STUDY PROTOCOL

Investigation of clinical, epidemiological, and genomic landscape of healthcare-associated infections in Malawi: a study protocol for a prospective longitudinal cohort

[version 1; peer review: awaiting peer review]

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v1 **First published:** 11 Dec 2024, **9**:722
https://doi.org/10.12688/wellcomeopenres.23240.1
Latest published: 11 Dec 2024, **9**:722
https://doi.org/10.12688/wellcomeopenres.23240.1

Abstract

Background

Healthcare-associated infections (HCAI) represent a pressing global health concern, with each country and healthcare setting facing distinct challenges. In African countries, the emergence of antimicrobial resistance (AMR), especially to third-generation cephalosporins amongst Enterobacterales (3GCR-E) is particularly severe given the widespread dependence on ceftriaxone as a first-line treatment for severe infections. The burden of HCAI is not yet adequately described. This study aims to address this gap in Malawi, by estimating the attributable mortality and morbidity associated with HCAI and associated AMR.

Methods

This is a prospective longitudinal cohort targeting three HCAI syndromes: surgical site infection, bloodstream infection, and catheter-associated urinary tract infection. We aim to recruit 600 adult (≥ 18 years) patients (300 with HCAI and 300 without HCAI) in three selected healthcare facilities in Malawi. Clinical variables are collected

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

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at enrolment, hospital discharge, and at day 30, 90 and 180 post-discharge using electronic case report forms. Mortality, extra length of hospital stay, and other health outcomes will be compared between patients with (drug-resistant or susceptible) HCAI and those without HCAI.

Discussion

The results of this study will contribute to understanding the burden of HCAI and AMR in Malawi. This information will help the infection prevention and control programme leads at facility level and policy-makers nationally, whilst providing regionally relevant insight into HCAI.

Plain language summary

Healthcare-associated infections (HCAI) are a major health issue worldwide, and different countries and healthcare systems face unique challenges. In African countries, the rise of bacteria that are resistant to antibiotics, especially those resistant to third-generation cephalosporins (commonly used drugs like ceftriaxone), is a serious problem. This is particularly concerning in countries like Malawi, where the impact of HCAI is not well understood.

This study aims to fill this gap by measuring the mortality and morbidity caused by HCAI in Malawi, including those related to antibiotic resistance. We will follow 600 adult patients from three hospitals—300 with HCAI and 300 without—focusing on three types of infections: surgical site infections, bloodstream infections, and catheter-associated urinary tract infections. We will collect health information when patients are admitted, discharged, and at 30, 90, and 180 days after leaving the hospital. The study will compare death rates, extra days spent in the hospital, and other health outcomes between patients with and without HCAI.

The findings will help healthcare providers and policymakers in Malawi better understand the problem of HCAI and antibiotic resistance, and support efforts to improve infection prevention and control in the region.

Keywords

Surgical site infection, Bloodstream infection, Catheter-associated urinary tract infection, Healthcare-associated infections, Antimicrobial resistance, Burden

This article is included in the Malawi-Liverpool Wellcome Trust Clinical Research Programme gateway.

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Author roles: Bunduki GK: Conceptualization, Investigation, Methodology, Project Administration, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Musicha P**: Methodology, Supervision, Writing – Review & Editing; **Kamchedzera W**: Writing – Review & Editing; **Bakali W**: Investigation, Writing – Review & Editing; **Ganiza TN**: Data Curation, Writing – Review & Editing; **Musopole O** : Writing – Review & Editing; **Musaya J**: Conceptualization, Methodology, Supervision, Validation, Writing – Review & Editing; **Feasey N**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome [206545/Z/17/Z] [223012/Z/21; to PM]; the NIHR Global Health Professorship [NIHR301627; to GKB, WK and NF].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Bunduki GK, Musicha P, Kamchedzera W *et al.* **Investigation of clinical, epidemiological, and genomic landscape of healthcare-associated infections in Malawi: a study protocol for a prospective longitudinal cohort [version 1; peer review: awaiting peer review]** Wellcome Open Research 2024, **9**:722 https://doi.org/10.12688/wellcomeopenres.23240.1

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Background

Healthcare-associated infections (HCAI) are a major global public health problem[1]. It has been estimated that more than 1.4 million patients are affected by HCAI worldwide at any time[2], although HCAI was not reported among the 282 causes of death in the 2017 Institute for Health Metrics and Evaluation Global Burden of Disease Study[3]. HCAI surveillance is a challenge in well-resourced healthcare settings; therefore, it may appear to be an impossible challenge for low-income countries, such as Malawi, to address. The magnitude of the problem is likely to be particularly large in situations where there are virtually no basic infection prevention and control (IPC) policies and guidelines and exacerbated by understaffing, poor infrastructure including water hygiene and sanitation (WASH), lack of antimicrobial prescribing policies, shortage of basic laboratory equipment, and limited funding for IPC consumables i.e. alcohol hand rub[4].

A recent systematic review reported that HCAI rates vary between 1.6 and 90% within African countries[5], however, there remains a paucity of data to guide local action. This is partly because the gold standard definitions of HCAI are difficult to achieve in low-income settings and there is a clear need to develop and validate sustainable HCAI definitions in Africa to support the implementation of routine HCAI surveillance and inform the implementation of context-appropriate IPC strategies. This study reported that resistance to multiple antibiotics was common among the reported bacteria; with 70.3% of Enterobacterales being third-generation cephalosporin-resistant (3GCR-E), and 70.5% of *S. aureus* being methicillin-resistant (MRSA)[5]. This was not a surprise as pathogens associated with HCAI are often antimicrobial resistant and a recent study estimated deaths and disability-adjusted life years (DALYs) attributable to and associated with antimicrobial resistance (AMR), finding an estimated 1.05 million deaths associated with bacterial AMR and 250,000 deaths attributable to bacterial AMR in the World Health Organization (WHO) African region in 2019. Third-generation cephalosporin-resistant *Klebsiella pneumoniae* and MRSA were shown to be the leading pathogen-drug combinations for deaths attributable to AMR[6]. These estimates were, however, generated based on scant clinical outcome data from Africa, especially for HCAI.

In Malawian hospitals, there is a narrow repertoire of antimicrobials available, hence, ceftriaxone is typically used as the first-line antibiotic, due to its spectrum and once-daily dosing regimen, given the limited diagnostics and nursing capacity[7]. However, this may lead to selective pressure on third-generation cephalosporin-resistant infections, and resistance to these has been reported to be associated with increased mortality and longer hospital stay in Malawi[8]. Despite the obvious reasons to use ceftriaxone, it is possible to safely reduce the use of these agents in the Malawian setting[7].

The prevalence of HCAI in Malawi remains undetermined, with a notable lack of comprehensive investigations into *S. aureus* and Enterobacterales within the aetiological framework of HCAI. We therefore hypothesise that the prevalence of HCAI is considerable, and HCAI aetiologies attributed to MRSA and 3GCR-E are associated with high morbidity and mortality in Malawi. This study therefore aims to estimate the burden (incidence, attributable and/or associated mortality, risk factors, excess length of hospital stay, and attributable DALYs) of surgical site infection (SSI), bloodstream infection (BSI) and catheter-associated urinary tract infection (CAUTI) in the southern region of Malawi.

Methods

Study settings

Malawi. Malawi, located in Southeastern Africa and bordered by Mozambique to the east, south, and southwest, Zambia to the west, and Tanzania to the north and northeast, with a projected population of 20,270,568 for 2024 (Malawi population projections 2018–2050 report) and is one of the world's poorest nations. Malawi grapples with significant infectious disease burdens, notably HIV/AIDS and malaria, impacting public health and overall well-being. The government, in collaboration with international organizations, has implemented various programs to address and manage these health challenges.

Malawi has four Central Hospitals, each located in one of the four largest cities, and 24 District Hospitals. This study will be conducted at Queen Elizabeth Central Hospital (QECH) in Blantyre, Zomba Central Hospital (ZCH) in Zomba, and Chikwawa District Hospital (CDH) in Chikwawa (Figure 1). These facilities were chosen for a comprehensive exploration of the burden of HCAI considering diverse patient populations, healthcare practices, resource levels, and geographic factors (urban and rural settings).

HCAI surveillance in Malawi encounters significant challenges, as assessments across healthcare facilities reveal critical deficiencies in surveillance practices and prioritisation of monitoring. Two-thirds of facilities lack professionals trained in basic epidemiology, surveillance, and IPC, exacerbating the issue. Furthermore, microbiology support is available in only about one-third of facilities, complicating both HCAI and AMR surveillance efforts[9].

Queen Elizabeth Central Hospital. Queen Elizabeth Central Hospital (QECH) is Malawi's largest urban government tertiary referral and teaching hospital, situated in Blantyre, the country's second-largest city. QECH serves as a crucial referral centre for complex medical cases from across the southern region. It provides tertiary services for the entire population of Southern Malawi which is projected at 7,922,339 (Malawi Population and Housing Census Report 2018). Despite having a capacity of around 1,350 beds, QECH often operates beyond its capacity[4]. Annually, approximately 10,000 adult and 50,000 paediatric patients are admitted[10].

Zomba Central Hospital. Zomba Central Hospital (ZCH) is one of the four Central Hospitals in Malawi. It is in the Southern region of Malawi and serves a population of roughly 4.3 million people. Apart from serving as a tertiary referral hospital in the Southeast Zone, it also works as a district hospital for the

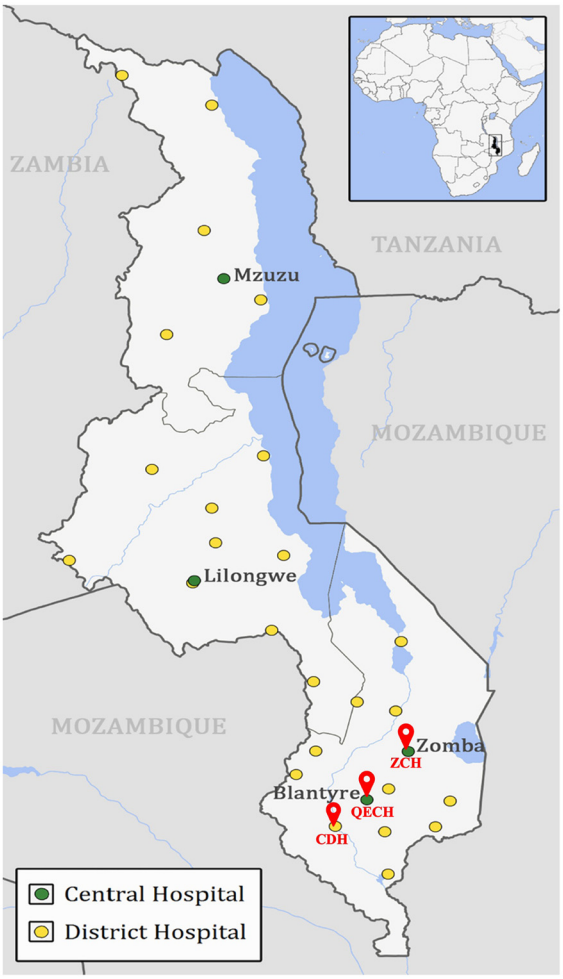

Figure 1. Map of Malawi showing Central and District hospitals and the selected study sites.

surrounding population of Zomba. ZCH has about 500 beds and four main clinical departments: internal medicine, surgery, paediatrics, and obstetrics and gynaecology.

Chikwawa District Hospital. Chikwawa District Hospital (CDH) is in Chikwawa district in the Southern region of Malawi, in the Shire Valley approximately 54 km away from Blantyre city. The district covers an area of 4,755 km² and has a population of 564,684 with the district hospital. CDH has about 250 beds. The district lies in a malaria-endemic area in Africa. The hospital setting provides both inpatient and outpatient services.

Malawi Liverpool Wellcome Programme. The Malawi Liverpool Wellcome Programme (MLW) is a research institute that provides a quality-assured blood culture service to febrile adult and paediatric medical patients at QECH, participating in the United Kingdom National External Quality Assessment Service (UK NEQAS). While samples from QECH and CDH are processed at MLW's microbiology laboratory, those from ZCH are initially processed at ZCH laboratory and later sent to MLW for re-identification and antimicrobial susceptibility testing.

Study design

This is a prospective longitudinal cohort conducted at QECH, ZCH, and CDH for twelve months recruiting individuals hospitalised for more than 48 hours in any of the surgical wards, adult medical wards or burns unit (Figure 2). Only QECH has a burns unit, while at ZCH and CDH burns patients are admitted within surgical wards. This cohort study helps generate reliable estimates of morbidity and mortality attributable to both HCAI and associated AMR.

In this study, the primary outcome of interest is mortality. The primary exposure of interest is HCAI. Consequently, a case is defined as a patient who develops HCAI, while a control is a patient from the same ward who does not develop HCAI. For the secondary analysis, we will stratify cases based on AMR status to examine its impact on the primary outcome. This approach allows us to not only assess the direct association between HCAI and mortality but also to evaluate whether AMR modifies this association.

A matched parallel cohort analysis will be done to assess the mortality and morbidity associated with and attributed to HCAI AMR using the WHO GLASS method for estimating attributable mortality of AMR[11]. This consists of comparing mortality and excessive length of stay in individuals with drug-resistant and drug-susceptible HCAI with those uninfected in both replacement and additive scenarios (Figure 3). The matched parallel cohort design ensures that cases and controls are comparable with respect to potential confounders present in the ward environment, thereby strengthening the validity of our findings.

HCAI case definitions, inclusion, and exclusion criteria

All adult patients (≥ 18 years) suspected of HCAI as defined by the WHO HCAI definitions tailored to low-income settings (Table 1) are included in the study. Patients previously included in the study can be enrolled again, so long as this represents a distinct, new episode of HCAI, with full resolution of the previous episode and a 30-day follow-up period completed before being eligible to be enrolled again. Uninfected patients, meaning those not suspected of having any targeted HCAI i.e. SSI, CAUTI or BSI, are also included in the study if they meet the matching criteria.

Patients with a repeated episode of the same HCAI within 30 days of a previous active HCAI are not eligible for inclusion as a new study patient – considering that this represents a recurrence of incompletely treated infection rather than a new disease episode. Outpatients are not included in this study. Participants lacking the capacity to provide informed consent are not included until they recover their capacity. Patients known to have or have had SSI, BSI or CAUTI at any point in their hospital admission are not eligible for inclusion as control.

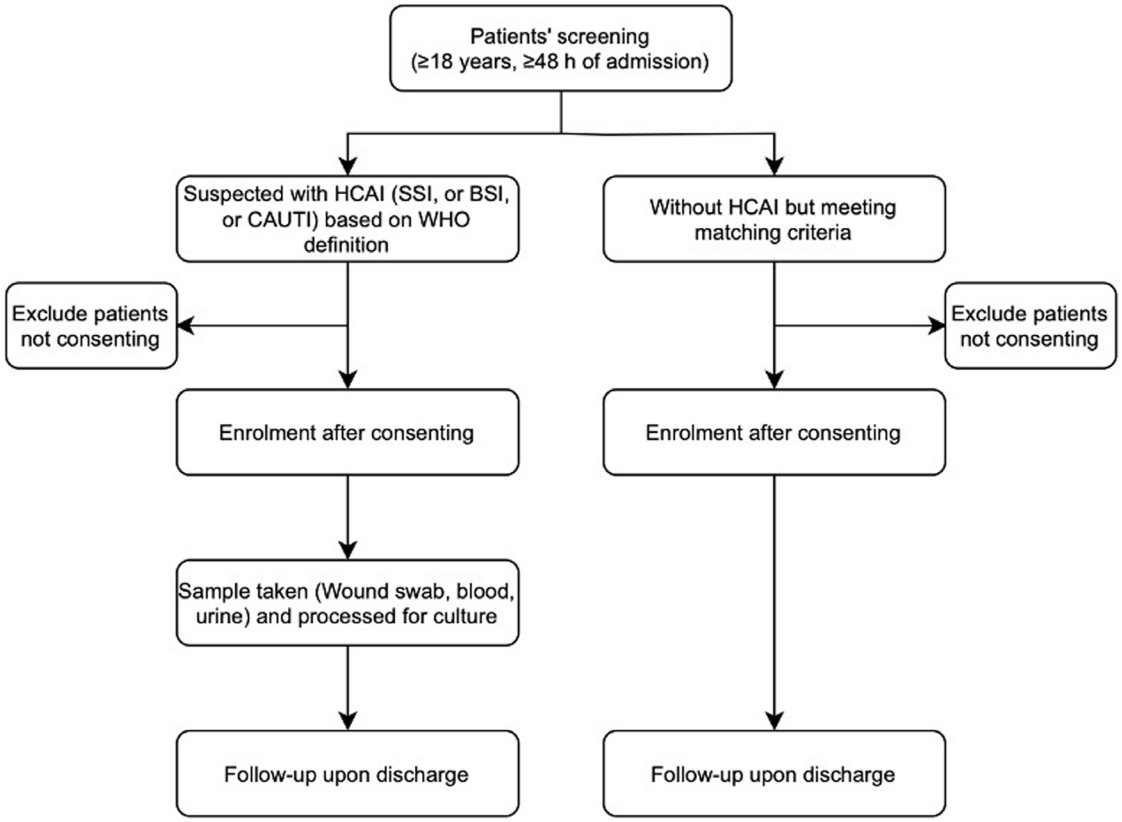

Figure 2. **Recruitment flowchart for the longitudinal prospective HCAI cohort.**

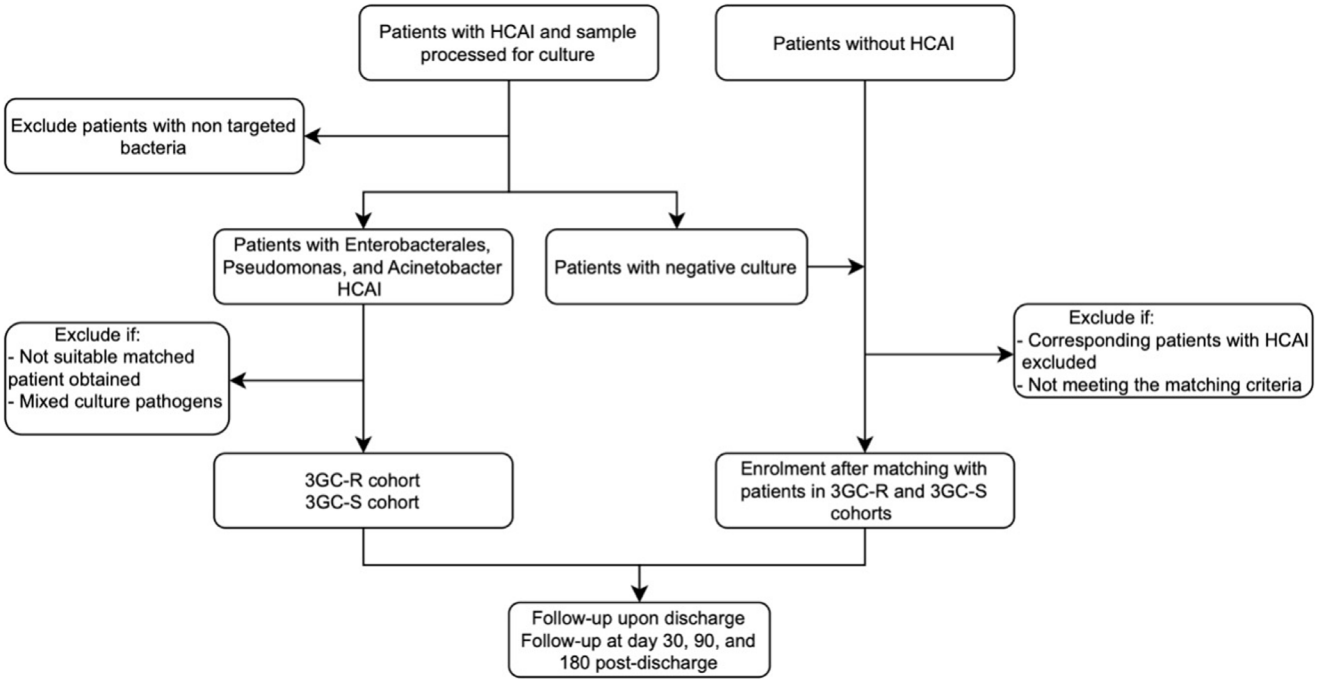

Figure 3. **Recruitment flowchart for the AMR matched parallel cohort.**

Patients screening, recruitment, and follow-up

Patients are screened and recruited between Monday and Friday, from 8 am to 5 pm. The HCAI active case findings are documented daily. Following informed written consent, a baseline questionnaire is used to capture demographic and clinical information of patients including data on any pre-hospital healthcare visit and medical history. Recent or current history of antibiotic exposure is also recorded. The electronic case report forms (eCRFs) are completed by gathering information from patients' medical files. Missing data are retrieved by medical note review, pharmacy notes and/or patient' interviews. In addition to clinical data, data related to health-related quality of life and economic burden are collected. However, the analysis of these data is considered in a separate protocol aiming to assess the economic burden of HCAI in Malawi.

Participants are followed up upon their discharge. Subsequent follow-ups are done at 30 days, 90 days, and 180 days post-discharge. The primary outcome is in-hospital mortality. Secondary outcomes are mortality at 6 months post-discharge

Table 1. HCAI case definitions.

A. Active HCAI	An HCAI is identified starting from day three after admission or on day one or two after admission, if the patient was discharged from another health care facility in the preceding 48 hours or if the SSI criteria were met at any time after admission (including previous surgery 30 days). An active HCAI present on the day of the survey is defined as follows: • An infection (one or more, among the three types of infections targeted in this thesis) is active when it meets the definitions as described below for each type. OR • Signs and symptoms were present in the past AND the patient is still receiving treatment for that infection on the survey date. The presence of symptoms and signs should be verified until the start of the treatment to determine whether the treated infection matches one of the HCAI case definitions.
B. Bloodstream infection (BSI)	**1. Confirmed BSI (BSI-A1)** One positive blood culture for a recognised pathogen. It excludes common skin commensals: coagulase-negative staphylococci, *Micrococcus* spp., *Propionibacterium* acnes, *Bacillus* spp., *Corynebacterium* spp.
	2. Confirmed BSI (BSI-A2) Patient has at least one of the following signs or symptoms: Fever (>38°C) OR chills OR hypotension (systolic pressure ≤ 90 mmHg) AND Two positive blood cultures for a common skin commensal(s) (from separate blood samples) within 48 hours. Common skin contaminants include coagulase-negative staphylococci, *Micrococcus* spp., *Propionibacterium* acnes, *Bacillus* spp., *Corynebacterium* spp.
	3. Suspected BSI – One positive blood culture (BSI-B) Patient has at least one of the following signs or symptoms: fever (>38°C) OR chills OR hypotension (systolic pressure ≤ 90 mmHg) AND Treatment for infection is instituted (i.e., on day of sample collection, physician documentation of antimicrobial treatment for suspected infection) AND One positive blood culture for a common skin commensal(s) (common skin commensals: coagulase-negative staphylococci, *Micrococcus* spp., *Propionibacterium* acnes, *Bacillus* spp., *Corynebacterium* spp.).
	4. Suspected BSI – Blood culture not done (BSI-C) Patient has at least one of the following signs or symptoms: fever (>38°C) OR chills OR hypotension (systolic pressure ≤ 90 mmHg) AND Treatment for infection is instituted (i.e., on day of sample collection, physician documentation of antimicrobial treatment for suspected infection) AND Blood culture not done, or no microorganisms detected in blood. Central vascular catheter-associated BSI (CVC-BSI) BSI-A1 OR BSI-A2 definition met AND Central vascular catheter in place ≤ two days prior to first meeting a component of the confirmed BSI definition.

C. Urinary tract infection (UTI)	**1. Microbiologically confirmed symptomatic UTI (UTI-A)** Patient has at least one of the following signs or symptoms with no other recognised cause: fever (> 38°C) OR urinary urgency OR increased urinary frequency OR dysuria OR flank pain OR supra-pubic pain OR tenderness AND Patient has a positive urine culture (≥ 10^5 microorganisms per mL of urine with no more than two species of microorganisms).
	2. Not microbiologically confirmed symptomatic UTI (UTI-B) Patient has at least two of the following signs or symptoms with no other recognised cause: fever (> 38°C) OR urinary urgency OR increased urinary frequency OR dysuria OR flank pain OR supra-pubic pain OR tenderness AND Patient has at least one of the following findings: positive dipstick for leucocyte esterase and/or nitrate OR pyuria with ≥ 10 WBC/mL or ≥ 3 WBC/high-power field or unspun urine OR microorganisms seen on Gram stain of unspun urine OR at least two urine cultures with repeated isolation of the same uropathogen (gram-negative bacteria or *S. saprophyticus*) with ≥ 10^2 colonies/mL urine in nonvoided specimens OR ≤ 10^5 colonies/mL of a single uropathogen (gram-negative bacteria or *S. saprophyticus*) in a patient being treated with effective antimicrobial agent for a UTI.
	3. Not microbiologically confirmed symptomatic UTI (UTI-C) Patient has at least two of the following signs or symptoms with no other recognised cause: fever (> 38°C) OR urinary urgency OR increased urinary frequency OR dysuria OR flank pain OR supra-pubic pain OR tenderness AND Clinician diagnosis of a UTI OR clinician institutes therapy for a UTI.
	4. Catheter-associated UTI (CA-UTI) UTI-A OR UTI-B OR UTI-C definitions AND Indwelling urinary tract catheter in place ≤ 2 days prior to first meeting a component of the confirmed UTI definition.
D. Surgical site infection (SSI)	**1. Surgical site infection type A (SSI-A)** Post-operative patients within 30-days following surgical procedure with evidence of SSI based on: microbiology (for positive culture) OR radiology (suggestive of infection) OR histopathologic criteria (for abscess or similar findings). Stratify by depth if information available as follows: - Superficial: an infection involves only skin and subcutaneous tissue of the incision, - Deep incisional: infection involves deep soft tissue (e.g., facia, muscle) of the incision, - Organ/space: infection involves any part of the anatomy (e.g., organs and spaces) other than the incision which was opened or manipulated during s surgical procedure.
	2. Surgical site infection type B (SSI-B) Post-operative patients within 30-days following surgical procedure with reopening of the wound for suspected infection OR abscess (or similar findings) found during direct examination or during reoperation (for deep and organ-scape SSI).
	3. Surgical site infection type C (SSI-C) Post-operative patients within 30-days following surgical procedure with evidence of purulent discharge at the incision or surgical site.
	4. Surgical site infection type D (SSI-D) Post-operative patients within 30-days following surgical procedure AND diagnosis of a SSI is made by the surgeon or attending physician or designee.

and length of stay. Discharge status "moribund" is recorded for participants who are discharged with the expectation they will die elsewhere. Post-discharge follow-ups are made by phone calls. If unable to contact the participants or their nominated representative on the due date, four further attempts are made within 15 days. Participants whose final follow-up outcome at 6 months is unavailable will not be included in the 6-month survival analysis. All the data are collected using standardised case report forms available in Zenodo (https://doi.org/10.5281/zenodo.13302811)[12] and in this protocol as extended data.

Participants suspected of having HCAI are matched to those without HCAI. The matching criteria are as follows:

- **Hospital and ward location at recruitment:** For example, if the participant with HCAI is currently in the Surgical ward at the time of enrolment into the study, the

matching participants must be recruited from the same ward of the same hospital. The participant physical location at the time when the sample for culture is collected is not relevant.

- **Consideration of time-dependency:** To avoid immortal time bias, the study is also considering the time-dependency of HCAI. This means that controls are matched in a way that ensures they have been in the hospital for a similar duration as the cases or, in the case of surgical procedures, have the same number of days since a matching surgical procedure.
- **Type and severity of HCAI:** The qSofa score is used for BSI, while the type of surgery is used for SSI and the indwelling urinary catheter for CAUTI.

The matching process aims to create a balanced comparison group for each HCAI case. This means that each suspected HCAI case is matched with an appropriate control based on the specified criteria. The matching is done at a 1:1 ratio.

Exploratory analysis of contribution of AMR to HCAI morbidity and mortality

For the purposes of this study, we have defined AMR as culture-confirmed resistance to the third-generation cephalosporin ceftriaxone, as this is the principal agent used as empirical therapy for suspected severe infection in hospitals in Malawi. We use a matched parallel cohort design for estimating AMR impact, with prospectively recruited patients with culture confirmed HCAI and individually matched patients without HCAI followed up in two cohorts: a third-generation cephalosporin-susceptible (3GC-S) cohort and a third-generation cephalosporin-resistant (3GC-R) cohort[11]. The 3GC-R status for Enterobacterales is defined by the resistance to cefpodoxime, with a cut-off breakpoint of <21 mm and for *Staphylococcus aureus* as resistance to cefoxitin with a cut-off breakpoint of <22 mm (thus an MRSA). *Pseudomonas* are intrinsically resistant to ceftriaxone. Patients with polymicrobial HCAI are included in the 3GC-R cohort if any infecting Enterobacterales species is resistant.

Once a participant with HCAI is identified (3GC-S or 3 GC-R), the research team identifies potentially eligible uninfected matches (current inpatients in the same ward, with the same age group, with the same admission to time-to-infection period or closest available, and clinical risk) at a 1:1 ratio. Patients are considered uninfected if there is no clinical suspicion of or confirmed target HCAI; thus, patients with other clinically suspected or laboratory confirmed forms of infection remain eligible for selection as matches. We include cases of HCAI with any species within the order Enterobacterales, *S. aureus, Acinetobacter* spp., and *Pseudomonas* spp. Patients with HCAI are excluded if no suitable matched patients are recruited.

Exposed patients will be compared to against matched, unexposed patients in regression models within each cohort (3GC-R and 3GC-S). These estimates will then be used to generate a resistant-versus-susceptible ratio-of-ratios to assess the effect of resistance to third-generation cephalosporins. Robust standard errors will be used to account for the matched design. Cox regression models will be used. We will estimate the hazard ratios (HRs) of the effect of HCAI on in-hospital mortality with censoring at competing event at discharge. We will also determine the effect on risk of discharge with censoring at inpatient death. The Kaplan-Meier survival curves and the log-rank test will be generated for different patient groups and HCAI syndromes. To assess the effect on overall in-hospital mortality, considering the competing event of discharge, we will use Fine and Gray extended Cox regression models. We will use generalised linear models with log link function to estimate the relative risk (RR) for 6-month mortality. Patients with unknown 6-month outcomes will be excluded from this analysis.

In this study, the excess mortality attributable to and associated with third-generation cephalosporin-resistant Enterobacterales HCAI are assessed.

Point prevalence surveys

In order to understand the scale of HCAI as a clinical entity in Malawi, we are conducting weekly point prevalence survey (PPS) using the WHO HCAI PPS tool. This will enable us to assess trends in HCAI in different departments (medical and surgical) of the three selected acute care hospitals in Malawi (QECH, ZCH, and CDH). These PPS are single day surveys, for which we are collecting metrics (total number of included patients, number of patients with HCAI, distribution of HCAI per syndrome type) for estimating weekly prevalence (Figure 4).

In brief, all patients admitted to the ward before or at 8 a.m. and not discharged from the ward at the time of the survey are included; in practice, this means that patients transferred in/out after 8 a.m. from/to another ward are not included. All day-cases (patients undergoing same-day treatment or surgery, patients seen at the outpatient department, patients in the emergency room, dialysis patients, and patients under 18 years of age) are excluded from the PPS. The total number of patients with ≥48 hours of admission is recorded as well as those with active HCAI as defined by WHO definition (Table 1).

Sample size calculation

Different scenarios were used to estimate the adequate sample size. These included the estimation of sample size powered to detect mortality from HCAI, MRSA HCAI, and 3GCRE HCAI. In general, there is insufficient existing data from Africa on HCAI on which to guide our sample size estimation. Therefore, in some scenarios, studies from European countries were used.

Recent African studies reported a 15% prevalence of HCAI[5] and 22.2% mortality[13]. Previous research found a mortality hazard ratio (HR) of 1.7 for adult patients with HCAI[14]. Another study reported a 30-day mortality rate of 10.8% in HCAI patients versus 4.1% in non-HCAI patients, with an HR of 1.4[15]. Due to poor availability of antibiotics, poor diagnostics, and underreporting of HCAI in Malawian hospitals, a higher HR of 3 was assumed for HCAI-associated mortality. A Cox

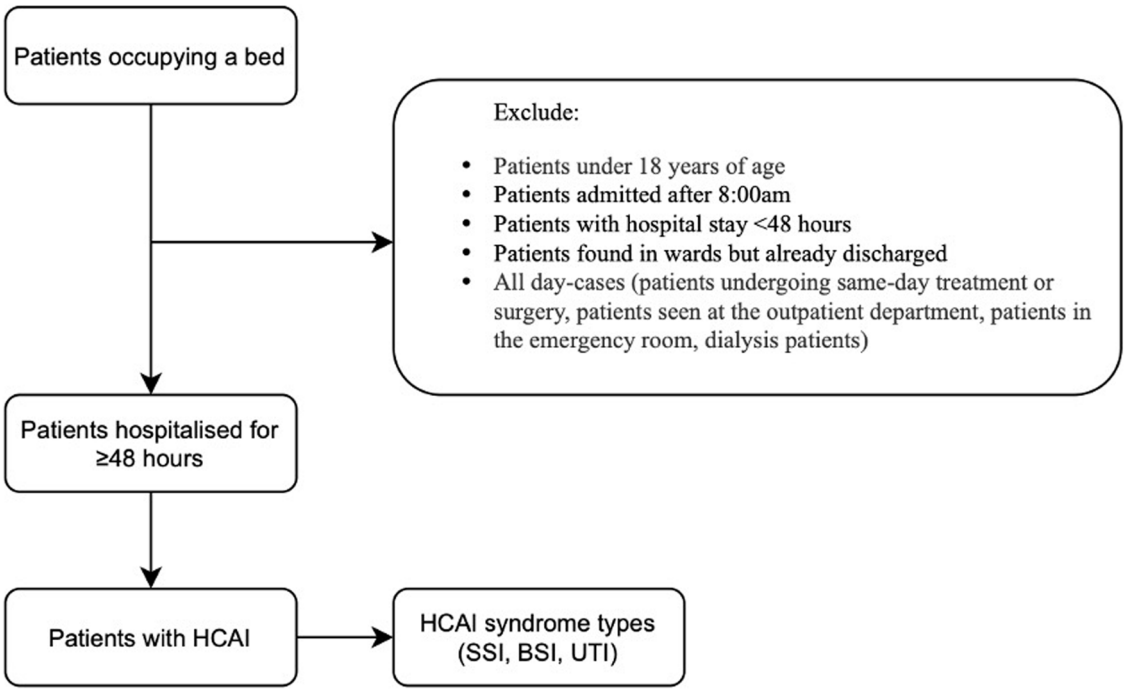

Figure 4. Recruitment flowchart for the point prevalence surveys.

Proportional Hazards model (2-sided equality) was used to calculate the sample size, setting HCAI prevalence and mortality at 15% and 22.2%, with 80% power, yielding a minimum sample size of 251.

HCAI MRSA prevalence in Africa varies widely, ranging from 12% to over 80%[16,17]. Few studies focus on MRSA HCAI and their mortality. One review reported a 26.5% one-month MRSA bacteraemia mortality versus 18.5% for MSSA[18], while another study found a 30-day mortality rate of 32.5% for MRSA HCAI compared to 7.9% for MSSA[19]. The HR for mortality due to MRSA HCAI was 1.79 compared to MSSA[14]. Using a 15% prevalence, 26.5% mortality, and an HR of 3, the sample size was calculated to be 223.

A study in eight sub-Saharan hospitals reported a 13.7% prevalence of HCAI third-generation cephalosporin-resistant Enterobacterales (3GCRE) vs 21.7% for susceptible strains, with a 30-day mortality of 39.6% for 3GCRE and 29.4% for 3GCSE, and a relative risk of 0.82[20]. Another study in Malawi found 80.1% prevalence of 3GCRE with a 45% mortality vs 34% for 3GCSE, with an HR of 1.44[8]. Using a 13.7% prevalence, 39.6% mortality, and an HR of 3, the sample size was calculated to be 190.

From the three scenarios, the largest value of the calculated minimum sample sizes was considered as the minimum study sample size as recommended by Hajian-Tilaki[21]. Hence, the minimum sample size was 251 participants. This sample size was increased by a design effect of 9% as recommended by the WHO-GLASS for studies that are conducted in multiple sites[11]. In addition, 10% was added to the sample size due to the lost to follow-up of some participants as recommended by the WHO-GLASS[11] and is consistent with a recent study done at QECH[8]. Therefore, the minimum sample size was 300 participants with HCAI. Each participant with HCAI is matched to a participant without HCAI in a ratio of 1:1. The sample size of participants without HCAI is therefore the same as the one with HCAI. Hence, the total sample size is 600 participants. This sample size is powered to estimate mortality attributed to HCAI and drug-resistant HCAI (either MRSA or 3GCR-E).

Diagnostic microbiological procedures

Having identified a suspected case of HCAI, the following diagnostic tests are undertaken to try to diagnose the aetiological agent of the infection and any associated antimicrobial resistance.

Blood culture

Under aseptic conditions, 7 to 10 mls of blood are taken for culture from adult patients with clinical suspicion of healthcare-associated BSI and inoculated into a BACT/Alert bottle for culture under aerobic conditions. Blood samples are collected before the administration of antibiotics if possible. Blood culture samples are sent to the laboratory as promptly as possible, preferably within 12 hours, they are not refrigerated, or frozen, or held at room temperature for more than a few hours. Samples are inoculated into single aerobic bottles and

incubated in an automated system (BacT/Alert®, BioMerieux Marcy-L'Etoile, France). Samples are processed per protocol in the ISO-accredited MLW diagnostic microbiology laboratory.

Urine culture

Approximately 10–20 mls of urine are collected from patients with clinical suspicion of CAUTI and with a dipstick test suggesting UTI (positive for leucocytes and/or nitrite). The sample is inoculated on CHROMagar™ orientation (a non-selective chromogenic agar medium for the isolation, differentiation, and enumeration of urinary tract pathogens) and incubated aerobically at 37°C for 16–18h.

Wound swab culture

The wound swab is taken on the surgical site wound using the Levine quantitative swab technique and inoculated on blood agar and CHROMagar orientation culture medium and incubated at 37°C for 16–18h. The Levine quantitative swab technique consists of:

- cleaning the wound with normal saline;
- pat dry the wound bed with sterile gauze;
- culture the healthiest looking tissue, excluding exudate, purulent, devitalised tissues;
- spin the end of the sterile applicator over a 1cmx1cm area for at least 5 seconds;
- apply sufficient pressure to the swab, causing tissue fluid to be expressed.

Bacterial identification

Bacteria are identified by their colony morphology and colour produced on the chromogenic agar. Pink colonies are assumed to be *E. coli*. For the differentiation between other members of the Enterobacterales, the analytical profile index 20E (API®-20E) (BioMerieux) is performed. Bacteria suspected as Staphylococci based on colony morphology are tested for catalase and the suspected *Staphylococcus* species are tested for coagulase for identifying *S. aureus*. Catalase-negative isolates presumed to be Streptococci and further tested as appropriate using Lancefield Antigens.

Antimicrobial susceptibility testing

Antimicrobial susceptibility is tested using the disc diffusion method (Oxoid™, UK) following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org). Enterobacterales bacteria are tested against ampicillin (10µg), cefpodoxime (10µg), meropenem (10µg), ciprofloxacin (5µg), gentamicin (10µg), and cotrimoxazole (25µg). For Gram-positive bacteria, the following antibiotics are tested: benzylpenicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), and cotrimoxazole (25µg).

MRSA, Extended-Spectrum Beta-Lactamase (ESBL), and Carbapenemase-Producing Enterobacterales (CPE) screening

Phenotypic methods are performed for the detection of MRSA, ESBL, and CPE strains according to EUCAST. An isolate is assumed to be MRSA by testing against cefoxitin (30µg) discs on Müeller-Hinton agar plates containing 2% NaCl, inoculated with broth suspension equivalent to 0.5McFarland. Discs are applied onto the plates and incubated at 35°C for 24h. The inhibition zones are measured and interpreted according to EUCAST guidelines, using a cut-off breakpoint of <22 mm. For the ESBL screening, a cefpodoxime disc (10µg) is used as it is the most sensitive individual indicator of cephalosporin for detection of ESBL production and is used for screening, with a cut-off breakpoint of <21 mm. CPE are screened using the meropenem (10µg) disc using a cut-off breakpoint of <28 mm as recommended by the EUCAST guidelines.

Inferring the clinical relevance of isolates

Inferring the clinical relevance of bacterial isolates in the context of HCAI is a critical step in ensuring appropriate treatment and infection control measures. It is possible for a participant to have a clinical syndrome consistent with a HCAI and for a pathogen to be isolated from the site, but for the pathogen to be an unlikely cause of that clinical syndrome observed (i.e. *E. coli* colonising an infected surgical site). Once the bacterium is identified to species level, it is placed in the context of the case and assessed to ascertain whether the organism is likely to have been responsible for the HCAI episode or simply colonising the wound or catheter bag or a blood culture contaminant following the assessment process described in Table 2.

A preliminary classification system was developed to categorize the impact of each positive culture in cases of SSI, CAUTI, and BSI among participants. This system was guided by the clinical observation that some patients recover from infections without receiving antibiotics specifically targeting the isolated organisms. The categories were initially developed by the principal investigators, specialist in infectious diseases and clinical microbiology, and included classifications such as definite or probable infection, possible infection, and definite or probable contamination, depending on clinical signs, culture results, and patient response to treatment. An expert panel, comprising five local healthcare professionals and a microbiologist specialist, was assembled to review and finalize these classifications. Discrepancies were discussed and resolved by consensus.

Recourse to this panel is generally unnecessary when well-known pathogens are isolated with clear clinical implications, such as *S. aureus* in SSI, *E. coli* and *K. pneumoniae* in patients symptomatic of CAUTI, or *S. aureus* and Gram-negative bacteria in BSI, where these organisms are likely to be the cause of infection. However, expert consultation may be required for cases involving potential contaminants, such as coagulase-negative staphylococci (CoNS) or Enterococcus species, or in cases with ambiguous clinical signs, mixed cultures, or asymptomatic bacteriuria. The panel's input becomes crucial when it's difficult to determine whether an isolate is pathogenic or merely a contaminant, or when transient bacteraemia or occult infections are suspected.

By following these steps, the panel systematically evaluate the clinical relevance of bacterial isolates in HCAI, also ensuring

Table 2. Classification of HCAI based on the likelihood of the isolate's contribution to clinical condition.

HCAI type	Isolate category	Description
SSI	Definite or probable cause of infection	- The isolate is clearly contributing to infection at the surgical site. Clinical signs of infection and positive culture confirm the need for treatment. - The isolate is likely contributing to infection at the surgical site, though not fully confirmed. Treatment was initiated based on clinical judgment.
	Possible cause of infection	The isolate could be contributing, but the patient improved without targeted antibiotic therapy or with an agent not expected to be effective.
	Definite or probable contaminant	The isolate is unlikely to be causing the infection.
UTI	Definite or probable cause of infection	- The isolate is clearly contributing to the patient's clinical condition, and treatment was deemed necessary based on symptoms and positive culture results. - The isolate is likely contributing to the patient's clinical condition, though there is not enough evidence to fully confirm. Treatment was deemed necessary.
	Possible cause of infection	The isolate may be contributing, but the patient improved without antibiotics or with an antibiotic not effective based on susceptibility testing.
	Definite or probable contaminant	- The isolate is unlikely to be contributing to the patient's condition and/or there is insufficient evidence to confirm its presence in the urinary tract.
BSI	Definite or probable cause of infection	- The isolate is contributing to the patient's clinical condition, and treatment was deemed necessary. - The isolate is likely contributing to the patient's clinical condition, but there is not enough evidence to confirm or rule this out. Treatment was deemed necessary.
	Possible cause of infection	The isolate could be a factor in the patient's clinical condition, but the patient showed improvement without the use of antibiotics that were expected to be effective based on antimicrobial susceptibility testing. As a result, it is not possible to definitively or probably confirm or rule out the contribution of the isolate to the clinical picture.
	Occult or transient bacteraemia	The isolate may have played a role in the patient's clinical condition; however, by the time the culture was performed, the patient had already shown improvement. Unlike the criteria for possible BSI, treatment was deemed unnecessary, though a repeat blood culture was recommended.
	Definite or probable contaminant	The isolate was unlikely to be contributing to the patient's condition and was of doubtful clinical significance

accurate diagnosis, appropriate treatment, and effective infection control measures.

Integrating Cohort and PPS data to estimate burden of HCAI in Malawi

PPS provide a snapshot of the HCAI burden at specific points in time, and by undertaking serial PPS, it is possible to describe trends in HCAI over time. The cohort study provides in depth assessments of the morbidity, mortality and healthcare costs associated with HCAI. By combining these two data sources, we aim to estimate the burden of HCAI across Malawi.

Using Bayesian hierarchical models, we will integrate PPS data and cohort data to estimate the annual burden of HCAI. Instead of simply extrapolating PPS data by multiplying point prevalence by total annual admissions, the Bayesian approach allows us to account for uncertainty and variability over time and across hospitals. We will use weekly PPS data as prior information to model the infection rates across hospitals and throughout the year, adjusting for seasonality if there are fluctuations in HCAI rates over time. To scale these estimates nationally across Malawi, we will gather data on total admissions from national health statistics or derive estimates based on the proportion of admissions in the three hospitals. Using the posterior distribution of HCAI prevalence from the Bayesian model, we will simulate national HCAI cases by applying these probabilities to the total annual admissions in Malawi. For estimating national HCAI-related mortality, we will use the case fatality rate (CFR) derived from the cohort study. This CFR will be applied to the posterior estimates of national HCAI cases to account for uncertainty and variability in the CFR across hospitals. To estimate the additional bed-days due to HCAI, we will compare the length of stay (LOS) between patients with and without HCAI, using

the cohort data. The difference in LOS will be multiplied by the total number of national HCAI cases, with Bayesian hierarchical modelling capturing uncertainty in the difference in LOS across hospitals.

Our model will be hierarchical, accounting for variations in HCAI rates between central and district hospitals, as well as urban and rural facilities. If necessary, we will apply model weights to adjust for different hospital sizes or admission rates. Additionally, we will simulate different scenarios by varying assumptions for HCAI prevalence, CFR, and LOS to estimate the burden under low, medium, and high prevalence conditions. This will allow us to explore the impact of changes in infection rates, mortality, and LOS across hospitals, offering a flexible framework for scenario analysis. By merging the detailed outcome data from the cohort study with the broad prevalence data from PPS, we will achieve comprehensive burden estimation scalable to the entire country.

Data management

We are collecting data using the mobile Open Data Kit (ODK) data collection platform running on password-secured Android tablets. Data are transmitted to the secure study server located within the MLW Research Offices over encrypted networks and stored in a secure password-protected SQLlite database hosted on the MLW data server.

We designed the eCRFs that had a built-in quality check (QC) process which aimed to minimize the rate of errors/missing fields and enhance data quality. Other quality control measures include running queries by the data manager that flag discrepancies in the form of missing values/records and outliers. The final data format is downloadable in Microsoft Excel, PDF, and Txt or CSV formats for importation into common statistical packages (SPSS, SAS, Stata, R). Data from analysed laboratory samples are managed by the secured laboratory management information systems (LIMS) based at MLW.

Quality control and quality assurance procedures

We are conducting this study following relevant regulations and standard operating procedures. Microbiological laboratory procedures are performed at the ISO15189 accredited MLW diagnostic microbiology laboratory. The study nurse research assistants and the laboratory technicians were trained in the protocols and relevant procedures before the start of the study. The team works closely with hospital staff to achieve the study goals while ensuring smooth continuation of care. Data quality is assured by training, validation steps built into data capture and visualisation tools, and central monitoring as described above in the data management plan.

Statistical analyses

Descriptive statistics will be used to provide a broad overview of participant characteristics and crude and adjusted case fatality rates for HCAI, per pathogen and resistance profile, followed by survival analysis using Cox proportional hazards models. For the estimation of AMR mortality, the crude case fatality rates for drug-susceptible HCAI will be compared to those for AMR HCAI using hazard ratios. The Kaplan-Meier survival curves and the log-rank test will be generated for different patient groups and HCAI syndromes. We will use the target trial emulation approach to estimates deaths and DALYs attributable to bacterial AMR HCAI. Excess length of stay in days will be calculated using proportional hazards models. The difference in expected length of stay will be estimated between the drug-resistant and susceptible states.

Dissemination

Results from this study will be presented internally within the KUHeS, QECH, ZCH, CDH, and COMREC 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.

Discussion

The global burden of HCAI is not precisely estimated. Due to comorbidities and the fact that HCAI are rarely reported as the primary cause of death, it is therefore difficult to estimate the number of deaths attributed to HCAI, nor to make the link to associated AMR.

Available evidence suggests the burden of HCAI in low-resource settings like Malawi is significant, yet there are huge data gaps, due to the lack of robust surveillance systems and standardised reporting mechanisms, hindering efforts to quantify the true burden of HCAI and identify high-risk groups and settings. While certain risk factors for HCAI are known globally, their relative contribution to HCAI in Malawi remains poorly understood, limiting the development of tailored interventions. Transmission dynamics of HCAI within and between healthcare facilities are poorly characterized, hindering effective IPC measures. Addressing these knowledge gaps through comprehensive research endeavours, such as this prospective longitudinal cohort study, is crucial for informing evidence-based interventions to improve patient safety and healthcare outcomes in Malawi. Bridging these gaps will enable the development of targeted strategies tailored to the context of Malawian healthcare settings, ultimately reducing the burden of HCAI on the healthcare system.

This study aims to investigate the morbidity, mortality, and genomic landscape of HCAI in Malawi. Through prospective recruitment and detailed characterization of HCAI, it will provide reliable data on its impact, including complications and disabilities caused by drug-resistant infections. The study will link microbiology results to clinical syndromes, contributing to a better understanding of HCAI AMR in Malawi. The findings will aid IPC program implementers and decision-makers in shaping policies and guidelines for HCAI interventions. This research will help reduce HCAI burdens, guide antibiotic use, and inform regional and global disease estimates.

Ethical approval, consent, and participant compensation

Ethical approvals were obtained from the College of Medicine Research Ethics Committee (COMREC) (P.11/22/3874, approved on 31/01/2023) based at the Kamuzu University of Health Sciences (KUHeS) and the Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee (LSTM REC 22–083, approved on 21/11/2023). LSTM provided sponsorship for this work.

The study procedures, risks and benefits, and confidentiality of data are adequately explained to participants, and they must have the capacity to consent. They are provided with written participant information leaflets and consent forms in either English or Chichewa. Those who are willing to participate in the study are given two copies to sign and date and keep one copy. Participants are free to withdraw their consent at any time without giving a reason. Recruited participants are compensated with 10,000 Malawian Kwacha. This study adheres to the Declaration of Helsinki.

Data availability

Underlying data

No data are associated with this article.

Extended data

Zenodo: Case report forms: Investigation of clinical, epidemiological, and genomic landscape of healthcare-associated infections in Malawi: a study protocol for a prospective longitudinal cohort. https://doi.org/10.5281/zenodo.13302811[12].

This protocol contains the following extended data:

- CRF longitudinal cohort
- CRF admission cost
- CRF denominator for HAI
- CRF lab report form
- CRF medical cost
- CRF patient follow up
- CRF EQ-5D-5L

Authors' contributions

GKB conceptualised and designed the study, project administration, writing the first draft of the protocol, review, and editing. GKB and WB wrote laboratory protocols, GKB and TNG designed the eCRF. PM contributed to the statistics design, writing-review and editing. WK, JM, and OM writing-review and editing. NF conceptualised and designed the study, project administration, overall supervision, funding acquisition, writing-review, and editing. All authors have read and approved the manuscript for submission.

Acknowledgements

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

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