STUDY PROTOCOL

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 1; peer review: 1 approved with reservations]

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Abstract

Introduction: Antimicrobial resistance is a global public health concern, but the problems are context specific, with each county or setting facing differing challenges. In 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 the 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 3GC-R bloodstream infection (BSI) in a large, urban hospital.

Methods: This study will investigate the burden of antimicrobial resistance by recruiting 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 3GC-S or 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. Mortality, direct and indirect costs and other health outcomes will be compared between patients with 3GC-R and comparable 3GC-sensitive 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. These classifications will then 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. Written

Open Peer Review

Reviewer Status ?

Invited Reviewers 1

version 1
13 Feb 2020

Report

1 Bieke Tack [], Institute of Tropical Medicine, Antwerp, Antwerp, Belgium
Jan Jacobs, Institute of Tropical Medicine Antwerp, Antwerp, Belgium

Any reports and responses or comments on the article can be found at the end of the article.
informed consent will be obtained from study participants or their parents/guardians. Results will be submitted to international peer-reviewed journals, presented at international conferences and shared with participating communities and collaborators.

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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Competing interests: No competing interests were disclosed.

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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 problem. 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 high.

Third-generation cephalosporin resistant Enterobacterales (3GC-R), have been identified by the World Health Organization (WHO) as critical priority pathogens on which national AMR programmes should focus their surveillance and reporting. 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 sepsis.

Median proportions of third-generation cephalosporin resistance (3GCR-E) amongst bloodstream Enterobacterales in sub-Saharan Africa (sSA) are high, approaching 15% in Escherichia coli and 50% in Klebsiella. 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 3GC-R amongst Enterobacterales. Between 2003 and 2016, ESBL 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 2005.

Knowledge gaps
Despite the rising prevalence of 3GC-R amongst key pathogens and a reliance on ceftriaxone for management of infection, the health impact of 3GC-R BSI in sSA has not been described. 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 costs, but only one published study from sSA has investigated health burden from AMR, finding a significant impact of 3GC-R on mortality. 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 outcomes.

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 debated. 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. 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-sensitive 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 contaminant. Consequently, Enterobacterales are routinely included in AMR surveillance studies without further consideration. 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 sensitive 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)\textsuperscript{15}. Blantyre is the second city of Malawi, with a population of 800,264 and is located in Blantyre district, population 995,000 (2018 census). 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 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. It has 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 cerebrospinal 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 > 37.5°C) or clinical suspicion of sepsis, severe sepsis or septic shock. In children, 1–2 ml 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.

Blood is inoculated into a single aerobic bottle using the automated BacT/ALERT system (bioMérieux, France). Enterobacteriales and Acinetobacter are identified to species level using Analytical Profile Index testing (API) (bioMérieux, France). Before March 2019, antimicrobial sensitivity testing (AST) was carried out as per British Society of Antimicrobial Chemotherapy (BSAC) guidelines\textsuperscript{16}, and from March 2019, as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines\textsuperscript{17}. 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 tables\textsuperscript{18}. The laboratory adheres to UK National External Quality Assessment Service (NEQAS)\textsuperscript{19}.

The absolute number of blood cultures collected fluctuates on an annual basis, but has approached 15000 per year since 2013. The most commonly isolated pathogens are non-typhoidal Salmonellae, Salmonella Typhi and Streptococcus pneumoniae (estimated minimum incidence ≥300/year) followed by the other Enterobacteriales, in particular E.coli and Klebsiella spp. (50–299/year)\textsuperscript{7}.

Participant selection and enrollment 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 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, their medical records will be collected for review.

At enrolment, a baseline questionnaire will be conducted, collecting demographic and clinical information including admission

### Table 1. Study inclusion and exclusion criteria.

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

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\textsuperscript{15} The World Bank. *World Development Indicators 2018*.

\textsuperscript{16} British Society of Antimicrobial Chemotherapy. *BSAC In Vitro Susceptibility Testing 2019*.

\textsuperscript{17} European Committee on Antimicrobial Susceptibility Testing. *EUCAST 2019*.

\textsuperscript{18} European Committee on Antimicrobial Susceptibility Testing. *EUCAST Breakpoints for Antimicrobial Susceptibility Testing*.

\textsuperscript{19} UK National External Quality Assessment Service. *NEQAS User Guide*. 

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\textsuperscript{7} Malawi College of Medicine. *Clinical Microbiology Laboratory Annual Report 2018*.
physiology, pre-hospital healthcare attendance, health-related quality of life (HRQoL) and a health-care utilisation survey. 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.

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 guidelines30. If a patient’s HIV status is unknown at the time of recruitment, 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. 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 sensitive. 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 2. 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 3.

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 3). 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 (got better with or without active antibiotics or died with or without active antibiotics).

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 4). 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 were lack of diagnostic resources frequently limit the ability to definitively confirm focus of infection.

Data analysis plan for primary outcomes
The effect of 3GC-R 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. 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 latent variable approach and a Bayesian paradigm. Taken together with BSI incidence estimates from Blantyre1, 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 morbidity4. To estimate length of hospital stay associated with 3GC-R, we will use multistate modelling, with time from hospital admission as the time scale31. 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...
**Table 2.** Participant vignettes: three example participants discussed at the consensus meeting, shown with final decisions on patient classification.

<table>
<thead>
<tr>
<th>Participant 1</th>
<th>26 year old female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
</tr>
<tr>
<td>S: ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, meropenem</td>
<td></td>
</tr>
<tr>
<td>R: ampicillin, cotrimoxazole</td>
<td></td>
</tr>
<tr>
<td><strong>Classification:</strong> Definite Gram-negative sepsis</td>
<td></td>
</tr>
<tr>
<td>2 weeks postpartum. Caesarian section done at Queen Elizabeth Central Hospital. Unwell for 10 days post-operatively: abdominal pain, and fevers.</td>
<td></td>
</tr>
<tr>
<td>Admission: Temperature 40.0°C. SIRS = Yes</td>
<td></td>
</tr>
<tr>
<td>Recruitment Day 7: Temperature 37.0°C. SIRS= Yes</td>
<td></td>
</tr>
<tr>
<td>Wound clean. No urinary catheters. Urine dipstick negative.</td>
<td></td>
</tr>
<tr>
<td>Antibiotics: 9 days ceftriaxone, 5 days ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td>Bloods Day 7: WCC 8.3 x10^9 L⁻¹, CRP 91 mg/L, Lactate 2.6,</td>
<td></td>
</tr>
<tr>
<td><strong>Outcome:</strong> Discharged alive</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant 2</th>
<th>72 year old male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
</tr>
<tr>
<td>S: ceftriaxone, chloramphenicol, gentamicin, ciprofloxacin, meropenem</td>
<td></td>
</tr>
<tr>
<td>R: ampicillin, cotrimoxazole</td>
<td></td>
</tr>
<tr>
<td><strong>Classification:</strong> Definite Gram-negative sepsis</td>
<td></td>
</tr>
<tr>
<td>Pre-hospital: Co-amoxiclav and azithromycin within 1 month of admission. Week 2 TB Treatment</td>
<td></td>
</tr>
<tr>
<td>Admission: Temperature 35.7°C, GCS 10. SIRS = Yes</td>
<td></td>
</tr>
<tr>
<td>Recruitment Day 5: Temperature 37.5°C. SIRS = No</td>
<td></td>
</tr>
<tr>
<td>Bloods Day 5: CD4 158 μL⁻¹, WCC 26.0 x10^9 L⁻¹, CRP &gt;120 mg L⁻¹, lactate 3.4 mmol L⁻¹, creatinine 862 mmol L⁻¹</td>
<td></td>
</tr>
<tr>
<td>Antibiotics: Ceftriaxone 24 hours</td>
<td></td>
</tr>
<tr>
<td><strong>Outcome:</strong> Died in hospital</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant 3</th>
<th>32 year old woman,</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
</tr>
<tr>
<td>S: chloramphenicol, gentamicin, amikacin, meropenem, co-amoxiclav</td>
<td></td>
</tr>
<tr>
<td>R: ceftriaxone, ampicillin, cotrimoxazole, ciprofloxacin</td>
<td></td>
</tr>
<tr>
<td><strong>Classification:</strong> Possible Gram-negative sepsis</td>
<td></td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>Bloods Day 6: WCC 6.3 x10^9 L⁻¹, creatinine 39 mmol L⁻¹, CD4 847 μL⁻¹, lactate 2.6 mmol L⁻¹, CRP 36 mg L⁻¹, urine dip negative</td>
<td></td>
</tr>
<tr>
<td>Repeat blood culture on Day 6 = negative, no antibiotics in between</td>
<td></td>
</tr>
<tr>
<td>Antibiotics: one dose of gentamicin on day 6, then discharged on nothing.</td>
<td></td>
</tr>
<tr>
<td><strong>Outcome:</strong> Discharged alive</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** SIRS, systemic inflammatory response syndrome; ART, antiretroviral therapy; WCC, white cell count; CRP, C-reactive protein.
Table 3. Classification scheme for Gram-negative blood cultures.

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite Gram-negative sepsis consequent upon cultured isolate</td>
<td>The blood culture isolate is contributing to the patient's clinical state and treatment was considered to be required.</td>
</tr>
<tr>
<td>Probable Gram-negative sepsis consequent upon cultured isolate</td>
<td>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.</td>
</tr>
<tr>
<td>Possible Gram-negative sepsis consequent upon cultured isolate</td>
<td>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.</td>
</tr>
<tr>
<td>Occult or transient bacteraemia</td>
<td>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.</td>
</tr>
<tr>
<td>Definite contaminant</td>
<td>The isolate has never contributed to patient’s condition and was very likely not present in the bloodstream</td>
</tr>
<tr>
<td>Probable contaminant</td>
<td>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.</td>
</tr>
</tbody>
</table>

Table 4. Clinically suspected focus of infection.

<table>
<thead>
<tr>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-focal</td>
</tr>
<tr>
<td>Central-line associated</td>
</tr>
<tr>
<td>Clear focus of infection</td>
</tr>
<tr>
<td>• Urinary tract infection</td>
</tr>
<tr>
<td>• CNS</td>
</tr>
<tr>
<td>• Skin and soft tissue</td>
</tr>
<tr>
<td>• Gastrointestinal (hepatobiliary)</td>
</tr>
<tr>
<td>• Gastrointestinal (non hepatobiliary)</td>
</tr>
<tr>
<td>• Cardiovascular system</td>
</tr>
<tr>
<td>• Respiratory tract infection (other than VAP)</td>
</tr>
<tr>
<td>• Reproductive tract infection</td>
</tr>
<tr>
<td>• Surgical site infection</td>
</tr>
<tr>
<td>• Bone and joint</td>
</tr>
<tr>
<td>• Post-operative, non-focal</td>
</tr>
<tr>
<td>• VAP</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

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

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 only22, 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 QECH22. The international market price will be used to estimate costs for all medication given23.

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 data. 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.

**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-up. 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 set.

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 bloodstream infections 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 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. 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 bloodstream infection. 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 BSI. 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 figures. 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.

**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 data, we will be able to provide a clear account of microbiological sampling criterial, 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 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

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.

References


15. World Bank. Reference Source


19. UK National External Quality Control. Reference Source


PubMed Abstract | Publisher Full Text

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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). 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

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.