STUDY PROTOCOL

Drivers of Resistance in Uganda and Malawi (DRUM): a protocol for the evaluation of One-Health drivers of Extended Spectrum Beta Lactamase (ESBL) resistance in Low-Middle Income Countries (LMICs) [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

In sub-Saharan Africa (sSA), there is high morbidity and mortality from

severe bacterial infection and this is compounded by antimicrobial resistance, in particular, resistance to 3rd-generation cephalosporins. This resistance is typically mediated by extended-spectrum beta lactamases (ESBLs). To interrupt ESBL transmission it will be important to investigate how human behaviour, water, sanitation, and hygiene (WASH) practices, environmental contamination, and antibiotic usage in both urban and rural settings interact to contribute to transmission of ESBL *E. coli* and ESBL *K. pneumoniae* between humans, animals, and the environment.

Here we present the protocol for the Drivers of Resistance in Uganda and Malawi (DRUM) Consortium, in which we will collect demographic, geospatial, clinical, animal husbandry and WASH data from a total of 400 households in Uganda and Malawi. Longitudinal human, animal and environmental sampling at each household will be used to isolate ESBL *E. coli* and ESBL *K. pneumoniae*. This will be complimented by a Risks, Attitudes, Norms, Abilities and Self-Regulation (RANAS) survey and structured observations to understand the contextual and psychosocial drivers of regional WASH practices. Bacterial isolates and plate sweeps will be further characterised using a mixture of short-,long-read and metagenomic whole-genome sequencing. These datasets will be integrated into agent-based models to describe the transmission of EBSL resistance in Uganda and Malawi and allow us to inform the design of interventions for interrupting transmission of ESBL-bacteria.

Keywords

Antimicrobial Resistance, Africa, One Health, Environment

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

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Introduction

Antimicrobial resistance (AMR) is a huge and complex global public health problem¹. It is a threat to health that reflects both the interconnectedness of humans, animals and the environment and humanity's dependence on antimicrobials². In sub-Saharan Africa (sSA), there is a high incidence of severe bacterial infection, frequently inadequate health system infrastructure to diagnose and treat bacterial disease, and widespread and uncontrolled availability of antimicrobials, which drives antibiotic use (ABU) in both human and animal sectors^{3,4}. There is also inadequate water, sanitation and hygiene (WASH) infrastructure to mitigate spread of environmentally dependent bacteria between humans, animals, and the environment⁵. This situation favours the transmission of AMR-bacteria, but the relative contribution of these different factors is uncertain.

The 3rd-generation cephalosporin (3GC) ceftriaxone is frequently the antimicrobial agent of first and last resort across much of sSA. 3GC resistant (3GC-R) enteric bacteria have rapidly emerged, largely due to acquisition of extended-spectrum beta lactamase (ESBL)-producing enzymes, resulting in infections that are frequently locally untreatable, due to unavailability of carbapenems or other reserve antibiotics⁶. ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are key examples of this. As low-income countries (LIC) in Africa have poor access to watch and reserve agents, it is critical to define the relative importance of different transmission routes of ESBL-producing enteric bacteria in order to develop interventions that will interrupt pathogen transmission and ultimately prevent drug resistant infections (DRI).

Uganda and Malawi are LIC with high incidence of neonatal sepsis and malaria, high prevalence of HIV, poorly regulated antimicrobial markets, and inadequate WASH infrastructure^{5,7–9}. Here, we present the protocol developed by the [Drivers of Resistance in Uganda and Malawi \(DRUM\) Consortium](#). DRUM will work in urban, peri-urban, and rural settings in Uganda and Malawi and focus on ESBL producing *E. coli* (ESBL-E) and *K. pneumoniae* (ESBL-K). These bacteria were selected as they belong to the same family and often share AMR phenotypes, however *E. coli* is typically considered to be both community-acquired and nosocomial, whereas *K. pneumoniae* is more often judged to be the archetypal nosocomial AMR pathogen¹⁰.

We will take an interdisciplinary, One-Health approach to assess how human behaviour, WASH practices, environmental contamination, and ABU in urban and rural locations within Uganda and Malawi contribute to the transmission of ESBL-E and ESBL-K between humans, animals, and the environment and how this transmission relates to strains isolated from the blood of humans with drug-resistant infection (DRI). We will collect demographic, geospatial, WASH, longitudinal clinical and molecular microbiological data, and integrate these data into agent-based models designed to estimate the impact of putative interventions on interrupting transmission.

Aim

In order to determine the critical points at which efforts to interrupt human AMR acquisition are likely to have the greatest

impact in Eastern Africa and beyond, we hypothesise that the household is a key setting in which ESBL enteric bacteria are transmitted. We therefore aim to identify risk factors for and infer drivers of ESBL-E and ESBL-K transmission in Uganda and Malawi at the household level. This is summarised in [Figure 1](#), created following a stakeholder meeting in Uganda in 2018 by [Design Without Borders](#).

Site selection

DRUM consortium members identified sites representing urban, peri-urban, and rural settings to enable variations in WASH behaviours, animal practices, ABU, and contamination with ESBL-producing bacteria to be contrasted. Additionally, sites were considered based on perceived acceptability of research within the communities and existing research capacity. Therefore, in Malawi, Ndirande (urban) and Chikwawa (rural) were selected because of the opportunity to utilize data from previous studies (i.e. detailed censuses) and prior research engagement, and Chileka (peri-urban) was selected due to local prior knowledge. We sought to achieve a comparable mixture in Uganda with varied socioeconomic status in Kampala (urban) and Hoima District (peri-urban and rural). Within these sites, recruitment polygons were drawn from local administrative wards ([Figure 2](#)).

Malawian site descriptions

Healthcare is free at the point of delivery in Uganda and Malawi, and this should be assumed unless otherwise stated.

Site 1: Ndirande, Blantyre, Malawi (Urban)

Ndirande is a large urban settlement with high-density housing 4 km from the geographical centre of Blantyre, the second city of Malawi^{11,12} and where 15% (109,164) of the Blantyre population resides¹³. Healthcare is provided by one large, government Health Centre (Ndirande Health Centre) and by the tertiary referral hospital for the Southern region, Queen Elizabeth Central Hospital (QECH), 2–6 km away^{11,14}. HIV prevalence in adults aged 15–65 is 18% and there is a high burden of typhoid and tuberculosis^{15,16}. The study polygon is 3 km², and our initial survey in April–May 2019 identified 8 secondary schools, 46 primary (or nursery) schools, 52 places of worship, 15 markets, 1 farm and 9 pharmacies within it.

Site 2: Chileka, Blantyre, Malawi (Peri-Urban)

Chileka is a peri-urban administrative ward on the northern outskirts of Blantyre city. Healthcare is provided by a government Health Centre (Chileka Health Centre), a small local private hospital (Mtengo-Umodzi) or admission to QECH 10–16 km away. The study polygon is 14 km², and our initial survey in April–May 2019 identified 3 secondary schools, 20 primary (or nursery) schools, 14 places of worship, 4 large farms and 6 pharmacies within it.

Site 3: Chikwawa, Malawi (Rural)

Chikwawa is a large district with a population of ~450,000, situated in the southern Shire valley and its border is 50 km from Blantyre¹⁷. It is a rural area, including a mixture of subsistence and large-scale sugar farming, and given its low-lying situation is historically prone to flooding¹⁸. Healthcare is provided by Chikwawa District hospital, 14 health centres and 26

Figure 1. Hypothetical model of related behaviours and the movement of AMR-bacteria in Uganda and Malawi. The schematic situates the household at the heart of the model, in which humans act in response to their environment within which bacteria are evolving in response to selective pressures around them.

Figure 2. Diagram of DRUM study sites. (a) We selected two geographic areas within Uganda including Kampala (red) and Hoima District (green). From these areas, polygons were created that mapped an urban setting (Kampala) and urban/rural setting (Hoima District). (b) We selected sites in two regions within southern Malawi including Blantyre (red) and Chikwawa District (green). Polygons were created and mapped for urban (Ndirande) and peri-urban (Chileka) settings within Blantyre and a rural setting within Chikwawa District.

community health care worker outposts¹⁷. We identified a 71 km² study polygon readily accessible from Blantyre by road, including villages engaged in research activity on the edge of Chikwawa town. Our survey in April-May 2019 identified 2 secondary schools, 9 primary (or nursery) schools, 29 places of worship, 3 markets, 11 farms and 1 pharmacy within the polygon.

Ugandan site descriptions

Site 4: Kampala, Uganda (Urban)

Kampala, the capital and largest city of Uganda has a metropolitan area population of 3.3 million people. Adult HIV prevalence is 6.9%¹⁹. The sampling frame comprises of 3 contiguous areas drawn in wedge shape (measuring 3.4 km² x 2.7 km² x 1 km²) with a spectrum of population density areas. These areas were loosely stratified relative to each other as being of low, medium or high socioeconomic status based on local knowledge. The smallest polygon closest to the centre is considered low, whilst the one furthest from the centre as medium and the middle one as high socioeconomic status.

Site 5: Hoima, Uganda (Rural and Urban)

Hoima, in the Western Region of Uganda, is the main municipal, administrative, and commercial centre of Hoima District and has a population of 122,700 people²⁰. HIV prevalence among adults aged 15–64yrs in the Mid-West Region of Uganda where Hoima is located is 5.7%¹⁹. The sampling frame comprises of two non-contiguous polygons of 3.6 km² and 7.6 km², the former incorporating Hoima town (peri-urban) and the latter (rural) being a few kilometres away from Hoima town and which has more animal and human cohabitation.

Methods

Household selection process

As DRUM will investigate AMR transmission at the household level, we chose a spatial design based on the “inhibitory with close pairs” approach²¹. This enables us to distribute primary sampling sites across the study area evenly, avoiding systematic biases that may occur when sampling on a regular grid. Secondly, “close-pair” points are added to the design to allow localised comparison of sample sites and therefore measurement of close-range correlation in AMR status. Thus, seventy percent of households will be sampled at a minimum inhibitory distance (MID) from all other points²² Using one inhibitory point at a time, the rest of the points, called close pairs, are randomly selected within a circle with a pre-determined close-pairs radius (CPR). The minimum distance for our design is 100 meters and the radius for each close pair is 30 meters. These values were chosen based on results from a spatial investigation of enteric pathogen *Salmonella* Typhi in Blantyre that showed a spatial correlation up to approximately 150 meters²³.

Depending on the richness of existing geospatial data within each study area, we will implement different versions of the algorithm in each area. In Ndirande (Malawi), where all households had previously been geolocated, direct random sampling

of households subject to the spatial constraints above is possible¹¹. In Hoima (Uganda), where OpenStreetMap (OSM) data appears complete, OSM-derived building locations can be chosen to identify potential households. In Chikwawa (Malawi), WorldPop population density rasters allow us to preferentially (though not exclusively) propose sampling sites in high population density areas thus avoiding field teams visiting vacant sites (www.worldpop.org/). In Kampala (Uganda) and Chileka (Malawi), apparent uniformity of the population density across the study area allows a simple spatially uniform proposal to be used. Two practical site-specific considerations are necessary. Firstly, for Chileka, the MID and CPR must be doubled due to the sparse population. In Kampala, the availability of a marked socioeconomic gradient within the study region allows stratification of the population by socioeconomic status, with households randomised within strata, but respecting our spatial design constraints across strata borders.

Proposed sampling locations are then translated into households by the data collection field teams. For instances where either no suitable household exists at the location or in the event that a household declines to participate in the study, a random direction is selected by the field team, and the closest consenting household in that direction is chosen.

Recruitment of households

We aim to enrol up to 100 households in each of the five sites. Households will be grouped into either “intensive” or “sparse”, with 15 intensive households pre-selected at random within each polygon, and all others allocated as sparse (Figure 3). Intensive households will undergo extensive WASH observations at the first and last visit, whereas “sparse” households will not undergo any WASH observations (Figure 3).

All households will be followed up at 3–4 time points over a period up to 6 months to provide longitudinal microbiological and WASH data. Household recruitment will be staggered over 12 months to assess seasonality of transmission of ESBL-bacteria. At each visit, households will be asked to respond to questionnaires to provide information at the individual and household level on ABU, health seeking behaviour and WASH behavioural practices. Microbiological sampling will be undertaken to determine the presence of ESBL *E. coli* and ESBL *K. pneumoniae* from human, animal and environmental samples.

Participant eligibility

Eligibility will be considered at the level of the household and individuals. Households will be required to exist within the boundaries of the study polygon and be able to provide a minimum of 12 samples at the baseline visit, inclusive of a minimum of 2 human stool samples from household members. Individuals will be required to speak either the predominant local language (Chichewa in Malawi or Luganda or Runyoro in Uganda) or English to provide informed consent, and not

Figure 3. DRUM household study design.

have confirmed or suspected acute infection at the time of recruitment.

Data collection

Case Report Forms (CRFs)

Study CRFs have been designed by an interdisciplinary working group of the DRUM consortium that included specialists in human health, animal health, food, water and environmental microbiology, WASH & Environmental health and medical anthropology. Questions were selected from pre-tested tools evaluating regional demographics, human and animal health, WASH infrastructure and behavioural practices, humans and animal ABU determinants and environmental exposures^{23,24}. These questions were inputted into CRFs that were tailored to the resident population, structured into either individual or household level, thematically separated into key drivers of AMR and translated into local languages (Table 1).

At the baseline visit, these CRFs will be completed to provide information at the individual and household level on human health, ABU, health seeking behaviour, structural and behavioural WASH practices and animal husbandry (Extended

data). At each follow-up visit, changes to human health, household practices and antibiotic exposure will be assessed (Extended data).

Longitudinal microbiological sampling

The consortium was asked to consider priorities for household sampling at the kick-off meeting at Liverpool School of Tropical Medicine (LSTM), UK (23/09/2018). We decided to focus on areas identified as hand-contact zones or where food handling occurred and also to include broader environmental sites that serve as important reservoirs of ESBL AMR. We established a consensus opinion for the microbiological sampling strategy based on a maximum of 20 samples per visit, inclusive of human and animal stool samples and environmental samples (see Figure 4).

Field sampling methods

Human stool will be self-collected in a 30 mL stool pot by participants. Animal stool samples will either be collected using rectal/ cloacal swabs (for poultry) or taken directly from the ground. Food samples will be placed in sterile Whirl-Pak® bags, and all water samples will be collected using sterile 500 ml Nalgene® BPA-free, polypropylene bottles.

Table 1. DRUM CRF themes and data capture.

	Individual Level Data	Household Level Data
Demographic	<ul style="list-style-type: none"> Participant Demographics 	<ul style="list-style-type: none"> Household Demographics Socio-Economic Information Household Head Information
Health	<ul style="list-style-type: none"> Health Status and Comorbidities Regular Medication Use Recent Illness 	<ul style="list-style-type: none"> Household Health Seeking Behaviour
Exposure Risk	<ul style="list-style-type: none"> Healthcare Exposure Travel and Residency Health Seeking Behaviour 	<ul style="list-style-type: none"> Visitors into the household
Antibiotic Usage	<ul style="list-style-type: none"> Antibiotic Usage 	<ul style="list-style-type: none"> Household Experience of Illness and Antibiotics
WASH	<ul style="list-style-type: none"> Hand-Washing Data 	<ul style="list-style-type: none"> Household WASH Infrastructure Toileting Behaviour Waste Management Water Usage and Management Washing and Bathing Practices Food Preparation and Hygiene Information. Hand-Washing Data
Environmental		<ul style="list-style-type: none"> Household Infrastructure Household Environment
Animal		<ul style="list-style-type: none"> Household Animal Husbandry Animal Health and Disease Prevention Drug Usage in Household Animals (including antibiotics)

Figure 4. DRUM microbiological sampling frame, used at household visits. Samples are inclusive of human stool, animal stool, and a selection of household environment and the broader external environment.

Household environmental sampling will be informed by the WASH observations to determine high-risk areas of environmental contamination. Environmental contact-surface samples and clothing samples will be collected with 3M™ Sponge-Sticks containing 10 ml sterile buffered peptone water (BPW) broth, and floor samples will be collected with the use of boot socks. Drain samples (defined as water in motion either in a constructed drain (dug or built) or moving on a surface) will be collected in a 30 ml universal container from within the household compound. Detailed descriptions of the sampling processes are included in the study SOPs (Extended material)

All samples will be issued a unique identification code, labelled, stored in ice chests at 2–8°C in the dark and transported to the laboratory, for processing within 24 hours, where possible.

Microbiological Methodology

Consistent with practice at the UK Health Security Agency Food Water and Environment (FWE) Microbiology Services, samples will initially be cultured in enrichment broth (BPW) to improve the recovery of Gram-negative organisms. The volumes of BPW added will depend on sample type and will be determined by either the manufacturer's advice (3M™ Swab-Samplers), expert opinion and SOPs from FWE (3M™ Sponge-Sticks, water filtration methods, food processing methods), previous local experience (stool processing) or from pre-testing and optimisation in the piloting phase of the study (river water processing, drain sample processing, boot socks).

Human stool, animal stool and environmental swabs will not require pre-processing steps. BPW will be directly added to the sample upon reception. Water and food samples will be pre-processed as follows:

- Water samples will be filtered through a sterile 0.45 µm cellulose-ester gridded membrane (VWR™) using a vacuum-based manifold, before adding to universal containers with 20 ml of BPW. In river water samples, a second sample will be processed in parallel, and the filter membrane will be stored at -80°C without the addition of BPW.
- Fruit will have enough BPW added to the Whirl-Pak® bag to cover before being massaged for a period of 30 sec to 3 min. The fruit will then be aseptically removed from the bag prior to incubation.
- Green leafy vegetables will be weighed and have nine times the weight of the food added in BPW to obtain a sample-to-diluent volume of 1:9, before being manually stomached for 30 sec to 3 min. Vegetables will be left inside the Whirl-Pac® bag while incubated.

Once the enrichment broth (BPW) has been added, all samples will be placed in an aerobic incubator at 37 ± 1°C for 18–24 hours. After incubation a 1.8 ml aliquot of the culture BPW will be stored at -80°C, and the remaining sample will be plated onto ESBL CHROMagar™ chromogenic agar (CHROMagar™, France). Plates will be placed in an aerobic incubator at 37 ±

1°C for 18–24 hrs and read for growth of ESBL bacteria, via the presence of either pink, blue or white colonies. Pink colonies and (indole positive) white colonies will be categorised as ESBL *E. coli* while blue colonies will undergo speciation for *K. pneumoniae*, using high resolution melt-curve (HRM) PCR, to identify ESBL *K. pneumoniae* isolates²⁵. ESBL isolates and plate sweeps of all positive samples will be stored at -80°C.

Samples will be stored at intervals during the microbiological processing to facilitate subsequent whole genome sequencing (WGS), including aliquots of the original sample (shotgun metagenomics); samples pre-enriched with BPW (limited-diversity metagenomics via mSWEEP/mGEMS), CHROMagar™ plate sweeps (limited-diversity metagenomics) and single colony picks (short-read and long-read sequencing)²⁶.

DNA extraction and Sequencing

DNA will be extracted from all ESBL-positive and a selection of ESBL-negative isolates, plate sweeps and pre-enriched BPW ESBL positive samples using the QIASymphony DSP Virus/Pathogen mini-kit® on the QIASymphony® (QIAGEN, USA) automated DNA extraction platform or manually extracted using the DNeasy® blood and tissue kit (QIAGEN, USA). Extracted DNA will be shipped to the Wellcome Sanger Institute (WSI, UK) under export licences issued following signature of Access and Benefit Sharing agreements in accordance with the Nagoya protocol.

DNA from single colony pick isolates and plate sweep samples will be whole genome sequenced on the Illumina X10 platform (Illumina Inc, California, USA) to produce 150bp paired end short reads. Preliminary analysis of these short-read WGS data will inform the identification of clusters from which representative isolates will be selected for long read sequencing on the MinION platform (Oxford Nanopore Technologies, UK) in order to generate hybrid, improved draft assemblies, and thus characterise mobile genetic elements (MGEs). Finally, shotgun metagenomic sequencing will be performed on up to 420 pre-enriched BPW samples on the Illumina HiSeq 4000 platform (Illumina Inc, California, USA) to investigate the microbial community composition and AMR gene pool or “resistome”.

WASH Evaluations

Household WASH, environmental health and food safety evaluations

Each recruited household will be asked to engage with a range of qualitative and quantitative data collection methods to gain an understanding of the contextual and psychosocial elements of their household, individual and habitual WASH practices as outlined in IBM-WASH²⁷. Questions will be asked of household members at the baseline assessment (combined with the household and individual CRFs), and a checklist and sanitation inspection form will be completed by a member of the study team at each visit to evaluate WASH infrastructure. Lastly, a household plan will be completed at baseline to contextualise the household infrastructure where specific activities take place (including perceived high-risk areas) and aid in analysis.

WASH practices will be assessed via checklist and structured observations at households and identified for “intensive surveillance”, at both the baseline and fourth visit. Observations will be undertaken on 3 consecutive days, for a period of 6 hours per day, with two morning sessions (6am-12pm) and one afternoon session (12pm-6pm) to describe WASH practice over the period of a day. The focus on early sessions has been chosen due to previous studies illustrating that key WASH activities occurred mainly in the mornings²⁸. Observations will be recorded by research staff and summarised in a structured format for content analysis to enable the identification of critical control points around WASH behaviours for faecal and environmental exposure.

Understanding WASH behavioural drivers

Psychosocial drivers of WASH practices will be explored using the Risks, Attitudes, Norms, Abilities and Self-Regulation (RANAS) Model, undertaken at up to 100 households in each region^{29,30}. The RANAS questionnaire design will be informed by the structured observations in intensive households and focus on hand hygiene, food preparation, waste management, water usage and environmental exposure. The RANAS survey will be conducted with 2 household members in each household, and where possible, will be directed to the household head (e.g. father) and one household worker (household staff member). RANAS data will be analysed using an ANOVA mean comparison to determine the differences between doer and non-doer contextual and psychosocial factors for potential targeted behaviours. The data from this survey will be used to inform potential behaviour change techniques which could be used to tackle high risk transmission areas identified in the agent-based model.

Assessment of broader environmental exposure

Transect walks of each region will be undertaken using an integrated approach to the collection and evaluation of environmental, WASH and microbiological data to understand the wider context in which household members are living. Based on the principles of the [SaniPath method](#), walks will be undertaken with community leaders using walking interviews, while collecting video footage and photographs and geolocating walk routes and sampling sites. Reference will be made to specific Shit Flow Diagrams (SFD), where available, which visually describe excreta flow in urban and rural settings, and data will be mapped to provide a spatial outline of potential pathways for faecal exposure^{31–33}. Wherever feasible, longitudinal data will be collected on study sites to assess the effects of seasonality. This novel adaption of the SaniPath tool will enable us to integrate environmental AMR data into urban and rural WASH exposure pathways.

Observations and structured checklists will be completed at 10 public and institutional settings within each of the five sites (n=50). This will be complemented by Focus Group Discussions (FGDs) and In-depth interviews (IDIs) with key informants (heads of household, primary caregivers, school children, market vendors, etc.) to explore perceptions of barriers and challenges to WASH posed by circumstances of daily life.

Spatial analysis and integration of datasets into an agent-based model

The initial approach will be to determine variables (as described above) that have strong associations with ESBL status using model-based statistical analysis. Spatial and temporal correlations will be accounted for using both geostatistical and agent-based modelling techniques to increase the precision of our inference, and hence insight into the main demographic and environmental drivers of transmission and carriage. Geostatistical models will initially be used as a phenomenological way of detecting such associations in the quantitative elements of our data. Findings from our qualitative components will then be used to inform the structure of an agent-based model. This will allow us to test different systems models of social and behavioural features of the population that may contribute to ESBL emergence, transmission, and colonisation/decolonisation of individuals.

Data management and analysis

In Uganda CRF data and laboratory data will be collected using REDCap (version 10.0.25). In Malawi CRF data will be collected using tablets with Open Data Kit software (ODK, 1.4.10) and laboratory data will be collected using Teleform Data Capture software (10.7). Initial transcription (where needed) and data cleaning will be performed within the local data centres in Uganda and Malawi, close to the data collection context. These data will then be pulled nightly from the local data centres to the University of Lancaster (UoL), UK and formalised into an SQL database to facilitate full record linking with RANAS and WASH study data, extract query construction, and final quality assurance ([Figure 5](#)). All data will be securely stored with restricted access to the study PIs and database administrators at Malawi-Liverpool Wellcome Trust (MLW, Malawi), IDI (Infectious Diseases Institute, Uganda) and Lancaster, and shared where required with specific members of the DRUM project team using a secured instance of Dataverse hosted on UoL servers.

Community engagement and involvement

Prior to study initiation and at regular intervals throughout the study, programme-wide community engagement and involvement will be held at study sites in Uganda and Malawi, including the convening of community advisory groups and meetings with the local leadership, district health offices and district executive councils. Findings will be shared with participants, communities and local government, including key stakeholders such as the Malawian Ministry of Health AMR technical working group, the University of Malawi and the Uganda National One Health Platform's national AMR Sub-committee of the One Health Technical Working Group within Makerere University College of Health Sciences (CHS) and College of Veterinary, Animal resources and Biosecurity (COVAB).

Ethics statement, regulatory approvals and governance

The protocol, participant information sheets, consent forms and data collection tools have been approved by the LSTM Research and Ethics Committee (REC, #18-090), College of

Figure 5. DRUM data management workflow.

Medicine REC, Malawi (#P.11/18/2541), Institutional Animal Care and Use Committee (IACUC), Uganda (Ref: SVAR-REC/18/2018), Joint Clinical Research Centre (JCRC) REC, Uganda (#JC3818) and Uganda National Council for Science and Technology (UNCST, #HS489ES).

In addition, administrative permissions have been granted from community leaders and support obtained from local community advisory groups. Sensitizations of study areas will be conducted prior to initiation and full informed written consent will be obtained from all participants recruited into the study, in their local language when required.

Study status

In Uganda and Malawi, household recruitment and follow-ups have been completed in line with this protocol. Observational, CRF and microbiological data have been collected, cleaned, and integrated into the SQL database. RANAS questionnaires and transect walks have been undertaken, and genomic and spatial analysis is underway. The available data has been fed into agent-based models, which are undergoing iterative developments and optimisation.

Conclusion

In settings where there is a high incidence of severe bacterial infection and inadequate WASH infrastructure, we will identify risk factors and infer drivers of ESBL-E and ESBL-K transmission in Uganda and Malawi at the household level.

This one-health study will also provide insights on how human behaviour, WASH practices, environmental contamination, and ABU in urban and rural locations within Malawi and Uganda contribute to the transmission of ESBL-E and ESBL-K between humans, animals, and the environment. By integrating this high-quality data into agent-based transmission models, we will be able to determine critical points at which efforts to interrupt human ESBL acquisition are likely to have the greatest impact in sSA and share this information with policymakers to co-produce future intervention strategies.

Data availability

Underlying data

No data are associated with this article.

Extended data

Zenodo: Case report forms (CRFs) used for the publication: Drivers of Resistance in Uganda and Malawi (DRUM): A protocol for the evaluation of One-Health drivers of Extended Spectrum Beta Lactamase (ESBL) resistance in Low-Middle Income Countries (LMICs), <https://doi.org/10.5281/zenodo.5855820>³⁴.

This project contains the following extended data:

- DRUM01 Participant Enrolment CRF.pdf
- DRUM02 Household Enrolment CRF.pdf

- DRUM03 Household WASH CRF.pdf
- DRUM04 Participant Follow-up CRF.pdf
- DRUM05 Household Follow-up CRF.pdf
- DRUM06 Human Stool Sample Collection CRF.pdf
- DRUM07 Animal Stool Sample Collection CRF.pdf
- DRUM08 Household Food Sample Collection CRF.pdf
- DRUM09 Household Environmental Sample Collection CRF.pdf
- DRUM10 Household Floor Sample Collection CRF.pdf
- DRUM11 Household Clothing Sample Collection CRF.pdf
- DRUM12 Household Water Sample Collection CRF.pdf
- DRUM13 River Water Sample Collection CRF.pdf
- DRUM14 Household Hand-Hygiene Audit CRF.pdf

Zenodo: Laboratory standard operating procedures (SOPs) used for the publication: Drivers of Resistance in Uganda and Malawi (DRUM): A protocol for the evaluation of One-Health drivers of Extended Spectrum Beta Lactamase (ESBL) resistance in Low-Middle Income Countries (LMICs), <https://doi.org/10.5281/zenodo.5855774>³⁵.

This project contains the following extended data:

- DRUM_SOP1_V2 Human and animal stool processing.pdf
- DRUM_SOP2_V2 Environmental sample processing .pdf
- DRUM_SOP3_V2 ESBL culture.pdf
- DRUM_SOP4_V2 K. pneumoniae identification.pdf
- DRUM_SOP5_V2 Storage.pdf

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

Acknowledgements

We would like to thank the Ugandan and Malawian coordinating staff (Uganda: Sylvia Nanono [IDI], Fred Isaasi [IDI]; Malawi: Taonga Mwapasa [University of Malawi]) and laboratory team members (Uganda: Fiona Amolo Oruru [IDI]; Malawi: Madalitso Mphasa [MLW], Winnie Bakali [MLW], Chifundo Salife [MLW] and Allan Zuza [MLW]) who made local adaptations to the CRFs and laboratory SOPs. We would also like to thank the wider [DRUM consortium](#) for their insights into these methods and the local communities for their acceptance of this study.

References

1. O'Neill J: **Tackling drug-resistant infections globally: Final report and recommendations**. Lond HM Gov Wellcome Trust. 2016. [Reference Source](#)
2. WHO: **Global Action Plan on Antimicrobial Resistance**. 2015. [Reference Source](#)
3. Mendelson M, Røttingen JA, Gopinathan U, *et al.*: **Maximising access to achieve appropriate human antimicrobial use in low-income and middle-income countries**. *Lancet*. 2016; **387**(10014): 188–98. [PubMed Abstract](#) | [Publisher Full Text](#)
4. Musicha P, Cornick JE, Bar-Zeev N, *et al.*: **Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study**. *Lancet Infect Dis*. 2017; **17**(10): 1042–52. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. WHO: **Technical brief on water, sanitation, hygiene (WASH) and wastewater management to prevent infections and reduce the spread of antimicrobial resistance (AMR)**. WHO. 2020. [Reference Source](#)
6. Laxminarayan R, Matsoso P, Pant S, *et al.*: **Access to effective antimicrobials: a worldwide challenge**. *Lancet*. 2016; **387**(10014): 168–75. [PubMed Abstract](#) | [Publisher Full Text](#)
7. Seale AC, Mwaniki M, Newton CR, *et al.*: **Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa**. *Lancet Infect Dis*. 2009; **9**(7): 428–38. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Dwyer-Lindgren L, Cork MA, Sligar A, *et al.*: **Mapping HIV prevalence in sub-Saharan Africa between 2000 and 2017**. *Nature*. 2019; **570**(7760): 189–93. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. WHO: **World Malaria report**. Geneva, World Health Organization, Global Malaria Programme; 2018. [Reference Source](#)
10. Podschun R, Ullmann U: ***Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors**. *Clin Microbiol Rev*. 1998; **11**(4): 589–603. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Darton TC, Meiring JE, Tonks S, *et al.*: **The STRATAA study protocol: a programme to assess the burden of enteric fever in Bangladesh, Malawi and Nepal using prospective population census, passive surveillance, serological studies and healthcare utilisation surveys**. *BMJ Open*. 2017; **7**(6): e016283. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. MacPherson P, Lalloo DG, Webb EL, *et al.*: **Effect of Optional Home Initiation of HIV Care Following HIV Self-testing on Antiretroviral Therapy Initiation Among Adults in Malawi: A Randomized Clinical Trial**. *JAMA*. 2014; **312**(4): 372. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
13. Kazembe LN, Mathanga DP: **Estimating risk factors of urban malaria in Blantyre, Malawi: A spatial regression analysis**. *Asian Pac J Trop Biomed*. 2016; **6**(5): 376–81. [Publisher Full Text](#)
14. Bates MJ, Mphwatiwa T, Ardrey J, *et al.*: **Household concepts of wellbeing and the contribution of palliative care in the context of advanced cancer: A Photovoice study from Blantyre, Malawi**. *PLoS One*. 2018; **13**(8): e0202490. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Ministry of Health, Malawi: **Malawi Population-Based HIV Impact Assessment (MPHIA) 2015–2016: Final Report**. Lilongwe, Ministry of Health. 2018. [Reference Source](#)

16. Choko AT, Desmond N, Webb EL, *et al.*: **The uptake and accuracy of oral kits for HIV self-testing in high HIV prevalence setting: a cross-sectional feasibility study in Blantyre, Malawi.** *PLoS Med.* 2011; **8**(10): e1001102.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Ewing VL, Tolhurst R, Kapinda A, *et al.*: **Increasing understanding of the relationship between geographic access and gendered decision-making power for treatment-seeking for febrile children in the Chikwawa district of Malawi.** *Malar J.* 2016; **15**(1): 521.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Nightingale R, Lesosky M, Flitz G, *et al.*: **Noncommunicable Respiratory Disease and Air Pollution Exposure in Malawi (CAPS). A Cross-Sectional Study.** *Am J Respir Crit Care Med.* 2019; **199**(5): 613–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Ministry of Health, Uganda: **Uganda Population-based HIV Impact Assessment (UPHIA) 2016-2017: Final Report.** Kampala: Ministry of Health; 2019.
[Reference Source](#)
20. <http://www.citypopulation.de/en/uganda/cities/> Accessed 27th May 2021.
21. Chipeta M, Terlouw D, Phiri K, *et al.*: **Inhibitory geostatistical designs for spatial prediction taking account of uncertain covariance structure.** *Environmetrics.* 2017; **28**(1): e2425.
[Publisher Full Text](#)
22. Diggle PJ, Giorgi E: **Model-based Geostatistics for Global Public Health: Methods and Applications.** Chapman & Hall/CRC Interdisciplinary Statistics, Chapman and Hall/CRC Press, London; 2019.
[Reference Source](#)
23. Gauld JS, Olgemoeller F, Nkhata R, *et al.*: **Domestic River Water Use and Risk of Typhoid Fever: Results From a Case-control Study in Blantyre, Malawi.** *Clin Infect Dis.* 2020; **70**(7): 1278–84.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Wieland B, Dione M, Alemu B, *et al.*: **AMUSE Livestock, version 2—Antimicrobial use in livestock production: A tool to harmonise data collection on knowledge, attitude and practices.** Nairobi, Kenya: ILRI. 2019.
[Reference Source](#)
25. Edwards T, Sasaki S, Williams C, *et al.*: **Speciation of common Gram-negative pathogens using a highly multiplexed high resolution melt curve assay.** *Sci Rep.* 2018; **8**(1): 1114.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Mäklin T, Kallonen T, Alanko J, *et al.*: **Bacterial genomic epidemiology with mixed samples.** *Microb Genom.* 2021; **7**(11): 000691.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Dreifelbis R, Winch PJ, Leontsini E, *et al.*: **The Integrated Behavioural Model for Water, Sanitation, and Hygiene: a systematic review of behavioural models and a framework for designing and evaluating behaviour change interventions in infrastructure-restricted settings.** *BMC Public Health.* 2013; **13**(1): 1015.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Morse T, Chidziwisano K, Tilley E, *et al.*: **Developing a Contextually Appropriate Integrated Hygiene Intervention to Achieve Sustained Reductions in Diarrheal Diseases.** *Sustainability.* 2019; **11**(17): 4656.
[Publisher Full Text](#)
29. Huber AC, Mosler HJ: **Determining behavioral factors for interventions to increase safe water consumption: a cross-sectional field study in rural Ethiopia.** *Int J Environ Health Res.* 2013; **23**(2): 96–107.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. Chidziwisano K, Slekiene J, Kumwenda S, *et al.*: **Toward Complementary Food Hygiene Practices among Child Caregivers in Rural Malawi.** *Am J Trop Med Hyg.* 2019; **101**(2): 294–303.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Peal A, Evans B, Ahilan S, *et al.*: **Estimating Safely Managed Sanitation in Urban Areas; Lessons Learned From a Global Implementation of Excreta-Flow Diagrams.** *Front Environ Sci.* 2020; **8**: 1.
[Publisher Full Text](#)
32. Collet S, Yesaya M, Tilley E: **SFD Report Blantyre, Malawi.**
[Reference Source](#)
33. Raj SJ, Wang Y, Yakubu H, *et al.*: **The SaniPath Exposure Assessment Tool: A quantitative approach for assessing exposure to fecal contamination through multiple pathways in low resource urban settlements.** *PLoS One.* 2020; **15**(6): e0234364.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Cocker D, Chidziwisano K, DRUM consortium CRF working group, *et al.*: **Case report forms (CRFs) used for the publication: Drivers of Resistance in Uganda and Malawi (DRUM): A protocol for the evaluation of One-Health drivers of Extended Spectrum Beta Lactamase (ESBL) resistance in Low-Middle Income Countries (LMICs) (Version 1).** *Zenodo.* 2022.
<http://www.doi.org/10.5281/zenodo.5855820>
35. Cocker D, Mphasa M, Oruru F, *et al.*: **Laboratory standard operating procedures (SOPs) used for the publication: Drivers of Resistance in Uganda and Malawi (DRUM): A protocol for the evaluation of One-Health drivers of Extended Spectrum Beta Lactamase (ESBL) resistance in Low-Middle Income Countries (LMICs) (2.0).** *Zenodo.* 2022.
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Article summary:

This manuscript by Cocker *et al.* describes a study protocol to investigate the drivers of antimicrobial resistance in two sub-Saharan countries. The study protocol focuses on the AMR representative extended spectrum beta-lactamase (ESBL) producing organisms. The protocol is a comprehensive One Health study employing a variety of data and samples from 400 households in Uganda and Malawi, and interdisciplinary research approaches to pinpoint the drivers of resistance, from human behaviour, through antibiotic use, WASH practices, and genomic epidemiology investigation of human, animal and environmental carriage of ESBL producers.

The background and motivation for this study are clearly stated and well justified. The protocol is clearly presented and logically structured. The range of methods is described with very good level of detail. Overall, this study protocol is bound to generate multidisciplinary data of significant quality and quantity, which in turn will contribute to improved understanding of the drivers of AMR and unravel potential ways of tackling these.

I have the following comments addressing a few specific points relevant to article format “study protocol” requirement of “sufficient details of the methods provided to allow replication by others”:

One of the motivations for this study was the burden due to “severe bacterial infection” “compounded by antimicrobial resistance”. Please describe how your study of carriage (from healthy individuals) and environmental isolates will link to clinical isolates and understanding of resistance transmission to/from/within clinical settings.

Genomic analyses will be done predominantly on culture-selected ESBL producing organisms. Is AMR and multidrug resistance going to be established by phenotypic antimicrobial susceptibility testing of the isolates? If so, is AST going to be evaluated with the identified genomic determinants of resistance? If not, why not? Please specify and/or add this in the protocol.

Further specific comments:**Introduction:**

- Statement “The 3rd-generation cephalosporin (3GC) ceftriaxone is frequently the antimicrobial agent of first and last resort across much of sSA.” Modify this statement so that you refer to 3rd generation cephalosporins as a class, rather than a single drug, or expand and explain why ceftriaxone is “of first and last resort across much of sSA” and support it with a reference.
- Add “genes encoding” in the statement “...due to acquisition of genes encoding extended-spectrum beta lactamase (ESBL)-producing enzymes...”.
- What is the incidence of neonatal sepsis and malaria? What is the prevalence of HIV in these countries?
- Consider modifying this sentence to “...focus on isolation and genomic characterisation of ESBL producing *E. coli* (ESBL-E) and *K. pneumoniae* (ESBL-K).”
- Specify if in the statement “We will take an interdisciplinary, One-Health approach to assess how human behaviour, WASH practices, environmental contamination,...” you are referring to “environmental contamination by enteric bacteria” or other.
- In the final sentence “...on interrupting transmission.” specify if you are referring to bacterial transmission, resistance transmission, or other.

Site selection:

- Why were there three sites defined in Malawi (sites 1, 2 and 3) but only two sites (sites 4 and 5) in Uganda? Was it due to the contiguous location of site 5 in Uganda? How is this going to be considered for future comparative data analysis (i.e. 100 households per site)?
- Consider using “animal husbandry practices” instead of “animal practices”, or just “husbandry practices” as farming areas are mentioned in Fig. 2.
- Do you refer to “environmental contamination” (of household or premises?) in “contamination with ESBL-producing bacteria”? Please specify this.
- Was the socioeconomic status of the sites in Kampala and Blantyre comparable? Is there information on housing density comparison between the equivalent sites in Uganda and Malawi? Can you expand on this a bit more?
- Did you mean “Blantyre, the second largest city of Malawi”?
- What was the estimated population of Chileka?
- What was the estimated population of the urban study site in Kampala?
- Site 5: Hoima – check the subheading and the text in this paragraph. Was Hoima town area defined as urban (subheading) or peri-urban (in the text)?

Methods:

- How was the “apparent uniformity of the population density across the study area” estimated in Kampala and Chileka?
- How was the data on socioeconomic gradient acquired for “In Kampala, the availability of a marked socioeconomic gradient within the study region”? Please use a reference if possible.
- In the statement “... and not have confirmed or suspected acute infection”, does this take HIV status into account?
- Figure 3 – please clarify if sampling procedure in Fig. 3 or in the main text is preferred, i.e. will 3-4 visits be done within 6 months (Fig) or 12 months (text); which is more representative of seasonality? Malawi has one rainy season, Uganda has two rainy seasons.
- In the statement “...sites that serve as important reservoirs of ESBL AMR.” – did you mean “ESBL-producing organisms”?
- Household environmental sampling – what size of surfaces were sampled and/or what exposure time was used for sampling (e.g. for boot socks)? Can you specify this please?
- Microbiological methodology order – to improve the flow of this section, specify pre-processing steps at first (e.g. filtration, centrifugation, homogenization etc.), then describe pre-enrichment (in BPW if needed), and then culture and isolation.
- Write out abbreviations in full at first use, e.g. buffered peptone water (BPW).
- Drain samples – specify if these are inner drains (e.g. in the kitchen or hand washing sinks) or outer drains (sewage, other).
- Manufacturers – manufacturers are written out for some consumables (e.g. VWR for water filtering, sampling bags) but not for other consumables. For consistency, decide on including all products and manufacturers or make these generic.
- Consistency in fruit and vegetable preparation for culture – what ratios and volumes of fruit and BPW will be used? Will peel of fruits be included for culture? What mass/weight of green leafy vegetables will be used?
- Terminology – choose either “massaged” or “manually stomached” if these refer to the same processes.
- Please check clarity of this sentence “Green leafy vegetables will be weighed and have nine times the weight of the food added in BPW to obtain a sample-to-diluent volume of 1:9”. Do you refer to weights or volumes? Did you mean the diluent (BPW) will have 9 times the weight/volume of the leafy vegetables?
- Storage of BPW cultures at -80°C – is any glycerol going to be added?
- Inoculation on CHROMagar – what volume of samples cultured in BPW will be plated on the

selective agar? It was not specified what volume of BPW would be used for pre-enrichment.

- Plate sweeps – these are mentioned for the first time after ESBL isolates. What cultures and plates will be used for plate sweeps, the same as those for isolated ESBL producers?
- ESBL isolation – how many suspected ESBL colonies will be selected from each sample? A single colony of each ESBL-E and ESBL-K if present or multiple?
- Original sample storage for metagenomics – how will these be stored? E.g. will you store filtered and concentrated water, will you store swabs or swabs homogenized in PBS?
- It is not necessary to mention metagenomic tools (mSWEEP/mGEMS) in sample storage section, and it would be more suitable for sequencing data analysis section.
- DNA extraction and sequencing – how will ESBL-negative isolates be acquired and selected? This should be specified in the microbiology section.
- What library preparation kit will be used for Illumina sequencing?
- Numbers of samples for metagenomic sequencing are specified. How many isolates and sweeps will be whole genome sequenced on an Illumina platform? How many or up to how many isolates will be Nanopore sequenced?
- Metagenomic resistome of PBP pre-enriched samples will be investigated. Will you investigate the resistome of CHROMagar plate sweeps? If so, please specify this.
- Spatial analysis and integration of datasets into an agent-based model. “The initial approach will be to determine variables (as described above)” please specify in which paragraph are the variables mentioned. While biological sampling and microbiological analysis are described in a great level of detail, this section is less evolved. What software, computational tools will be used? What geostatistical models will be used? How will qualitative components be evaluated and chosen for ABM?
- As this manuscript is a study protocol and investigating “the transmission of ESBL-E and ESBL-K between humans, animals, and the environment” was one of the study objectives, it would be valuable to add a section on genomic epidemiological methods to investigate the transmission (although, this may be further detailed in publication of the study results).

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

Yes

Is the study design appropriate for the research question?

Yes

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

Partly

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

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, bacterial pathogens, antimicrobial resistance, ESBL, TB, Streptococcus, WGS, genomics, East Africa

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

Reviewer Report 20 September 2022

<https://doi.org/10.21956/wellcomeopenres.19440.r52055>

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Catrin E. Moore

St George's, University of London, London, UK

Overview:

The protocol describes an interdisciplinary study using a One Health approach which will characterise *E. coli* and *K. pneumoniae* strains carrying the extended spectrum beta lactamase resistance in the environment, animals and being carried by people in 400 households in Uganda and Malawi. The study provides a baseline examining the consequences of human behaviour, WASH practices, environmental contamination, and antibiotic use using longitudinal clinical and molecular microbiological data, set in urban and rural locations in both countries, before the introduction of relevant interventions. The study aimed to identify risk factors for and infer drivers of ESBL-*E. coli* and ESBL-*K. pneumoniae* transmission in Uganda and Malawi at the household level.

The protocol is well written and all encompassing, describing a very holistic study to examine the drivers of antibiotic resistance in two low- and middle-income countries. I look forward to reading the results which will be published soon.

I have some specific questions, separated by sections in the protocol:

Introduction:

- Please explain what you mean or provide a reference when you say “The 3rd-generation cephalosporin (3GC) ceftriaxone is frequently the antimicrobial agent of first and last resort across much of SSA”.
- You state that low-income countries in Africa have poor access to watch and reserve antibiotics, either provide a reference for this statement or explain this further with an additional sentence.

- What is a high incidence of sepsis and malaria? Please quantify.

Site descriptions:

- The site descriptions differ markedly between Malawi and Uganda, I would like to see a similar breakdown of the sites in Uganda as described in Malawi – the number of schools, farms, places of worship, hospitals and pharmacies etc.

Some clarification is required on the data collection questions on the Case Report Forms:

01: Please write CHAM (facility) in full the first time.

02: Please write CPPC in full the first time.

If a patient received more than one antibiotic, how was the formulation (oral, syrup etc) recorded?

03: Please add handwashing facilities (HWF) first time in the document.

13: How did you determine the cloudiness of the water colour to ensure robustness?

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

Yes

Is the study design appropriate for the research question?

Yes

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

Yes

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

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, AMR, burden of AMR, antimicrobial use.

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