. Given the huge numbers of patients presenting to the hospital daily, it is unfortunately not logistically possible to have a screening log for all potentially eligible patients presenting to the Emergency Department, nor would a screening log adequately capture the reasons a clinician chose to culture, or not to culture any given patient. This is a potential limitation of the data which we will have the opportunity to discuss following analysis.

P5 Clinical follow-up: both in the paragraph on recruitment and follow-up procedures, I miss data about treatment guidelines and collection of treatment data (including correct dosage in children). If I understand it correctly, not only the patients treated with ceftriaxone will be enrolled? Is there any classification according to (available and affordable) treatment alternatives in case of ceftriaxone-resistance? Is there a hospital policy about choice of second- and third-line antibiotics?

It is correct that all patients will be enrolled, regardless of treatment as this is an observational study with no treatment interventions. We have added sentences to the recruitment and follow-up paragraphs, to clarify that information on treatments administered will be collected throughout the patient's hospital stay and this information may be incorporated into outcome models if appropriate. The lack of available alternatives to ceftriaxone means that policies on second- and third-line antibiotics cannot currently be incorporated into antibiotic guidelines. We have added a sentence to the introduction to explain this.

P7 Table 3: I suggest to take into account the issue of healthcare-associated infections, for which certain *Enterobacteriales* (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter...*) and *Acinetobacter* may be more likely to be involved (transient bacteremia; pseudo-bacteremia) than for instance *E. coli* which is more likely to be endogenous.

Thank you for this suggestion, we agree this is an important component of the study. Information on epidemiological attribution of infections (community vs healthcare-associated) will be collected as part of the study so we will be able to take these factors into account during the classifications. We have clarified that we will collect this information with the following sentence in Methods. *Pre-hospital healthcare information will be used to classify infections into community acquired or healthcare associated.*

P7 Table 4: some of the "clear focus" may be difficult to assess – for instance "Urinary Tract Infection" and the "Respiratory tract infection" and "VVAP"

We agree that this will be a challenge in the setting of limited diagnostic resources. Hence why we have asked the panel to provide consensus on *suspected*, rather than definite focus, as per the paragraph on page 7 which reads. *The panel will be asked to classify into likely rather than definite focus, because of a desire not to overclassify non-focal sepsis in a setting where lack of diagnostic resources frequently limit the ability to definitively confirm focus of infection.*

P6 – P7: Clinical care and follow-up: it is not clear at which moment the patients will be assessed and by who: if the assessment is done by a trained panel or experts, one can expect sensible and relevant therapeutic and diagnostic actions during the hospital course (e.g. extra cultures at surgical debridement, additional x-ray...). They may influence patient outcome. How will this be handled?

Patients will be assessed by the study PI (a specialist in infectious diseases) or a clinical member of the study team (clinical officer or junior doctor). Whilst diagnostic and therapeutic recommendations may therefore be made, as this is an observational study, there will be no specific interventions made as part of the study. As each patient is reviewed by a trained clinician, any influence of this on patient outcome will be consistent across the study cohort and should not therefore introduce bias to one patient group over the other. We have clarified this with the following paragraph:

All participants will be reviewed as soon as possible after recruitment, by the study PI (a specialist in infectious diseases) or a clinical member of the study team and discussed with the PI. If required, recommendations on treatment and diagnostics will be made but as this is an observational study, the care of the patient will be directed by the clinical team responsible for the patient.

Table 1: Not clear why Acinetobacter is included – in terms of habitat and behavior, it is different from Enterobacteriales as well as from the other Gram-negative fermentative rods.

During the set-up period for the study, it became clear that 3GC-R *Acinetobacter* spp. were an emerging problem at QECH, particularly amongst neonates. *Acinetobacter* are closely related to *Enterobacteriales*, often sharing similar AMR profiles, in particular resistance to ceftriaxone which is the basis of this study. Given their importance as a nosocomial pathogen, we decided to include these patients in the cohort. However for analysis purposes we may decide to separate *Acinetobacter* from components of the analysis. We have clarified this as a footnote to Table 1.

P3: the "counterfactual" approach is not well described for naïve readers – this should be addressed as it is key to the approach.

We have described this approach in more detail and added additional references:

The counterfactual assumption is that death would not have occurred if the organism causing the infection had been drug-susceptible (or if there had been no infection). This assumption allows for comparison between patients with resistant and susceptible bacterial infection.

"Blood cultures which are positive over a weekend will be identified on Monday morning" meaning that a 2-day delay in identification and antibiotic susceptibility testing will occur and that directed treatment will be delayed – this can be important in situations where empiric treatment did not include effective treatment against Enterobacteriales – is this an issue or is ceftriaxone a part of the standard empiric treatment?

The blood cultures are reported in real time from the clinical laboratory, including over the weekend, and clinicians can access these results daily. Any weekend delay refers only to recruitment of participants and not routine clinical care. The study team will identify weekend cultures for the purposes of recruitment on a Monday morning, but there would be no expectation of treatment delay. We have adjusted the sentence as follows:

*Blood cultures which become positive over a weekend will be identified by the study **team for recruitment** on a Monday morning.*

Minor comments

Sensitive: suggest to replace by "Susceptible".

We have made this change.

Study Site: for reasons of representativeness, comparison and generalizability, some national demographic and health data could be provided (aside from the GDP).

We have expanded this paragraph to include further health data.

Study Site: why are only the admission data for the MJC supplied (and not those for the entire hospital?)

We now include admission data for the hospital.

Study Site: I suggest to give an overview of the (estimated) distribution adult versus children blood culture samples as well as community versus hospital-associated infections.

We provide a breakdown of adult versus paediatric blood culture samples. Data on community versus hospital associated infections are currently unknown at QECH.

Diagnostic bacteriology: blood volumes sampled are (too) low: this is a reality in low-resource settings but its implications should be discussed – it is possible that mainly patients with high bacterial loads will be selected and included.

We agree that this is a potential source of bias in the study and although we are limited by space to discuss this in more detail, we have added the following sentence:

The volume of blood taken for cultures in children in this setting is often low, potentially introducing bias to the study and selecting for patients with high bacterial load. This will be noted as a potential limitation

Diagnostic bacteriology: not clear which antibiotics will be tested and of ESBL testing is

done.

We have added an addition table (now Table 2) to describe the AST and a paragraph on ESBL testing.

Clinical presentation P5: not clear what is meant with "admission physiology": vital signs, clinical signs and symptoms, illness severity,...?

For clarity, we have changed this to admission vital signs.

The Abstract might be restructured to give more essential and detailed study information such as the (i) concrete classification of the role of the isolated bacteria and (ii) the healthcare economic data.

We have restructured the abstract to make it clearer and more detailed.

Abstract: Some abbreviations not explained previously (3GC-S) and there is some inconsistency (3GCR-E versus 3GC-R) - probably it will be easier if no abbreviations are used.

We have provided expansions for 3GC-R, 3GCR-E and 3GC-S.

In line with the Keywords, "Africa" (L3) might be changed to "sub-Saharan Africa".

We have made this change.

References: Some references are (too) briefly cited (e.g. ref. 15 "World Bank). Suggest to add websites + date of access where appropriate.

We have made these changes

Consistency of abbreviations or unusual abbreviation: 3GCR-E versus 3GC-R and DRI – only used a few time in the text.

We have made these consistent but left them in the text as they are now standard abbreviations in the literature.

Style: P4 and elsewhere: paragraph of methods mixes past, future and present tense.

We have proof read the manuscript to remove any inconsistencies

Competing Interests: No competing interests were disclosed.