An investigation into the role of human, animal and environmental factors on transmission of ESBL *E. coli* and ESBL *K. pneumoniae* in southern Malawian communities

Thesis submitted in accordance with the requirements of the Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy by Derek Cocker

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For Sarah

Collaborators and colleagues

This thesis is the result of my own work. Given its interdisciplinary approach and integration within the drivers of resistance in Uganda and Malawi (DRUM) consortium, many people have contributed to the methodology, data collection and statistical analysis. The following lists the roles of collaborators and colleagues who have assisted me throughout this period.

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Abstract

In sub-Saharan Africa (sSA) there is a high prevelance of gut colonisation with antimicrobial resistant (AMR) bacteria and a high morbidity and mortality from drug-resistant infections. Given the reliance on third generation cephalosporins (3GCs) in human health, two of the most important AMR bacteria found in these settings include the extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae, *E. coli* and *K. pneumoniae*. These bacteria are present in the guts of humans and animals and also within the wider environment. In LMICs, the key factors that lead to community ESBL colonisation are unclear, and I hypothesise that within low-income settings, ineffectual household water, sanitation and hygiene (WASH) practices and a paucity of WASH infrastructure contribute to ESBL contamination of the household environment and pollution of the riverine and community environment via inadequate management of faecal sludge. Interactions between humans, animals and environmental reservoirs of ESBL bacteria in these settings promotes the acquisition, maintenance and spread of ESBL *E. coli* and ESBL *K. pneumoniae*, ultimately resulting in increased levels of gut carriage of these drug-resistant organisms. To that end, in this thesis I present the results from two observational studies undertaken in southern Malawi designed to broadly assess key One-Health risks for human carriage of ESBL *E. coli* and ESBL *K. pneumoniae* in Malawian communities.

Firstly , within a large household-centred study I found a paucity of household WASH infrastructure and access to materials to enable safe toileting, adequate sanitation or effectual hand-hygiene and waste management in urban, peri-urban and rural communities, paralleled by behavioural proxies that may increase the risk of bacterial transmission, such as household attitudes to water usage, food-hygiene, open defaecation, and handwashing. Microbiological surveillance of the households illustrated a staggeringly high prevalence of ESBL colonisation in humans and animals, alongside ESBL contamination of the household and broader environment (i.e. rivers and drains). Risk factor analysis highlighted the importance of the wet season alongside differences in WASH and animal factors between urban, peri-urban and rural settings that lead to differing AMR prevelance and regional risk profiles. Lastly, in addition to the high levels of ESBL bacteria found within the river networks, I identified elevated levels of antibiotics and other resistance driving chemicals within urban rivers, suggesting that the riverine system may be a key ecological niche for AMR in this setting.

In summary, within this thesis, I highlight the key role that WASH infrastructure and behaviours play in driving human carriage of ESBL bacteria in communities of southern Malawi and identify key differences in risks of ESBL colonisation from urban, peri-urban and rural settings. Therefore, I propose that future interventions and policy designed to interrupt ESBL AMR transmission should adopt a One-Health approach, consider the integration of community-based WASH interventions, and be cognisant of regional differences in AMR-prevalence, making adaptions wherever possible which are tailored to the local population for maximal effect.

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Presentations and publications

Publications

Cocker D, Sammarro M, Chidziwisano K *et al.* 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: awaiting peer review]. *Wellcome Open Res* 2022, **7**:55

Oral presentations

One-Health AMR and WASH: Is the a***poo***calypse coming? UNC Water and Health Conference, Chapel Hill, North Carolina, USA. Oct 2021.

High levels of extended spectrum beta-lactamase (ESBL) contamination are found in Malawian households with basic water, sanitation and hygiene (WASH) access. ASTMH, Washington, USA. Nov 2021

Drivers of Resistance in Uganda and Malawi: The DRUM consortium. UKRI AMR Event series: AMR and One-Health after COVID-19. Nov 2021

Posters

One-Health drivers of ESBL resistance in Malawian households. ECCMID (online), July 2021.

Abbreviations

- 3GC: Third-generation cephalosporin
- 3GC-R: Third-generation cephalosporin resistant
- ABU: Antibiotic usage
- AIC: Akaike information criterion
- AMR: Antimicrobial resistance
- AMS: Antimicrobial stewardship
- ARG: Antimicrobial resistance gene
- ARV: Antiretroviral
- BPW: Buffered peptone water
- BSI: Blood stream infection
- CPR: Close-pairs radius
- DRI: Drug resistant infection
- DRUM: Drivers of resistance in Uganda and Malawi
- CAI: Community acquired infection
- CI: Confidence interval
- CRF: Case report forms
- CrI: Credible interval
- CoM: College of Medicine
- COMREC: College of Medicine research and ethics committee
- COVID: Coronavirus disease
- CPT: Co-trimoxazole preventative therapy
- ESBL: Extended-spectrum beta-lactamase
- ESBL-E: Extended-spectrum beta-lactamase *E. coli*
- ESBL-K: Extended-spectrum beta-lactamase *K. pneumoniae*
- ETEC: Enterotoxigenic *E. coli*
- FAO: Food and agricultural organisation for the United Nations
- FGD: Focus group discussions
- FWE: UK health security agency, food, water and environment microbiology service
- GPS: Global positioning system
- HAI: Hospital acquired infection
- HGT: Horizontal gene transfer
- HIC: High-income country

- HIV: Human immunodeficiency virus
- HRM: High-resolution melt-curve
- HWF: hand-washing facility
- IDI: In-depth interview
- IPC-MS: Inductively coupled plasma mass spectrometry
- IQR: Interquartile range
- LIC: Low-income country
- LOQ: Limit of quantification
- JMP: Joint monitoring program
- LMIC: Low- and middle-income country
- LSTM: Liverpool school of tropical medicine
- MDG: Millennium development goal
- MEM: Mixed-effect multivariate model
- MGE: Mobile genetic element
- MID: Minimum inhibitory distance
- MLST: Multilocus sequence type
- MLW: Malawi-Liverpool Wellcome clinical research program
- MK: Malawian kwacha
- NCD: Non-communicable disease
- OIE: World organisation for animal health
- OR: Odds ratio
- OSM: Open Street Map
- PC: Principal component
- PCA: Principal component analysis
- PCR: Polymerase chain reaction
- PES: Polyethersulfone
- PNEC: probable no-effect concentration
- POCIS: Polar organic chemical integrative sampler
- PrProb: Predicted probability
- QECH: Queen Elizabeth Central Hospital
- RANAS: Risks, attitudes, norms, abilities and self-regulation
- SD: Standard deviation
- SDG: Sustainable development goal

- SOPs: Standard operating procedures
- SRWB: Southern Region Water Board
- sSA: sub-Saharan Africa
- ST: Sequence type
- SWARM: Study of water, sanitation and hygiene and antimicrobial resistance in Malawi
- TB: Tuberculosis
- UPLC, LC-MS/MS: Ultra-performance liquid chromatography coupled with tandem mass spectrometry
- USEPA: United states environmental protection agency
- UTI: Urinary tract infection
- WASH: Water, sanitation and hygiene
- WGS: Whole genome sequencing
- WHO: World health organisation
- WWTP: Wastewater treatment plant

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Chapter 1:

Introduction

"Whoever wishes to investigate medicine properly, should proceed thus: in the first place to consider the seasons of the year, and what effects each of them produces for they are not at all alike, but differ much from themselves in regard to their changes. Then the winds, the hot and the cold, especially such as are common to all countries, and then such as are peculiar to each locality. We must also consider the qualities of the waters, for as they differ from one another in taste and weight, so also do they differ much in their qualities."
Hippocrates 4 Century AD

1.0. Chapter summary

Within this chapter I review the public health challenge of antimicrobial resistance (AMR) through a "One-Health" lens, with a focus on low-and middle-income countries (LMICs), specifically African ones. I then evaluate the importance of water, sanitation and hygiene (WASH) and environmental hygiene in contributing to the acquisition, maintenance and transmission of AMR, concentrating on the extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae *E. coli* and *K. pneumoniae*. Lastly, I draw this information together to provide a conceptual framework that forms the rationale for my thesis and explain my hypothesis and how it will be tested throughout this thesis.

My contributions to this chapter and those of others are included in Table 1.0.

Table 1.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted by the PhD candidate.

Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborator, Tracy Morse.

1.1. AMR and antibiotic usage

Bacterial AMR occurs due to changes to bacterial structure or function, resulting in the drugs used to treat infections becoming less effective (1). This is a natural phenomenon, whereby changes arise from mutations generated in response to host or environmental selection pressures, and has been well documented since the introduction of penicillin in the 1940s (2). The majority of antibiotics available are derived from chemicals produced by bacteria or fungi to protect themselves from microbes in the ecosystem, with only a small minority synthetically derived in the laboratory (3). After the "golden antibiotic era" few novel antibiotics have been produced and currently there are limited antibiotics in the clinical (4) or the pre-clinical pipeline (5), leaving us with a finite number of antibiotics available for use. There are many mechanisms in which bacteria have evolved to become resistant to antibiotics (6–9), and resistance mechanisms exist for all currently available antibiotics (10). This is compounded by accumulation of resistance mechanisms in the same organism, rendering bacteria resistant to multiple antimicrobials (11,12).

Effective antimicrobials are required in human and animal heath for the prevention and treatment of bacterial disease and to help safeguard the practice of routine surgery or chemotherapy (13,14). This places antimicrobials firmly at the core of modern medicine. Therefore, global rises in AMR are starting to greatly impact on morbidity and mortality, especially in low income settings (15–18). In this regard, it is estimated that without efforts to curb the rising trend in antimicrobial resistance, up to 10 million people annually will die from AMR causes by 2050, most profoundly impacting LMICs, with an associated economic impact comparable to the 2008/9 financial crisis (13). While these estimates are disputed, the magnitude of the threat posed by antibiotic resistance is well accepted, and novel methods are currently being used to better determine the true burden of disease (19–22). Estimates of the AMR disease burden in animals are unavailable because of a lag in global animal health surveillance metrics alongside a complexity in inferring outcomes from resistance. Nevertheless, it was estimated that 131,109 tons of antibiotics were used in animals in 2013, and this figure is expected to rise to 200,235 tons by 2030 (23–25). The true effect of antibiotic usage in animals on human health is uncertain, however there is a consensus that antimicrobial use contributes to the

overall burden of AMR (26,27), and hence there has been a recent drive for policies that streamline antibiotic use in animals for the health and wellbeing of both animals and humans (14,28–31). Reasons for ABU in animals commonly includes growth promotion as well as disease prevention and treatment (25,32–34), although the use of antibiotics as growth promoters in livestock has been banned in the European Union since 2006 (34). Antibiotics consumed by animals and humans reach the environment through the excretion of waste (urine or faeces), and this leads to contamination of groundwater, soil, crops and plants, which is in turn fed back into the human and animal chain through a variety of mechanisms (35–37).

Antibiotic usage is only one of several factors that drive AMR and given the importance of antibiotic prescription in the treatment of bacterial disease there is an ethical balance to be struck between access and restriction (38–43). Within humans, 3rd-generation cephalosporins (3GCs) are frequently the first line antimicrobial agent of choice in the treatment of severe gram-negative bacterial infections, especially in LMICs (44). 3GC resistant (3GC-R) enteric bacteria have rapidly emerged, largely due to acquisition of ESBL-producing enzymes, resulting in infections that are frequently untreatable in low-income countries, due to unavailability of carbapenems or other reserve antibiotics (21,22,45,46). As a result of the expansion of ESBL and carbapenem resistance, Enterobacteriaceae such as *E. coli* and *K. pneumoniae* have been labelled as critical on the WHO priority pathogen list (47). Within this thesis I will be focussing on ESBL AMR within these two key organisms, in the low-income country setting of Malawi.

1.2. International AMR policy

Due to overwhelming evidence of the threat posed by AMR, in 2014 the UK government commissioned the first report on global issue of rising AMR, commonly referred to as the O'Neill report (13,48). This was a hugely impactful piece of work which contributed to increased international support for action and has been accompanied by responses from the World Health Organization (WHO) (14) the Food and Agricultural Organization (FAO) of the United Nations (30), the World Organisation for Animal Health (OIE) (31), and the European Union (49). A global action plan was developed by WHO in 2015 (14), which stated that all member states should have a national action plan within 2 years, and while this ambitious timeframe has not been achieved, to date there has been the creation of over a 100 national action plans by a range of high income, middle income and low income countries (50).

There has, however, been criticism of the governance, strategy, and scope of these documents, particularly in relation to adoption of One-Health goals and a lack of consideration for health inequities in low-income settings (51,52). Despite the acceptance of the importance of human, animal and environmental health there is still a focus on human health, with limited targets or goals in the animal sector, and rarely any meaningful incorporation of environmental targets or actors (53). It has been proposed that this is, in part, a consequence of chronic underfunding in environmental microbiology capacity and research, leading to a lack of understanding of AMR in the environment and resulting in an absence of evidence-based mitigation strategies (36). Funding issues are also pertinent to AMR strategies in LMICs, and without external financial support or a global focus on capacity strengthening, LMICs are unable to effectively implement action across the human, animal and environmental sectors (51,54). A better understanding of how humans, animals and the environment interact to facilitate AMR transmission in LMICs is clearly needed and this begins with the generation of microbiological surveillance data on key priority pathogens, embedded within a framework that contextualises the specific risks of life in low-income settings.

1.3. The role of One-Health in AMR

One-Health is a modern phrase for an ancient concept that recognises that the health of people is strongly interconnected with the health of animals and the shared environment (55–57). Since Hippocratic times there has been a historical precedent in the need for physician advocacy within the realm of One-Health, and texts taken from "*On airs, waters, places"* advises the doctor to serve his patient best by paying attention to the environment including the quality of the waters and the soils (58,59). Recently, there has been an evolving use of the term "One-Health", from the integration of animal and human medicine to the current day incorporation of human and animal health within the framework of environmental health (55,60,61). Changes to the definition have resulted from a heightened awareness within the scientific and political community in the need for interdisciplinarity and a holistic response to understanding complex systems, to enable effective solutions for a range of critical global health issues. These efforts have been largely focused on new and emerging zoonotic diseases, due to the prominence of recent viral epidemics (i.e. Ebola, Zika and SARS-CoV2), historical collaborations between human and animal health sectors and the absence of an environmental voice within the political narrative (60,62,63). However, One-Health has now become broader in its scope, and is widely accepted to incorporate other global health threats such as antimicrobial resistance, food safety and environmental contamination, alongside non-communicable diseases such as mental health, ecotoxicology and the effects of urbanisation (49,60,64–67).

AMR is one of the issues which is considered most likely to benefit from a One-Health approach, given that it has clear connections to human, animal and environmental health domains, and solutions requires collective action from a range of specialists and authorities (13,68,69). Therefore One-Health approaches are now the foundation on which a number of AMR research activities, policies and international action plans are built (14,50).

1.4. Current knowledge of ESBL carriage and disease epidemiology in sub-Saharan Africa from a One-Health perspective.

1.4.1. The microbial context

Enterobacteriaceae are a family of gram-negative facultative anaerobes, which includes the species *E. coli* and *K. pneumoniae*. These are nearly ubiquitous bacteria, and can be responsible for a broad range of intestinal and extraintestinal infections in humans and animals (70). These bacteria have been selected as they 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 pathogen (71). However, these bacteria are also present within the broader environment, including surface waters such as lakes, rivers and groundwater, soil and plants, and these ecological niches provide a gene pool with a far greater diversity than that of the human or animal microbiota (72).

Bacterial classification is complex; for example, *E. coli* can separated into >190 serogroups based on its surface antigens (73) or seven broad phylogenetic groups (A0, A1, B1, $B2_2$, $B2_3$, D1 and D2) (74). Given the propensity for these bacteria to adapt to a range of host and environmental conditions, several highly adapted *E. coli* or *K. pneumoniae* clones have emerged, with specific virulence factors that produce pathogenic *E. coli* or hypervirulent *K. pneumoniae* (75). These virulence attributes may either be encoded on genetic elements that can be mobilised into other strains to create novel combinations or be locked into the chromosome. In *E. coli*, the most evolutionary successful combinations of these virulence factors form “pathotypes” which can cause specific disease in healthy individuals (76), and within *K. pneumoniae*, alterations to the thick polysaccharide coat can facilitate evasion of the host defences and can change the propensity for disease leading to hypervirulent strains (77). These adaptions highlight some of the mechanisms by which these bacteria can become pathogens.

E. coli and *K. pneumoniae* infections are commonly treated with antibiotics, including, but not limited to, ß-lactams (penicillins, cephalosporins or carbapenems), fluoroquinolones and aminoglycosides. They have evolved many mechanisms to survive the toxic effects of antibiotics, including decreased uptake or removal of antibiotics through alterations in efflux or membrane permeability, target modification or replacement, and inactivation, frequently via enzymatic methods (7,8). ß-lactamases are the enzymes that hydrolyse the active ß-lactam ring of ß-lactam antibiotics, and the production of these enzymes is the most common mechanism for ß-lactam resistance identified in gram negative bacteria, including Enterobacteriaceae (78). Extended-spectrum ß-lactamases are widely defined as ß-lactamases that confer resistance to penicillins, first-, second- and third-generation cephalosporins and aztreonam and can be inhibited by ß-lactamase inhibitors such as clavulanic acid (79).

Characterisation of ß-lactamases is made on the basis of functional (Bush-Jacoby) or structural (Ambler) information (80,81) (**Table 1.1**). The Ambler system sorts ß-lactamases into classes A, B, C and D based on protein homology, with class A, C and D denoting serine-ß-lactamases and class B denoting metallo-ß-lactamases (78). The Bush-Jacoby system sorts ß-lactamases into four main groups, with other multiple subgroups according to functional similarities. In the Bush-Jacoby system ESBLs belong to group 2be (Ambler group A) or 2d (Ambler group D). These include the enzyme families TEM, SHV, CTX-M, PER, VEB or in the case of 2d, OXA (80). Amp-C ß-lactamases, located in Bush-Jacoby group 1 (Amber group C) can also confer resistance to ß-lactam antibiotics, but they are not inhibited by clavulanic acid (82). Within this thesis, I will focus on third generation cephalosporin resistance (3GC-R) caused by ESBL-production of group 2be and 2d enzymes within the Enterobacteriaceae species *E. coli* and *K. pneumoniae*.

Table 1.1. Enzymatic classification of ESBLs. Adapted from (78,80)

Representative enzymes	Bush Jacoby group *(2009)*	Ambler class	Distinctive substrates
E. coli AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1	1	C	Cephalosporins
GC1, CMY-37	1e	C	Cephalosporins
PC1	2a	A	Penicillins
TEM-1, TEM-2, SHV-1	2b	A	Penicillins, early cephalosporins
TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1	2be	A	Extended-spectrum cephalosporins, monobactams
TEM-30, SHV-10	2br	A	Penicillins
TEM-50	2ber	A	Extended-spectrum cephalosporins, monobactams
PSE-1, CARB-3	2c	A	Carbenicillin
RTG-4	2ce	A	Carbenicillin, cefepime
OXA-1, OXA-10	2d	D	Cloxacillin
OXA-11, OXA-15	2de	D	Extended-spectrum cephalosporins
OXA-23, OXA-48	2df	D	Carbapenems
CepA	2e	A	Extended-spectrum cephalosporins
KPC-2, IMI-1, SME-1	2f	A	Carbapenems
IMP-1, VIM-1, CcrA, IND-1 L1, CAU-1, GOB-1, FEZ-1	3a	B (B1/B3)	Carbapenems
CphA, Sfh-1	3b	B (B2)	Carbapenems

E. coli and *K. pneumoniae* can acquire ESBL resistance vertically or horizontally through transfer of mobile genetic elements (MGEs) independently of cell division, and this can occur in a variety of intestinal and extraintestinal environments (83). A globally important example of this is the rise of ESBL-*E. coli* (ESBL-E) infections due the acquisition of IncFI, IncI, and IncK plasmids associated with CTX-M enzymes, often in *E. coli* ST131 (39,84,85). Resistance genes can be transferred between

chromosomes and plasmids and between different plasmids, and therefore it is important to consider the degree to which AMR is a problem of movement of ESBL genes, MGEs, or bacteria species and subtype, and the degree to which the relative importance differs between bacteria.

Whole genome sequencing (WGS) data offer unprecedented resolution when attempting to type bacteria in order to investigate transmission of bacteria or MGEs between bacteria circulating in the human and animal guts and the environment and shines a light on the degree to which these compartments are interconnected (86). Therefore, it will ultimately be important to large scale sequencing of isolates collected in this study to add granularity to the findings.

Despite the propensity for bacteria to share genetic information, there are variations seen in the epidemiology of ESBL-E and ESBL-resistance *K. pneumoniae* (ESBL-K) in terms of species cross-over, carriage rates, disease burden and associated risk factors (38,41,87–89). In the next sections I explore the epidemiological landscape of ESBL-E and ESBL-K in more detail.

1.4.2. The human context.

The global burden of disease from AMR is vast, and depending on the measurement index used is either the third or twelfth leading cause of death annually in humans; with 929,000 deaths attributable, and 3·57 million deaths associated with AMR (90). Within this, 50,000-100,000 deaths are attributed to 3GC-resistant *E. coli*, and 25,000-50,000 deaths are attributed to 3GC-resistant *K. pneumoniae* (90). Contributing factors include a person's age and geographical location, with infants and the elderly most at risk, and the highest all-age death rates seen in low income settings (90,91). Estimation of the burden of disease attributed to ESBL-E and ESBL-K in sub-Saharan Africa (sSA) is hampered by inadequate clinical and microbiological surveillance and inadequate data reporting frameworks (46,90). Nevertheless, recent estimates place the prevalence of ESBL in blood stream infections (BSIs) in sSA at 18.4% for ESBL-E and 54.4% for ESBL-K (46). Locally in Malawi, 61.9% of BSIs are either 3GC or fluroquinolone resistant, and there has been an increasing trend of ESBL resistance seen in *E. coli* and *Klebsiella* spp. BSI isolates, with 30.3% of *E. coli*, and 90.5% of *Klebsiella* spp. now 3GC resistant (16). ESBL-producing bacteria are implicated in a range of other conditions including adult and neonatal sepsis (92–95), urinary tract infections (UTIs) (96,97) and intrabdominal infections (98) amongst others, however, there is even less clinical data for these syndromes in the African context.

Gut mucosal colonisation with ESBL Enterobacteriaceae is thought to precede invasive infection (88,99,100), and the prevalence of ESBL gut colonisation varies widely between geographical location (46,101). Global estimates range between 3-8% in Europe, 3-4% in North America, and are as high as 46% in SE Asia (101). In sSA ESBL carriage rates have been reported between 5-84%, with a median of 31% (101). However, the prevelance of colonisation also depends on the setting (i.e. community vs hospital), and this information is rarely captured. These data are key if we are to quantify the interrelationship between community and hospital ESBL transmission. Current figures of the community carriage rate with ESBL bacteria from sSA populations range between 5-59%, with a pooled estimate of 18%. This increases to 32% for samples taken at hospital admission and up to 55% for hospitalised inpatients (101). Longitudinal community-based studies are needed to evaluate community prevelance more accurately, with a focus on the role of different community structures and the effects of urbanisation.

An important factor within AMR transmission is the spread from person to person and transmission of ESBL-K between hospitalised patients in close proximity, whereas ESBL-E has shown less propensity for transmission between patients (102,103). This is contrasted in the community setting, where evidence exists that both ESBL-E and ESBL-K transmission occurs within households, and that this in turn is influenced by household density (87). This highlights the importance of within-household transmission in the community setting and that ESBL-E and ESBL-K may have different ecological niches (87,104–106). The dynamic interaction between healthcare settings and the community is less well described, especially in Africa.

Other than hospital exposure, risk factors for community ESBL colonisation include previous antibiotic usage, indwelling devices (i.e. catheters), foreign travel, prior colonization, increasing age, chronic disease, living in overcrowded households and contact with animal and environmental sources (38–40,87,107–110). Whilst there has been a great deal of focus on the risk from antibiotic usage, there is less data to quantify the risks associated with animal and environmental exposures, especially within LMIC settings. These factors are likely to play an important role in the acquisition, maintenance and transmission of ESBL resistance within the community and deserving of future research.

1.4.3. The animal context.

In HICs, human-human transmission is estimated to account for two thirds of community ESBL-E, with the other third coming from non-human causes, such as animals and the environment (83). Given

these estimates, community ESBL-E transmission may not be self-maintaining without transmission to and from non-human sources (83). The role of animals in the transmission of ESBL in LMICs is likely to be distinct to high-income settings. In Africa, 250–300 million people depend on livestock for their income and livelihood (111), and given this precarity, antibiotics are often used for growth promotion and treatment of disease, with rates up to 97% having been reported (112). Within LMICs the proportion of transmission of ESBL bacteria that is from animals and the environment to humans has not been modelled, but this is expected to be significantly higher than in high income settings, due to the complex and close relationship between animals and humans and the shared environment.

Akin to human health, antimicrobial use in animals is directly linked to higher rates of AMR (23,26,32,113). Antibiotic usage in animals is often unregulated, particularly in LMICs (28,111), and global antibiotic consumption in animals, including sSA is expected to rise substantially by 2030 (114,115), fuelled by the demand for meat production. ABU in these settings is dependent on access, cost, local veterinary services and socio-cultural choices (111,116). Currently there is no standardized framework for animal ABU within LMICs (117) and despite efforts to improve monitoring, regulation and stewardship, governmental action is still lagging in this sector (117–119). Development of macroeconomic approaches (120) and regulation of AMU in animals is required to address the wider issue of AMU, although it is recognised that implementation will be difficult in LMICs.

The close proximity of animals and humans is important for transmission, and in sSA, animals frequently live inside the household or compound (121). Frequent exposures to animal faeces have been reported in these settings (121), and this in turn leads to faecal-oral transmission of ESBL bacteria through direct contact with faecal matter or contamination of hands, food, and water sources. Subsistence farmers have reported to be colonised with the same bacteria as their animals (122), and it is likely that community members that share their household environments with animals would also be colonised with the same bacteria, however data on this is lacking from LMICs.

In HIC settings, livestock animals including cattle (86,123), pigs (124,125) and poultry (86,126) alongside domestic animals such as cats (127,128) and dogs (127–129) are shown to be colonised with ESBL bacteria to varying levels, depending on the country and context (i.e. abattoir works, farmers or retailers). In livestock, sequence types (STs) 131, 10 and 88 are commonly found, with CTX-M-1 / CTX-M-15 genes detected (86), especially in chickens (130,131) and pigs (86,132). These STs and ESBL genes have also been identified in other livestock species and domestic animals (127,133). Clonal complexes such as ST131 *bla*CTX-M-15 are of global importance to human ESBL-associated disease,

and the presence of these in animal populations demonstrates that the animals may serve as a reservoir for human AMR (134). However, a large number of other STs and ESBL genes have been reported in animals (86,125), and the true relationship between animal and human colonisation is the subject of extensive ongoing research, with many citing host-specific niches and minimal transmission between the animal-human axis. Examples of this include pooled analysis from an extensive collection of ESBL-E isolates in the Netherlands (135) and sequencing on livestock samples from the UK (136,137) which both failed to demonstrate any close epidemiological linkage between the genes or plasmid replicon types in human and animal populations. Equally, in relation to ESBL-K, the biggest One-Health study to date found no link between circulating clades of ESBL-K in humans and animals (138). It is important to note that these studies are all from HICs, and the prevelance of ESBL in livestock and domestic animals from LMICs, and sSA in particular is less well known. As evidence grows for the prevelance of ESBL-producing bacteria in animals, and genomic libraries increase, better assessment of the importance of animal-human transmission will be possible.

In the African context, the CTX-M group predominates, especially in poultry (139). Evidence from East Africa illustrates widespread pan-species ESBL-E colonisation in domestic and farm animals, including CTX-M-15 genes and plasmids of international concern; with the highest rates found in dogs (39.2%) and pigs (33.1%) (140,141). ESBL-E colonisation in poultry has been reported from many sites, including Ghana (142), Nigeria (143), Tanzania (140) Kenya (144) Uganda (145) and Zambia (146). Colonisation rates vary by setting and low sample numbers and methodological inconsistencies between the studies hamper us from making meaningful comparisons. Currently there are no published ESBL rates amongst poultry, livestock or domestic animals in Malawi.

Close proximity of humans and animals is important for transmission, but so is the species, the nature of the interaction and shared environment. For example, in abattoir workers in South Africa and Cameroon who slaughtered pigs, the greatest risk for ESBL colonisation came from external contact with poultry, not pigs (147), illustrating the differences in risks to human health posed by individual animal species. Poultry are a species of particularly concern, because they have the highest prevelance of ESBL colonisation globally (148), share the same clones that are found in human disease (149) and frequently co-habit with people in a shared household environment (150). Furthermore, they are regularly given antibiotics for growth promotion or disease prevention (113,148), especially in LMICs (117), and consume animal feed that contains antibiotics (151) or heavy metals (148), which co-select for AMR (36,152). When considering the impacts of animals in LMICs attention should be drawn to

the species in question, ABU and husbandry practices, the frequency and nature of interactions and the role of sanitation and hygiene measures governing animal waste management.

1.4.4. The environmental context.

Research and policy have long focussed on healthcare facilities and human or animal antibiotic exposures (1), whilst the environmental component has until recently been overlooked (153). Contamination of water, soil, food and household environments by AMR organisms may allow for the maintenance and spread of AMR, however the degree to which this is the case is unknown (36,154). Environmental contamination with ARB (i.e. ESBL-E and ESBL-K) that are able to colonise both humans and animals pose the highest overall risk (155), and specific environments can themselves serve as reservoirs for ARB or ARG (36,156). ESBL-E, ESBL-K and ESBL ARGs are frequently found in the environment, particularly surface waters such as lakes (157–160) or rivers (36,161–167). Evidence of their presence in these environments exists in LMICs (168–174), including sSA countries (32,140,175–177), but the rates reported vary substantially by site and source type, and to date, there is no documentation of environmental ESBL-E or ESBL-K rates from Malawi. There is also a dearth of studies reporting epidemiological and temporally linked clinical ESBL isolates with environmental samples. Where they do exist, they frequently employ a wide range of study designs and have very low sample numbers (168,178–181). So, although the high prevalence of ARB and ARG in the environment is well documented, the precise role that environmental niches play in the transmission and stable acquisition of AMR pathogens to humans still remains uncertain.

Metagenomic analysis has been undertaken to better determine the inter-relationship between AMR in humans and the environmental resistome (182–184). The resistome comprises of all the AMR genes and their precursors that are present in an environment, whether from pathogenic or non-pathogenic bacteria (185). Studies from high-income and low-and-middle income sites show that environmental resistomes are structured by ecological gradients, and ARGs in LMICs have been shown to cross habitat boundaries, most likely due to the excreta management strategies employed (183). Whilst these metagenomic studies provide information on the relative abundance and diversity of AMR in various habitats and permit broad-level assessment of the relationships between the human microbiota and environmental resistomes, they are unable to delineate the bacterial species which harbour specific ARG, and therefore how these environments relate to clinical disease. So, although

functional metagenomics is an excellent research tool, it does not provide us with the information needed to link the environment to human AMR-associated disease epidemiology (161,182,186–191). One-Health studies that capture temporally and geographically linked human, animal and environmental metadata are urgently needed, that undergo a range of short-read, long-read and metagenomic analyses.

The origin of AMR is ancient, evidenced by the presence of ARGs in permafrost predating the Anthropocene epoch and perhaps relates to mechanisms designed to evade destruction by toxic substances, such as antibiotic release by environmental bacteria in response to competition for nutrients (192). Antibiotics are not the only toxic substance to bacteria, and genetic mutations that confer advantages to specific environmental conditions such as the presence of heavy metals lead to indirect co-selection for AMR (72). Resistance genes are, however, commonly associated with a fitness cost, thus ARGs are prone to de-selection in nature (193). Within the aquatic environment, such as rivers or surface waters, the drivers of AMR selection include antibiotics, metals and biocides (36). The presence of antibiotics, alongside other key resistance-driving chemicals (i.e. biocides and heavy metals) in these aquatic environments promotes HGT and alters microbial communities, contributing to the dissemination of ARGs and subsequently poses downstream risks to human and ecological health (194–196). In certain settings, this is compounded by pollution from inadequate treatment of industrial, domestic, and agricultural waste, enhancing the resistome in the environment (197). Research on the distribution and ecological risks of resistance-driving chemicals in urban rivers from LMICs is scarce, particularly in sSA. A key knowledge gap is therefore the baseline metrics and seasonal trends in the presence and continuum of antibiotic residues from waterways within these settings to start to quantify these risks.

When considering potential environmental reservoirs of ARG, it is important to contextualise site-specific human and animal exposures and key WASH factors. For example, LMICs often have inadequate sanitation facilities and sub-optimal waste management practices (198), which leads to increased contamination of river and surface waters by human and animal faeces (199). Interactions with these types of environments are enhanced in LMICs due to paucity of WASH infrastructure (200), and exposures to untreated sewerage from surface waters put the local population at risk of AMR transmission (201). It will be crucial to study these key environmental niches in LMIC settings and the sanitation factors that drive the dissemination of ARB and ARG if we are to develop contextualised solutions to combating antimicrobial resistance.

Seasonal effects on the environment are of particular importance in LMICs. Alterations to water supply can drive behavioural changes that stimulate the use of unprotected water sources such as rivers, thereby placing the local population at increased risk of transmission (202–204). Increased rainfall in the wet season leads to the overflow of human and animal sewerage into the riverine environment (205,206). This is compounded by poor drainage systems which further heightens the susceptibility to flooding, thereby increasing the frequency of these events (207,208). Urban settings in sSA are particularly vulnerable to these threats, with dense populations and a paucity of WASH infrastructure, leading many to highlight them as potential hotspots for environmental AMR (209). The advent of open drain flooding in urban areas has been shown to parallel with increased rates of enteric infections, especially in children (210). Interactions with rivers are also known to increase rates of non-AMR enteric disease. River water use and exposures have been noted to be a risk factor for Typhoid disease in Nepal (211), Vietnam (212) and locally in Blantyre, Malawi (213). This in turn leads to an increase in antibiotic usage and further selects for AMR. With the advancement of climate change, flooding events are expected to increase in frequency and intensity (214) and LMICs will suffer the highest burden of effects as a result of climate insecurity (215).

1.5. Water, Sanitation and Hygiene and AMR

There has been international support linking the AMR agenda to WASH priorities (209,216), including most recently via WHO guidance on WASH and AMR published in 2020 (198). WASH infrastructure is a key barrier to unfettered interaction between human, animals and the environment. WASH inadequacies, whether infrastructural or behavioural increase these interactions. Globally, WASH inadequacies are heavily concentrated in LMICs. Currently, 2 billion people are estimated to lack access to basic sanitation such as improved pit latrines or private toilets, and over 600 million people practice open defecation (198). Poor sanitation levels directly correlate with higher levels of AMR (217), and increased prevelance of diarrhoea (218). Diarrhoeal disease is frequently treated with antibiotics and it is estimated that a 40% reduction in WASH-associated ABU would be achieved by implementing safe WASH practices and infrastructure in LMIC communities (13).

From the 3.4 billion people who have safely managed sanitation (as defined by WHO and Joint Monitoring Program [JMP]), only 2/3 have toilets connected to sewerage networks where waste is treated, and ~1/3 use toilets where excreta are managed in situ (219). The management of sewerage in situ impacts household exposure routes through direct contamination of faecal material (220) or sludge management issues including bioaerosol dispersal of enteric pathogens from pit emptying

(221,222) or soil and groundwater contamination from bacterial and chemical pollutants (223). Waste streams, particularly in urban slums can also be influenced by the use and disposal of grey water (defined as wastewater of domestic use, excluding that pertaining to toilets), and in peri-urban regions of LMICs grey water accounts for ~75% of the total domestic water consumption (224). Grey water is frequently contaminated by human faeces, most notably from households that are washing nappies (223–225), and where evidence exists, it commonly contains ESBL enterobacteriaceae (225). Leaching of grey water and pit latrine excreta into the groundwater and rivers subsequently contaminates drinking water and can lead to faecal-oral ingestion of AMR bacteria. Within the WASH community, faecal-oral exposure routes are commonly described in an F-diagram (121,226). When considering the routes from acquisition of AMR bacteria in our setting, adaptions are likely to be needed that incorporate the importance of animal faecal risks and environmental exposures (**Figure 1.1**).

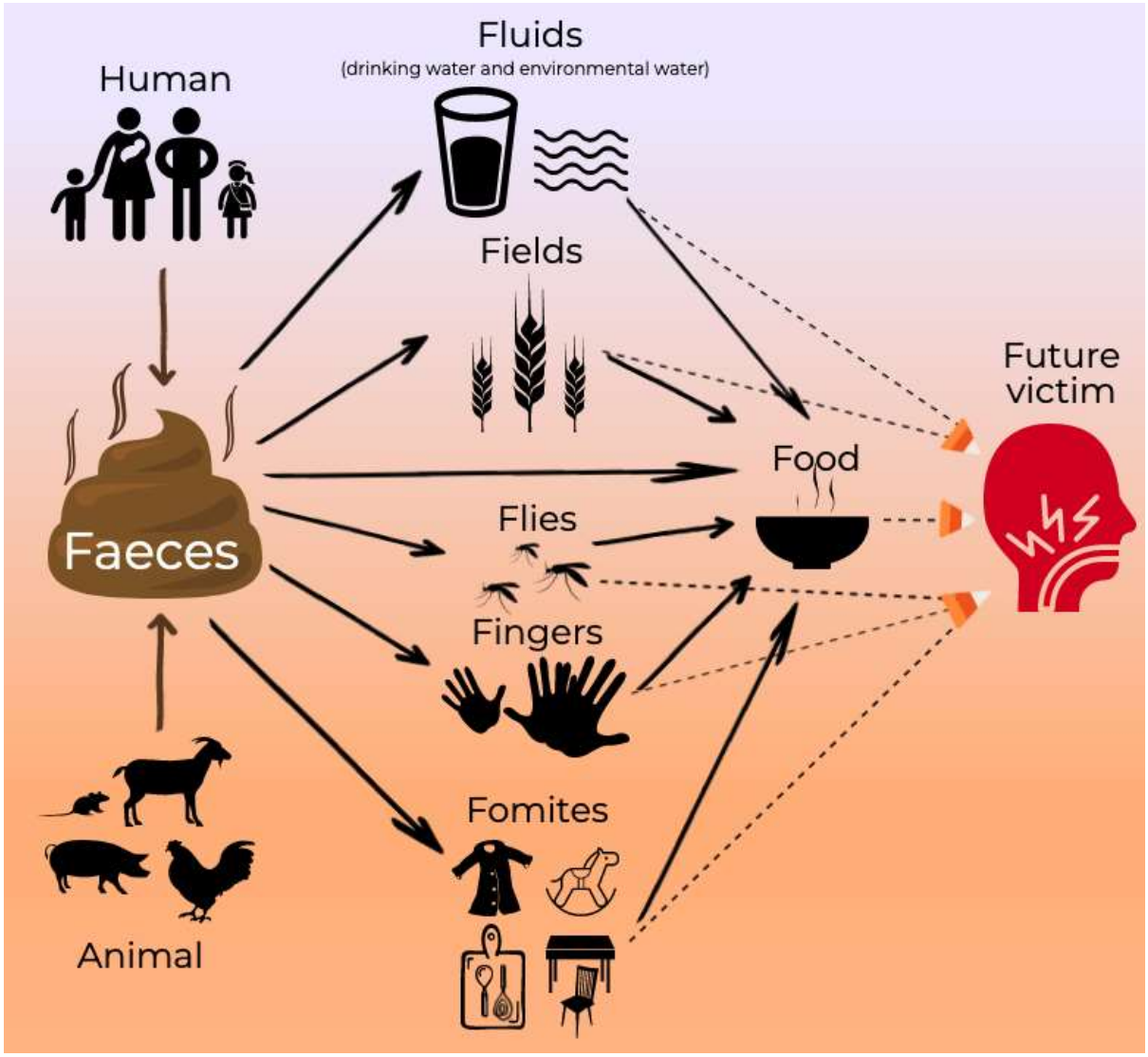

Figure 1.1. Adapted F-diagram, illustrating the routes of household faecal exposures from humans, animals and the environment (121,226).

Drinking water provides a key risk if not managed effectively, and unsafe water use is associated with an increase in the transmission of faecal oral pathogens (219,227). Safe water is in short supply, and at present 2 billion people consume water from a source contaminated with faeces (198,219). 785 million people do not have access to a basic drinking water service (defined as an improved water source accessible in 30mins) and 144 million people are dependent on surface water, such as lakes or rivers (198,219). However, unsafe water usage is considered to be highly prevalent in sSA, and JMP estimates that within Malawi 86% of people have basic water access, with the rest having either limited, unimproved or use surface water as a primary drinking water source (228). Unsafe water sources frequently have *E. coli* contamination (229) and many examples of ESBL-E contamination in water sources exist, especially from low-income settings or displaced populations where water insecurities are an everyday threat (211,230–232). There are differences noted in the regional qualities and risks of water sources from country-country and between urban vs rural settings, especially in sSA (209,229,233,234), so it will be important to contextualise these risks based on local infrastructure, socio-cultural practices and access.

In sSA, where water insecurities are common, drinking water is typically collected from non-household sites (i.e. boreholes or public kiosks), brought back in containers and stored on premises. Post-collection contamination of source water by *E. coli* and ESBL-E is commonly identified (235–237), and the levels of contamination reported in the literature fluctuate by site (237) and are subject to seasonal changes (235). The mode of contamination is thought to be related to inadequate hand-hygiene alongside contact with animal or environmental sources, and some benefits have been identified by covering water storage containers, thereby limiting environmental contamination (237,238).

Hand-hygiene plays an important role in safe water and sanitation and is frequently suboptimal in sSA. Globally, 3 billion people lack basic handwashing facilities at home, 1.6 billion have limited facilities lacking soap or water, and 1.4 billion have no facility at all. Rural areas in countries like Malawi have substantially less hand-washing facilities that those in urban settings, and this reiterates the importance of regional contextualisation (228). Hand-hygiene interventions in LMICs have been modelled for their impact on community ESBL carriage and early findings suggests that, targeted household hand-hygiene interventions could help prevent transmission of ESBL-E to a greater degree that alterations to local ABU (106). In practice, hand-hygiene interventions have been difficult to implement effectively. Work undertaken in Malawi found that a high level of knowledge on

appropriate hygiene practices was not reflected in observed habits (239) and observed levels of hand-hygiene are frequently lower than those that are reported (240). Therefore, we should consider the local barriers to key practices and psychosocial factors that influence decision-making in these contexts (239,241).

Food can be another source for AMR transmission. Food and water purchased from local vendors in these settings often are contaminated with ESBL bacteria (242,243). If safe produce is obtained, food prepared in the household under unhygienic conditions, with inadequate hand-hygiene can become contaminated with pathogens (244) or AMR bacteria including ESBL-E (135,137,245,246). This is further compounded by faecal contamination of kitchen utensils, water and other items used in cooking practices as a result of inadequate hand-hygiene, especially in LMICs (241,247,248). Animal contact with food items and utensils adds an additional risk for AMR transmission, and non-human contaminations should be evaluated in these settings (121).

Lastly, contaminated raw and ready to eat foods that are uncooked prior to eating (245), or post-cooked food contamination (249) are common sources of bacteria that lead to diarrhoeal disease. This is of particularly concern for children in low-income settings (250), where in the under 5s up to 70% of diarrhoea-inducing pathogens are thought to be acquired from food produce (241). In this cohort, epidemiological data indicates that food is more important than water for transmitting bacteria that leads to diarrheal disease (251). Together, vendor-associated food-hygiene and household practices can lead to direct risks of ESBL acquisition or pathogens that cause diarrhoea and subsequent antibiotic prescription. Whilst benefits from WASH interventions on diarrhoeal disease are still unclear (252–255) it is likely that measures that reduce bacterial transmission of faecal-oral pathogens would also reduce transmission of AMR bacteria.

1.6. Conceptual framework for the community transmission of AMR in Malawi.

Various drivers of AMR have been postulated and these have been framed within clinical, epidemiological, social, political and environmental narratives (1,3,256). From the evidence reviewed in this chapter we can see that the community setting is an important site for ESBL AMR transmission in LMICs such as Malawi. It is likely that in low-income settings, WASH factors play a central role in environmental ESBL contamination that leads to onward risks for the local population.

I therefore hypothesise that in Malawi, ineffectual household WASH practices and a paucity of WASH infrastructure leads to ESBL contamination of the household environment along with pollution of the riverine and community environments from faecal sludge containing ESBL bacteria. Human and animal interactions with these environmental reservoirs promotes the acquisition, maintenance and spread of ARB and ARGs, thereby facilitating the transmission of AMR-pathogens including ESBL-E and ESBL-K to humans, ultimately resulting in gut carriage of drug resistant organisms in these settings. Furthermore, variations between urban, peri-urban and rural infrastructure and behavioural practices are likely to result in regional differences in ESBL prevelance within humans, animals and the environment as a result of individual-level and household-level factors. Therefore, it will be important to describe the household-level risks associated with ESBL carriage from these unique geographic perspectives to look for key similarities and differences in WASH, animal husbandry and environmental exposures in parallel with assessments of individual-level factors such as age, sex, co-morbidities, ABU and healthcare exposure.

To interrogate this hypothesis, I will undertake a One-Health study in households from urban, peri-urban and rural Malawi that assesses the prevelance of ESBL-E and ESBL-K colonisation of humans, co-located animals and the household environments, in conjunction with ESBL-E and ESBL-K contamination of the broader local environment including the drainage and riverine networks. This will be augmented by individual-level and household-level datasets that capture a broad range of potential risk factors for ESBL AMR transmission, and a river water study in urban Blantyre to evaluate the key drivers and ecological risks within the aquatic environment.

These data will later be input into agent-based models and undergo genomic analysis to further characterise the drivers of ESBL AMR in our setting, but these analysis will not be included as part of this thesis. Details of the study design and methods are discussed in Chapter 2, and broad-level site descriptions including population demographics, healthcare and common disease epidemiology are contained in Appendix i.

This work is nested within the DRUM Consortium, an MRC “AMR in a Global Context” award and thus able to incorporate interdisciplinary approaches and opinions within the study design, data capture and analysis. This has enabled me to work with specialists in the relevant disciplines to select appropriate analysis plans and contextualise the findings, in an effort to maximise the validity of the interpretations made from a One-Health context.

1.7. Thesis structure and overview

Below I briefly describe the overview of the thesis and summarise the methodologies used and high-level findings in each chapter as an aide memoire (**Figure 1.2**).

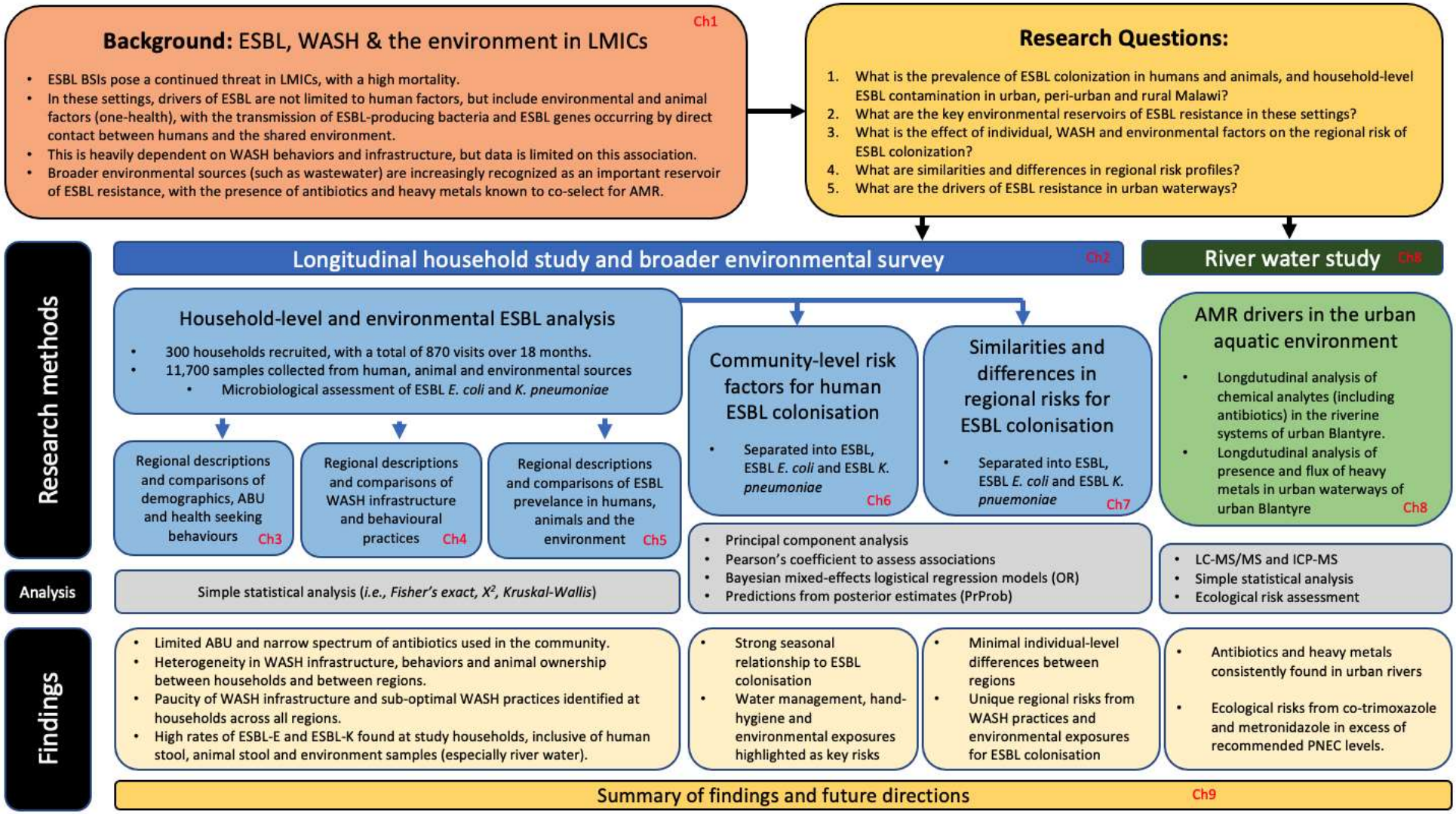

Figure 1.2. Overview of PhD chapters

Appendix

Appendix 1i. Detailed setting description including population demographics, healthcare and common disease epidemiology in southern Malawi.

Malawi has a population of 18 million, is split into 3 regions (north, central and southern) and has a life expectancy of 64 years. With an estimated population of 7,750,629, southern Malawi comprises of a few dense urban conurbations surrounded by rural areas (257). Malawi is less urbanised than similar African nations, with 83% of people living in rural areas (2). The city boundaries of Blantyre encompass 26 administrative wards and has a population of 800,264 (259).

Medical care in Blantyre is provided by health centres in the community and through a large tertiary hospital, Queen Elizabeth Central hospital (QECH). In Blantyre the HIV prevalence is 18% (260). The majority of adult admissions to QECH are HIV positive, with up to 34% newly diagnosed at presenting admission. A study of 892 sequential adult patients admitted to QECH in June-December 2014 showed a 69% prevalence rate for HIV (261). Trends in medical admissions have reflected the outcome of ART rollout, with fewer HIV related complications and the advent of increased presentation of non-communicable diseases (NCDs) and their complications (262,263). High levels of TB bacteraemia (264), pulmonary and extrapulmonary TB, Typhoid and enteric diseases have also been noted (265,266).

Whilst the incidence of BSIs has reduced since the rollout of ART in 2004, a study at QECH in 2009-2010 illustrated that 90% of patients presenting with BSIs were HIV positive (267). In adult attendees at QECH, the incidence of sepsis was 1772 per 100,000 person years and inpatient mortality for patients admitted with sepsis and severe sepsis was 23.7% (95% CI, 22.7-24.7%) and 28.1% (95% CI, 26.1-30.0%) respectively (268). The prevalence of ESBL BSIs in sSA is high, yet an accurate understanding of the burden of morbidity and mortality is unknown (46). A recent systematic review of 40 studies across 12 countries indicated the prevalence of 3GC resistance in *E. coli* BSIs was 18.4% (IQR 10.5-35.2) and *K. pneumoniae* of 54.4% (IQR 24.3-81.2) (46). In 2014, there was an ESBL *K. pneumoniae* outbreak identified on Chatinka ward (93). From February to November 2014, 75% of all paediatric *K. pneumoniae* BSIs were from neonates admitted to this ward. A retrospective WGS investigation of isolates obtained from 2010-2015 identified a discreet outbreak of the MDR ST340 clone (93).

Reliance on Ceftriaxone and other 3GCs at QECH is high (44). Historically there has been limited antimicrobial stewardship (AMS) or infection prevention and control (IPC) measures, and while success was noted following introduction in a pragmatic AMS campaign at QECH in 2016-2018, even after a reduction of 26.5% in 3GC prescribing, there was still a 53.6% rate of use (44). At present there is no AMS or IPC support, and in the absence of ongoing efforts it is unclear whether rates of 3GC remain low. Our anecdotal experience is that, in QECH, 3GCs are widely prescribed for both appropriate and inappropriate indications.

Chapter 2:

Methods for a One-Health observational study of ESBL prevalence in Malawi, focussing on households and the broader environment.

2.0. Chapter Aim

In this chapter I provide an overview of the Malawian sites, and illustrate the methodological approaches taken to household selection and recruitment, alongside a detailed description of the sampling frame, microbiological methods, data collection tools and SOPs.

2.1. Outline and contributions

This has been written in the format of a protocol paper, accepted by Wellcome Open, which takes an interdisciplinary One-Health approach to identifying the key drivers of AMR within Malawi. The detailed microbiological methods were adapted from previously published SOPs and methods provided by the UK National Institute for Health Protection Food Water and Environment (FWE) laboratory and have been locally adapted.

This manuscript summarises the combined work of work strands 2,5 and 6 of the DRUM consortium (*https://www.drumconsortium.org/*). It therefore describes the recruitment in Uganda, alongside genomic analysis and agent-based modelling approaches that will not be included in my thesis. The modelling and genomic sections provide a framework for how the Malawian data obtained within this thesis will be developed by other members of DRUM, consequently these sections were not primarily written by myself (**Table 2.0**). I wrote all other sections and undertook laboratory optimisation including implementing quality assurance from sample to bench, to incorporation of result in the DRUM database (see Chapter 5). Within the manuscript I have included links to the detailed microbiological methods and questionnaires used, and these are accessible online via Zenodo, on *https://doi.org/10.5281/zenodo.5855774* (269), and *https://doi.org/10.5281/zenodo.5855820* (270) respectively. References cited in the text of the manuscript have been placed at the end of the thesis.

One of the key successes to this project has been its truly interdisciplinary approach, and throughout the conceptualisation and iterative methodological processes, advice and opinion was sought from experts in human health, animal health, food, water and environmental microbiology, WASH &

environmental health, and medical anthropology from within the DRUM consortium. This manuscript is a culmination of those efforts, and I would like to both thank and acknowledge them for their contributions.

Chapter-specific statistical modelling techniques (Chapters 6 and 7), and antibiotic residue analysis (Chapter 8) will be covered in more detail within the relevant chapters.

Table 2.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	Sections of this paper that are primarily drafted by the PhD candidate include: • Abstract, introduction, aim, site selection, methods (apart from household selection and DNA extraction), data management and analysis, community engagement, ethics, study status, conclusion and extended data. The included SOPs and CRFs were primarily written and optimised by the PhD candidate.
Contributions from external partners and DRUM consortium collaborators	Sections **not** primarily written by the PhD candidate include household selection, DNA extraction and spatial analysis. • The section on household selection was primarily written by Melodie Sammaro. • The section on DNA extraction and sequencing was primarily written by Patrick Musicha. • The section on spatial analysis and agent-based modelling was primarily written by Chris Jewell. Document review was provided by all authors. Laboratory flow and processing was conceptualised by a DRUM laboratory working group, including: Nicola Elviss, Henry Kajumbula, Nicholas Feasey, Patrick Musicha and the

	PhD candidate. Specific guidance for details of the laboratory processing SOPs was sought from Nicola Elviss (UKHSA), and for the HRM PCR SOP from Rachel Byrne (LSTM). Rachel Byrne also completed optimisation of HRM PCR techniques within her MSc and these results assisted with the development of microbiological pipeline. The SOPs were reviewed by all listed SOP co-authors. The DRUM CRF working group contributed to the generation of the CRFs, and this included: Melodie Sammarro, Kondwani Chidziwisano, Shevin Jacob, Henry Kajumbula, Lawrence Mugisha, David Musoke, Andrew Singer, Rebecca Lester, Catherine Wilson, Chris Jewell, Tracy Morse, Clair Chandler, Eleanor MacPherson, Simon Alderton, Barry Rowlingson Rachel Tolhurst, Nicholas Feasey and the PhD candidate. Data management and quality assurance pipelines alongside electronic CRFs were generated by Barry Rowlingson, Lumbani Makhasa, Clemens Masasa, Stevie Amos and (primarily) the PhD candidate. Conceptualisation of the study was a culmination of all members of the DRUM consortium listed as authors.

2.2. Overview of microbiological methodologies and rationale

Water, food, environmental and stool samples were obtained using different equipment, dependant on the sample type. Water was collected in 1L Nalgene containers, food was collected in Whirl-pac bags, environmental samples were collected using 3M swabs and stool was collected either with a rectal/cloacal swab or a 30ml stool container. Samples were then processed according to their type (i.e. water, food, environmental or stool) and these methods are detailed in the paper (section: Microbiological methodology) and expanded on within the SOPs. A pre-enrichment step in buffered peptone water (BPW) was then undertaken to increase the chance of recovery of gram-negative

bacteria, followed by plating and growth on ESBL CHROMagar™ media to select for ESBL bacteria. ESBL *E. coli* was determined by chromogenic agar and indole testing, and ESBL *K. pneumoniae* was determined by chromogenic agar and high-resolution melt-curve (HRM) PCR. DNA was extracted on all isolates in conjunction with a selection of plate sweeps (chromogenic agar) and BPW samples for onward genomic analysis which is not included in this thesis.

Prior to the start of the study considerations were made in the microbiological pipeline for (a) the benefit of pooling and screening of samples vs individual sample screening, (b) the use of HRM PCR techniques (either via ESBL gene or bacterial species) vs ESBL culture as a first step after enrichment, and (c) the choice of ESBL media. In relation to pooling, given the high degree of ESBL positivity seen on piloting, pooling was not identified as a pragmatic or financially sensible option, therefore, individual sample testing was opted for. In relation to the use of HRM PCR vs ESBL culture on chromogenic agar, given the absence of pooling and the need for bacterial isolates for onward genomic testing, as a consortium we opted for culture-based techniques as the step after enrichment. It should also be highlighted that on piloting the screening of samples with ESBL genes (TEM/SHV/CTX-M) using HRM PCR methods, these were found to be less sensitive than ESBL culture (CHROMagar™ media), particularly on environmental samples (personal comms, Rachel Byrne). Lastly, in relation to the choice of ESBL media, CHROMagar™ media was chosen given its high reported sensitivity for ESBL *E. coli* and ESBL *K. pneumoniae* (362,363), its cost, simplicity, the absence of the need for quantitative data, and because we had previous local experience of using this media to good effect (300).

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)

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Keywords

Antimicrobial Resistance, Africa, One-Health, Environment

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.

Introduction

Antimicrobial resistance (AMR) is a huge and complex global public health problem (13). It is a threat to health that reflects both the interconnectedness of humans, animals and the environment and humanity's dependence on antimicrobials (14). 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 (16,271). There is also inadequate water, sanitation and hygiene (WASH) infrastructure to mitigate spread of environmentally dependent

bacteria between humans, animals, and the environment (198). 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 antibiotics (272). 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 (92,198,273,274). 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 pathogen (71).

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*.

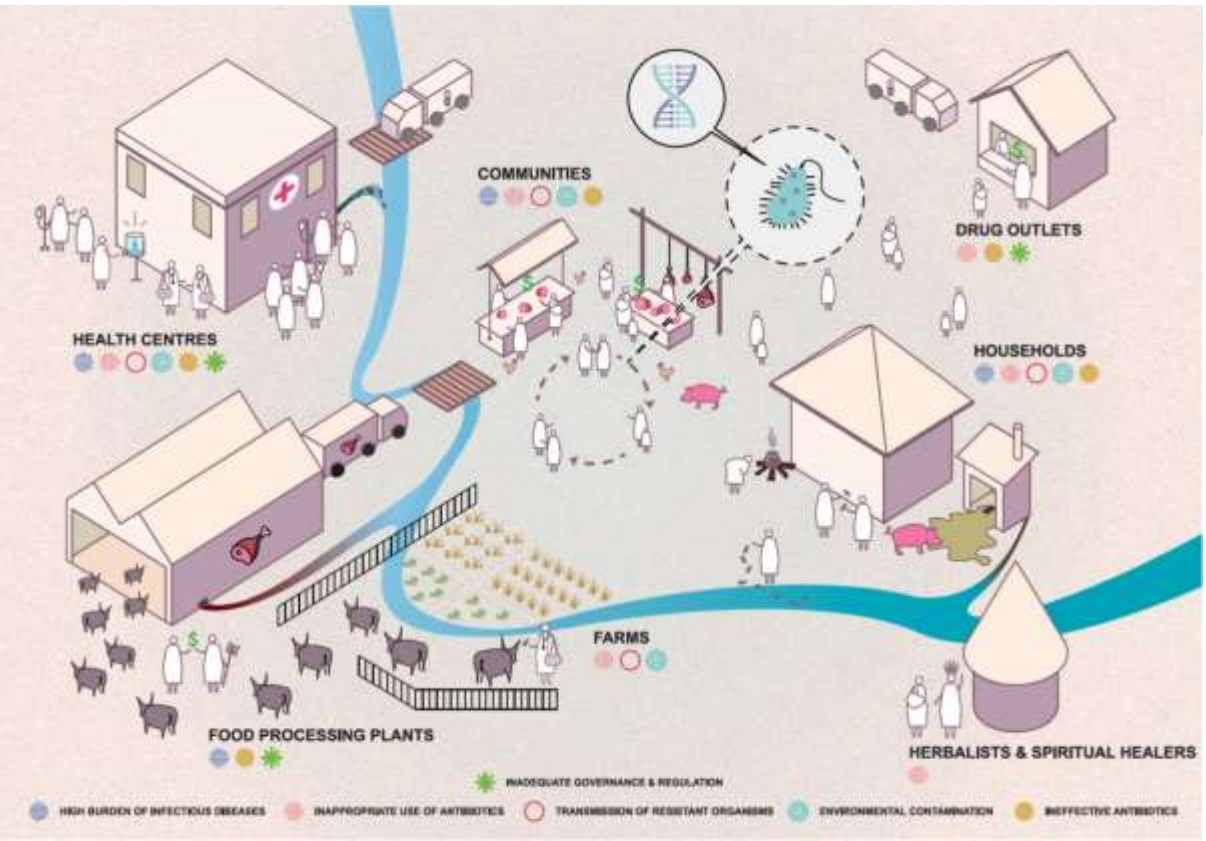

Figure 1. Hypothetical model of related behaviours and the movement of AMR-bacteria in Uganda and Malawi. The schematic situates the household at the heart of the model, in which humans act in response to their environment within which bacteria are evolving in response to selective pressures around them.

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).

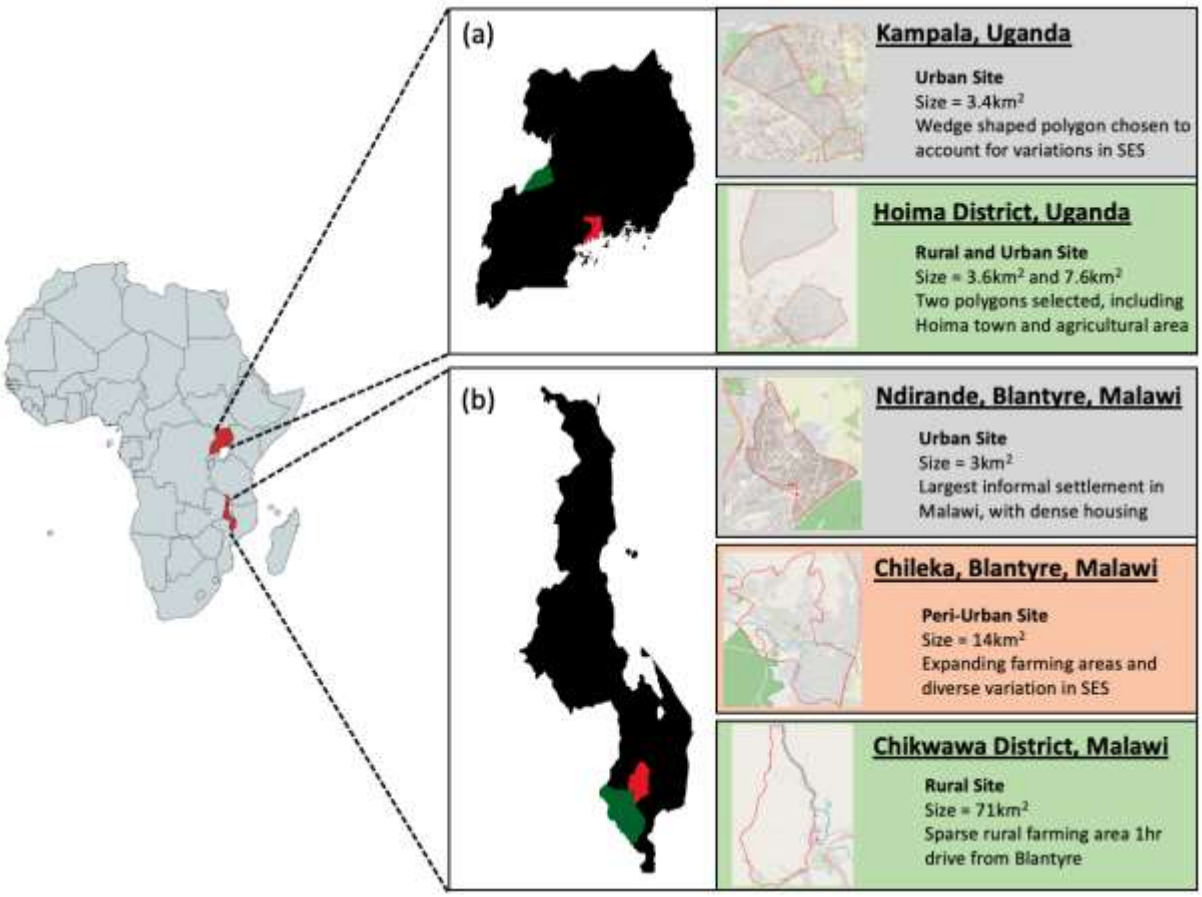

Figure 2. Diagram of DRUM study sites. (a) We selected two geographic areas within Uganda including Kampala (red) and Hoima District (green). From these areas, polygons were created that mapped an urban setting (Kampala) and urban/rural setting (Hoima District). (b) We selected sites in two regions within 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.

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 (259,275) and where 15% (109,164) of the Blantyre population

resides (276). Ndirande is geographically situated on a mountainside directly next to the city centre, supplied by 2 main rivers that run from the top of the mountain through the centre of the district and converge into the Mudi. Open drains flow directly into the rivers, which are frequently contaminated with plastic waste. 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 (275,277). HIV prevalence in adults aged 15-65 is 18% and there is a high burden of typhoid and tuberculosis (260,278). The study polygon is 3 km^2, 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. Chileka is a flat area with a mixture of households, light industry and farms (beef/pig/poultry). Household plots are typically larger in size than Ndirande, and the river system is formed of a complex network of small tributaries that flow into a main river which feeds back into the Shire downstream of Blantyre city. Akin to Ndirande, open drains also flow directly into the river network. 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 km^2, 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 Blantyre (279). 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 flooding (280). It is supplied by the large Shire river and is hotter than Blantyre, with a less developed sewerage network (T Morse personal comms). Healthcare is provided by Chikwawa District hospital, 14 health centres and 26 community health care worker outposts (279). We identified a 71 km^2 study polygon readily accessible from Blantyre by road, including villages engaged in research activity on the edge of Chikwawa town. The polygon is directly next to Chikwawa boma, and therefore the local hospital (Chikwawa District hospital) is located 300m from the southern-east tip of the polygon. Furthermore, given the climactic conditions, smaller rivers are only present in the wet season, and therefore, few rivers were included

within the polygon. 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% (281). The sampling frame comprises of 3 contiguous areas drawn in wedge shape (measuring 3.4 km^2 x 2.7 km^2 x 1 km^2) 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 people (282). HIV prevalence among adults aged 15-64yrs in the Mid-West Region of Uganda where Hoima is located is 5.7% (281). The sampling frame comprises of two non-contiguous polygons of 3.6 km^2 and 7.6 km^2, 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" approach (283). 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 points (284) 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 meters (213).

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 possible (275). 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.

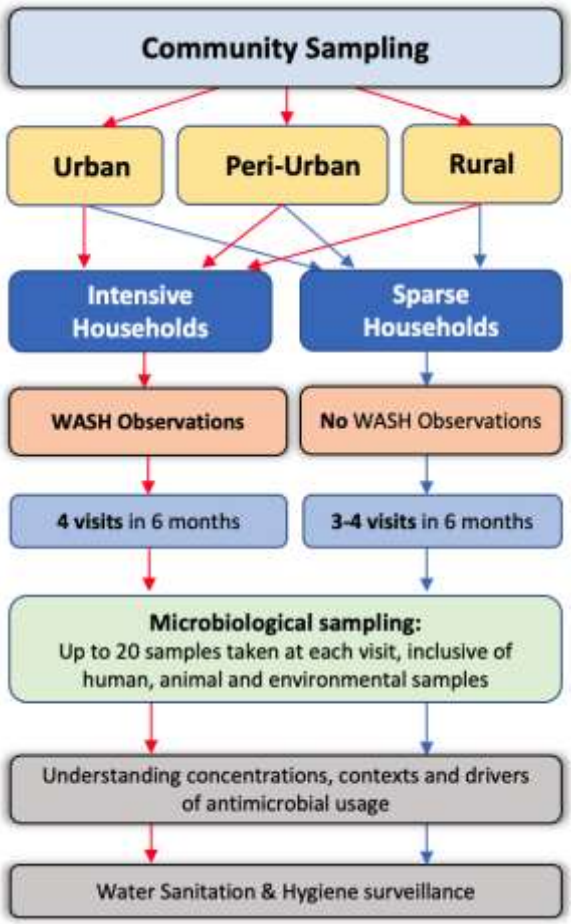

Figure 3: DRUM household study design.

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

Data collection

1. **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 (213,285). 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**).

	Individual Level Data	**Household Level Data**
Demographic	· Participant Demographics	· Household Demographics · Socio-Economic Information · Household Head Information
Health	· Health Status and Comorbidities · Regular Medication Use · Recent Illness	· Household Health Seeking Behaviour
Exposure Risk	· Healthcare Exposure · Travel and Residency · Health Seeking Behaviour	· Visitors into the household
Antibiotic Usage	· Antibiotic Usage	· Household Experience of Illness and Antibiotics
WASH	· Hand-Washing Data	· Household WASH Infrastructure · Toileting Behaviour · Waste Management · Water Usage and Management · Washing and Bathing Practices · Food Preparation and Hygiene Information. · Hand-Washing Data
Environmental		· Household Infrastructure · Household Environment

Animal	· Household Animal Husbandry · Animal Health and Disease Prevention · Drug Usage in Household Animals (including antibiotics)

Table 1: DRUM CRF themes and data capture.

2. 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).

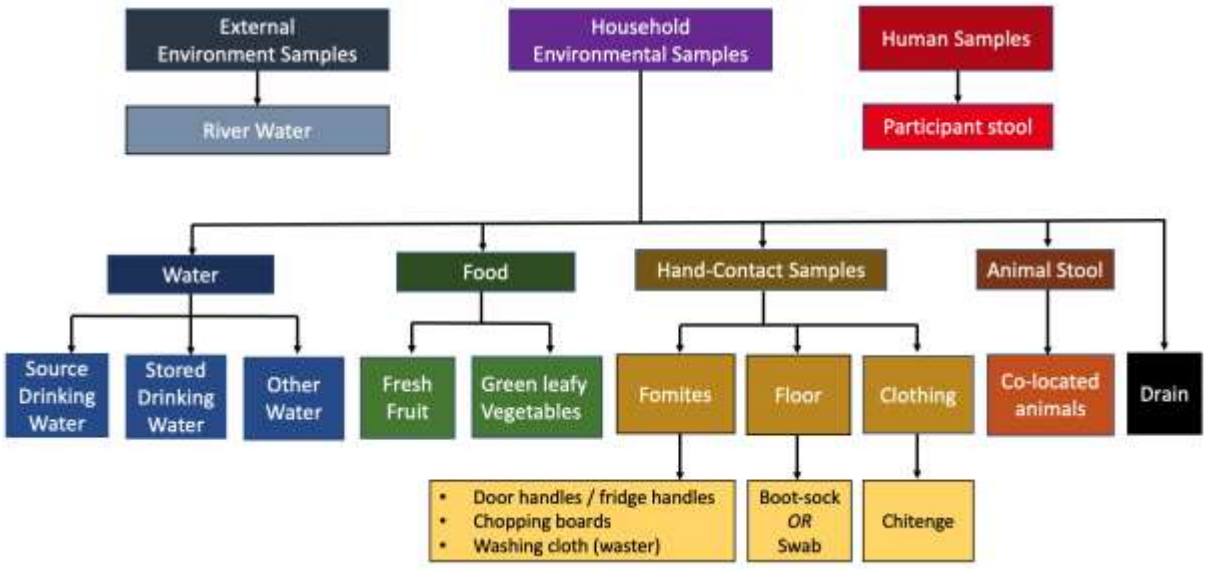

Figure 4. DRUM microbiological sampling frame, used at household visits. Samples are inclusive of human stool, animal stool, and a selection of household environment and the broader external environment.

2.1 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.

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^{o}C in the dark and transported to the laboratory, for processing within 24 hours, where possible.

2.2 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* isolates (286). 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) (287).

2.3 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".

3. WASH Evaluations

3.1 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-WASH (288). 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 mornings (239). 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.

3.2 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 (241,289). 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.

4. 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 (290–292). 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.

5. 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.

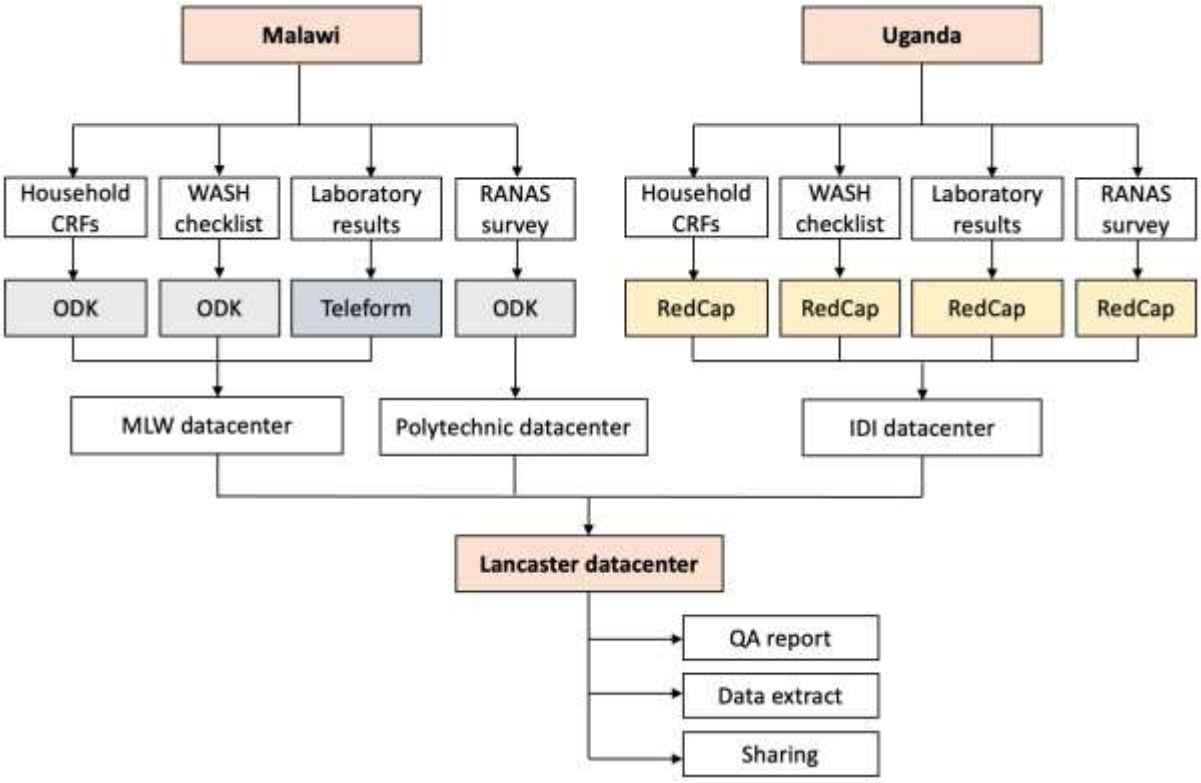

Figure 5. DRUM data management workflow.

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 Medicine REC, Malawi (#P.11/18/2541), Institutional Animal Care and Use Committee (IACUC), Uganda (Ref: SVARREC/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 questionaries 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 underdoing 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.

Grant information

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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* (270).

This project contains the following extended data:

- DRUM01 Participant Enrolement CRF.pdf
- DRUM02 Household Enrolement 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* (269).

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

Chapter 3:

Comparison of demographic, health, antibiotic usage and health seeking behaviour data from study households in urban, peri-urban and rural Malawi.

3.0. Chapter summary

Within this chapter I have outlined the selection process of households recruited into the household study in Malawi, evidencing that they are representative of urban, peri-urban and rural settings. Using data obtained from the CRFs, I have provided a detailed description of the household characteristics and participant demographics, evaluating regional differences in household density, age composition and socioeconomic status that could serve as confounders in future analysis and assessed whether there are regional differences in the underlying health status of household individuals, which may impact on human antibiotic exposure. This is followed by an evaluation of the regional attitudes to antibiotic usage and health seeking behaviour, alongside a description of recent antibiotic usage within household participants. Finally, given the scarcity of available animal data in LMIC settings, I have assessed whether there are regional differences in animal ownership, husbandry practices, access to animal healthcare services and antibiotic use in animals co-located at study households, which may be important when assessing antimicrobial resistance rates seen in animals, humans and the household environment.

Data from the 300 households recruited illustrates similar household density between the regions (mean 4.5), with households in the rural setting on average poorer than those in the urban or peri-urban setting. The median age of household members was 18yrs, and participants were invariably in good health with few co-morbidities or recent hospital admissions; with an adjusted HIV prevalence of 14.0% across the study cohort. Antibiotic exposure in the study cohort was predominantly limited to oral amoxicillin, co-trimoxazole and metronidazole and associated with episodes of illness, irrespective of diagnosis. ABU was higher in the rural site compared to other regions and in children under 5, as an age group.

Animal ownership was commonplace, with 58.7% of households owning an animal, highest in the rural site. Poultry was the most frequently owned animal, and the species present at households varied by setting, with larger livestock animals more often seen in the rural area, and domestic animals seen in the urban and peri-urban sites. Preventative measures were employed to reduce episodes of animal

illness, and when animals became unwell households would rarely seek specialist advice or give medication, and therefore I found limited recent ABU exposure in animals included within the study.

My contributions to this chapter and those of others are included in Table 3.0.

Table 3.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted and analysed by the PhD candidate
Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborator, Tracy Morse. Statistical advice was sought from Chris Jewell. Data collection was aided by study staff, including: • Witness Mtambo, Gladys Namancha, Suzgo Mkandawire, Steria Chisesele, Dyson Rashid, Odetta Duwa, Lughano Ghambi, Chiyembekeso Palije and Fletcher Nangupeta

3.1. Polygon derivation and household recruitment

As described in Chapter 2, the study was designed to focus on the level of the household and obtain a representative sample of urban (Ndirande), peri-urban (Chileka) and rural (Chikwawa) households from within Malawi. An outline of the polygons, and key structures within them are included in **appendix 3.i**. From the pre-selected 100 household geolocations in each site, 15 were characterised into “intensive” households and 85 into “sparse” households. 50 households within the 85 “sparse” households were prioritised for longitudinal follow-up. The minimal acceptable numbers of households recruited was 65 households in each region, inclusive of 15 intensive and 50 sparse, with an ideal recruitment of 100 households, inclusive of 15 intensive and 85 sparse (**Figure 3.0**). Due to a COVID-19 enforced institutional shutdown on studies operating at MLW, the study was paused from May 2020 to August 2020. During this period household follow-ups were suspended and resumed

once COVID safety measures had been deployed, and local agreements were in place. As a result of these delays, in each region, only the 65 households originally prioritised for longitudinal follow-ups (15 “intensive” and 50 “sparse”) were able to have the full complement of 4 visits (1 baseline and 3 follow-up), and the other 35 households underwent a single baseline visit. Below we describe the selection process, geolocation, and recruitment of these households in each region.

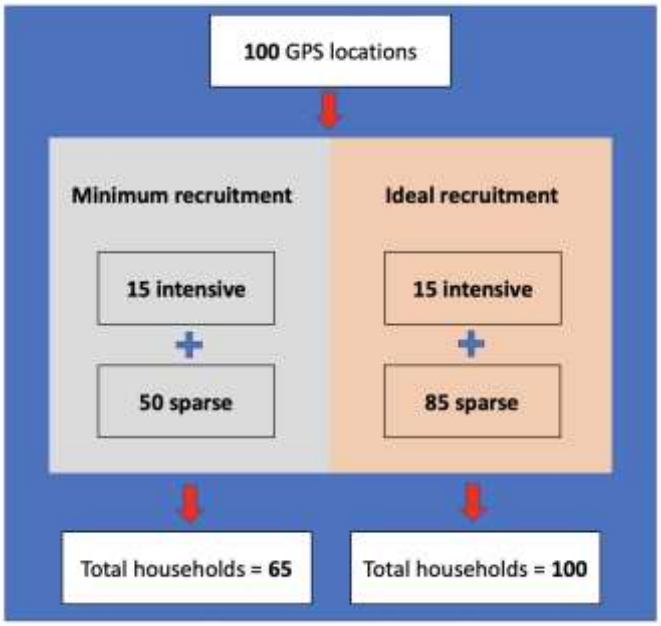


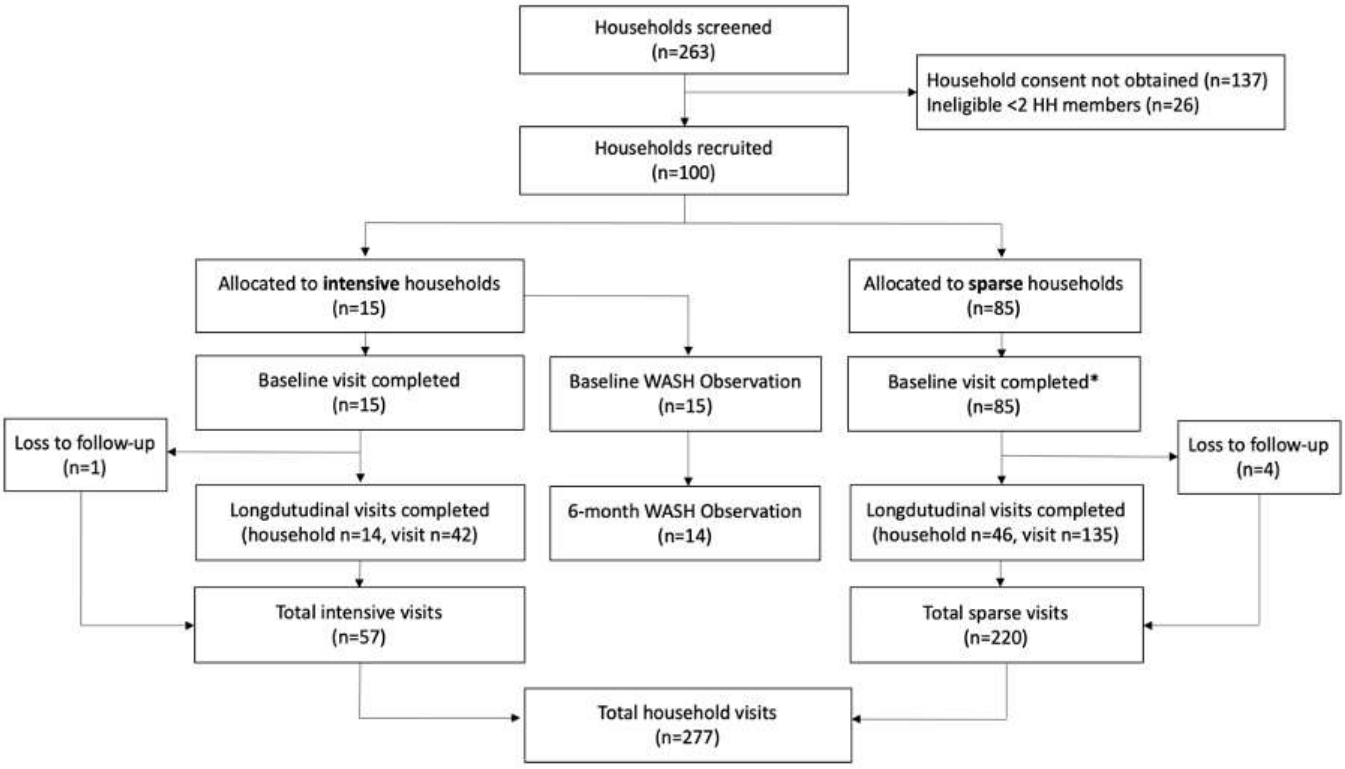
Figure 3.1. Schematic of acceptable household recruitment numbers of sparse and intensive households for each region.

3.2. Urban, peri-urban and rural household recruitment and geolocation

Between May 2018 and October 2020, 263 households in Ndirande (**Figure 3.1**), 229 households in Chileka (**Figure 3.2**) and 119 households in Chikwawa (**Figure 3.3**) were screened, to enable to the recruitment of 100 households into the study from each site. In each region, households were classified into either “intensive” or “sparse”, as per the study protocol (Chapter 2), and geolocated within the study boundaries (**Figures 3.4-3.6**).

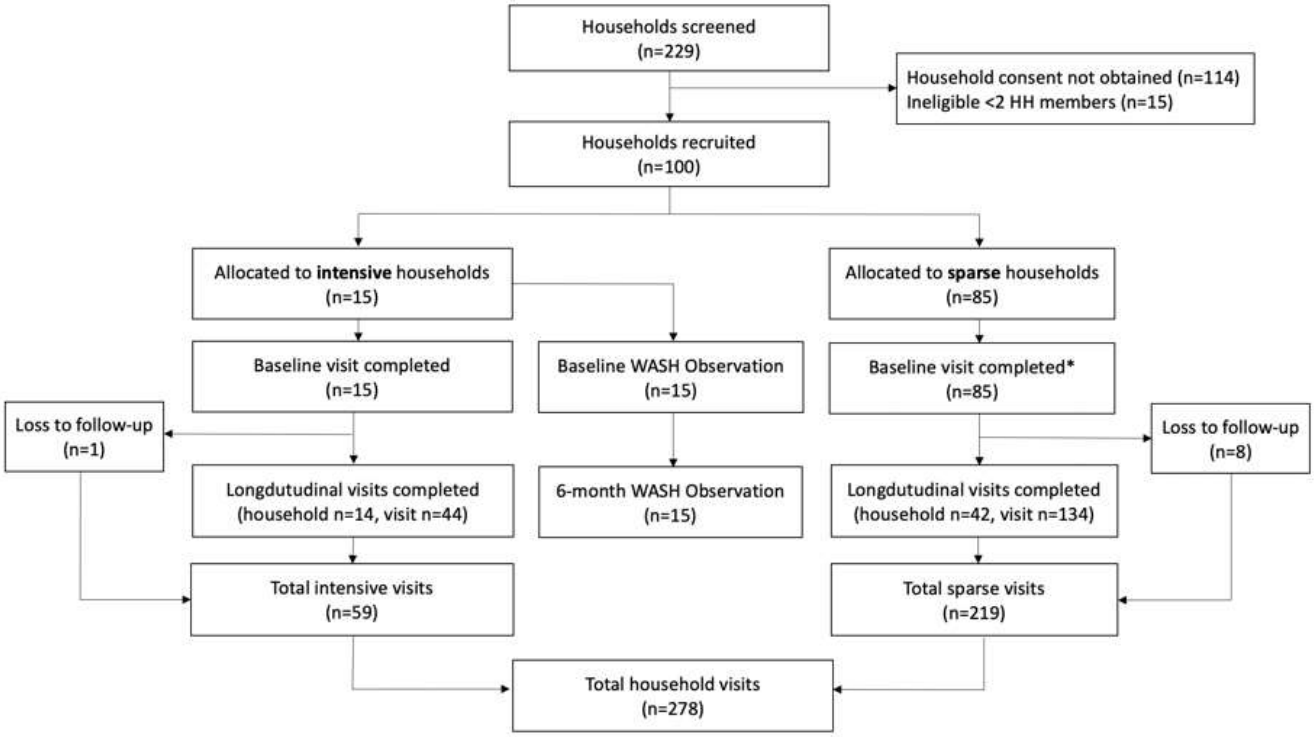
In Ndirande, 137 households screened did not consent to take part in the study and 26 households did not meet the inclusion criteria (needing at least 2 household members). A total of 5 households (1 “intensive” and 4 “sparse”) were lost to follow-up during the study period, all of which were due to relocation. This provided us with baseline data for the urban 100 households and microbiological sampling from 277 visits (57 at “intensive” households and 220 at “sparse” households) within the

urban region (**Figure 3.1**). In Chileka, 114 households screened did not consent to take part in the study and 15 households did not meet the inclusion criteria. A total of 9 households (1 "intensive" and 8 "sparse") were lost to follow-up during the study period. 1 household withdrew consent and the other 7 households relocated outside of the study boundaries. This provided us with baseline data in each of the urban 100 households recruited and microbiological sampling from 278 visits (59 at "intensive" households and 219 at "sparse" households) within the peri-urban region (**Figure 3.2**). In Chikwawa, 9 households that were screened did not consent to take part in the study and 2 households did not meet the inclusion criteria. A total of 2 households (1 "intensive" and 1 "sparse") were lost to follow-up during the study period, both related to relocation outside the study boundary. This provided us with baseline data in each of the rural 100 households recruited and microbiological sampling from 286 visits (56 at "intensive" households and 230 at "sparse" households) in the rural region (**Figure 3.3**). Due to COVID associated logistical restraints and reduced staff numbers, in the rural region individual level CRFs were not completed at the final 35 households, which represents individual-level data loss on 135 participants.



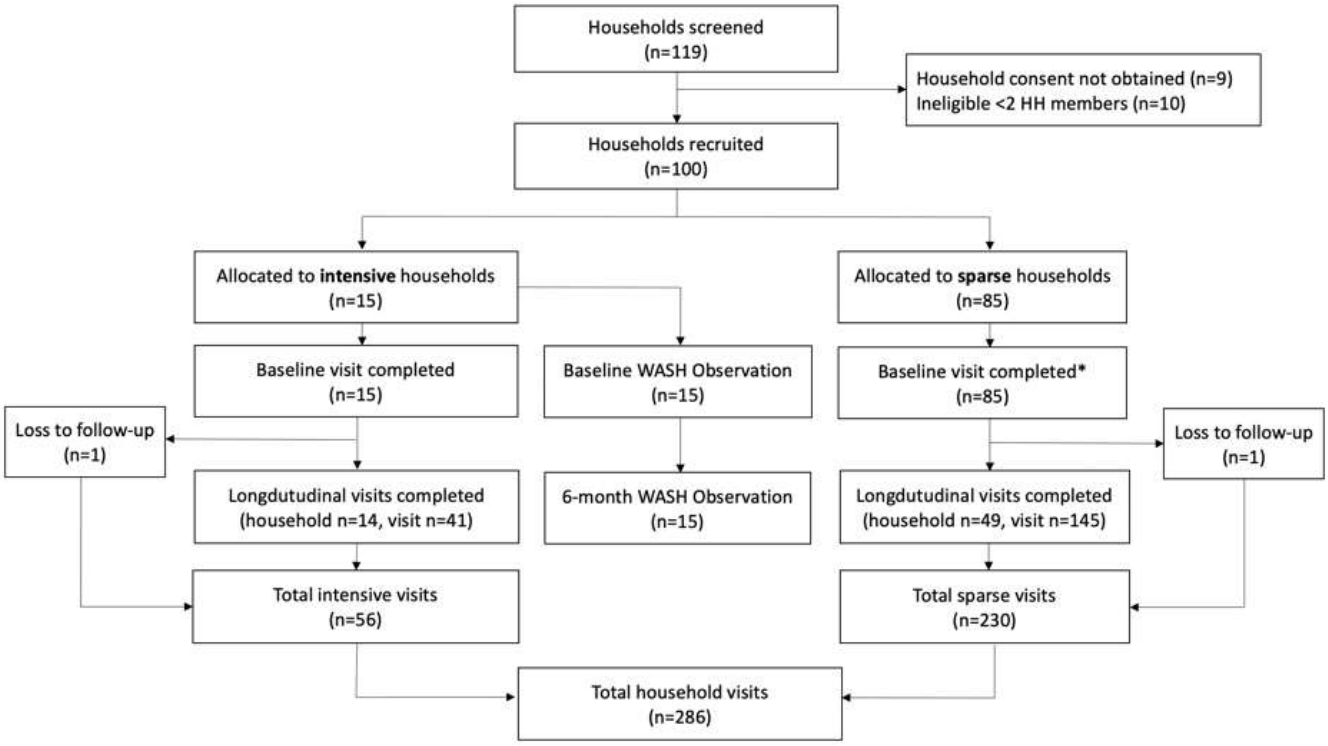
**COVID-19 safety concerns led to 35 sparse households only undergoing a baseline visit*.

Figure 3.2. CONSORT chart for urban households, classified into "sparse" or "intensive", and illustrating loss to follow-up and total visits.



**COVID-19 safety concerns led to 35 sparse households only undergoing a baseline visit*.

Figure 3.3. CONSORT chart for peri-urban households, classified into "sparse" or "intensive", and illustrating loss to follow-up and total visits.



**COVID-19 safety concerns led to 35 sparse households only undergoing a baseline visit*. These 35 households were also not able to complete individual level CRFs.

Figure 3.4. CONSORT chart for rural households, classified into "sparse" or "intensive", and illustrating loss to follow-up and total visits.

Figure 3.5. Map of urban polygon detailing the GPS selection process and allocation of sparse or intensive households. (a) Boundaries of urban polygon. (b) Geolocation of 100 GPS points [black] for household identification, using pairwise design within the urban polygon. (c) Random selection of 65 GPS points [red] chosen within the initial 100 allocated to have longitudinal follow-up (minimum of 4 visits). (d) Random selection of 15 households [white] within the 65 households allocated to "intensive" households (where WASH observations will be performed). (d) Final GPS point selection for household recruitment, categorised into "intensive" (white) and "sparse" (red or black) households, with sparse households sub-categorised for priory to longitudinal follow-up (red), and longitudinal follow-up if possible (black). (e) The GPS locations of "actual" households recruited into the study, including 15 "intensive" (yellow) households and 85 "sparse" (purple) households.

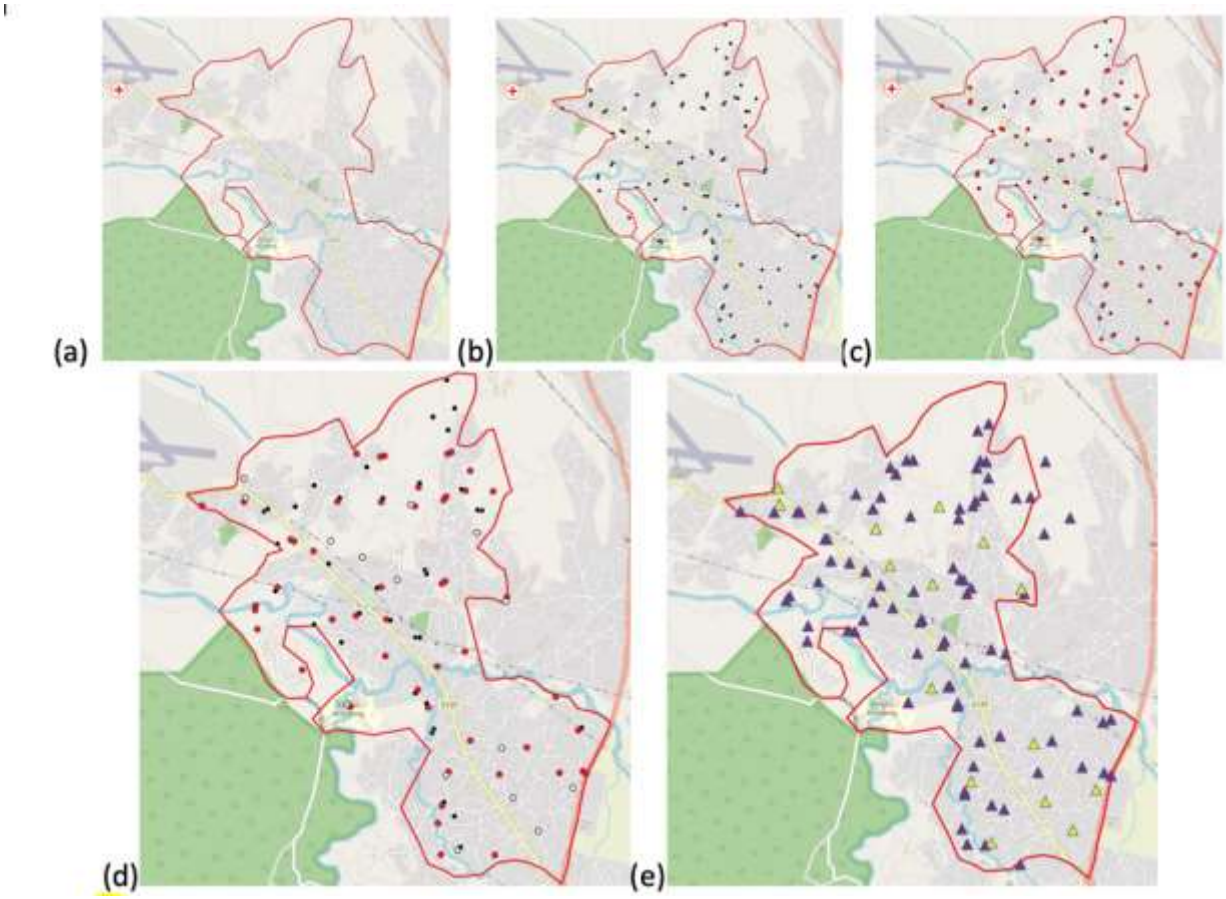


Figure 3.6. Map of peri-urban polygon detailing the GPS selection process and allocation of sparse or intensive households. (a) Boundaries of urban polygon. (b) Geolocation of 100 GPS points [black] for household identification, using pairwise design within the urban polygon. (c) Random selection of 65 GPS points [red] chosen within the initial 100 allocated to have longitudinal follow-up (minimum of 4 visits). (d) Random selection of 15 households [white] within the 65 households allocated to "intensive" households (where WASH observations will be performed). (d) Final GPS point selection for household recruitment, categorised into "intensive" (white) and "sparse" (red or black) households, with sparse households sub-categorised for priory to longitudinal follow-up (red), and longitudinal follow-up if possible (black). (e) The GPS locations of "actual" households recruited into the study, including 15 "intensive" (yellow) households and 85 "sparse" (purple) households.

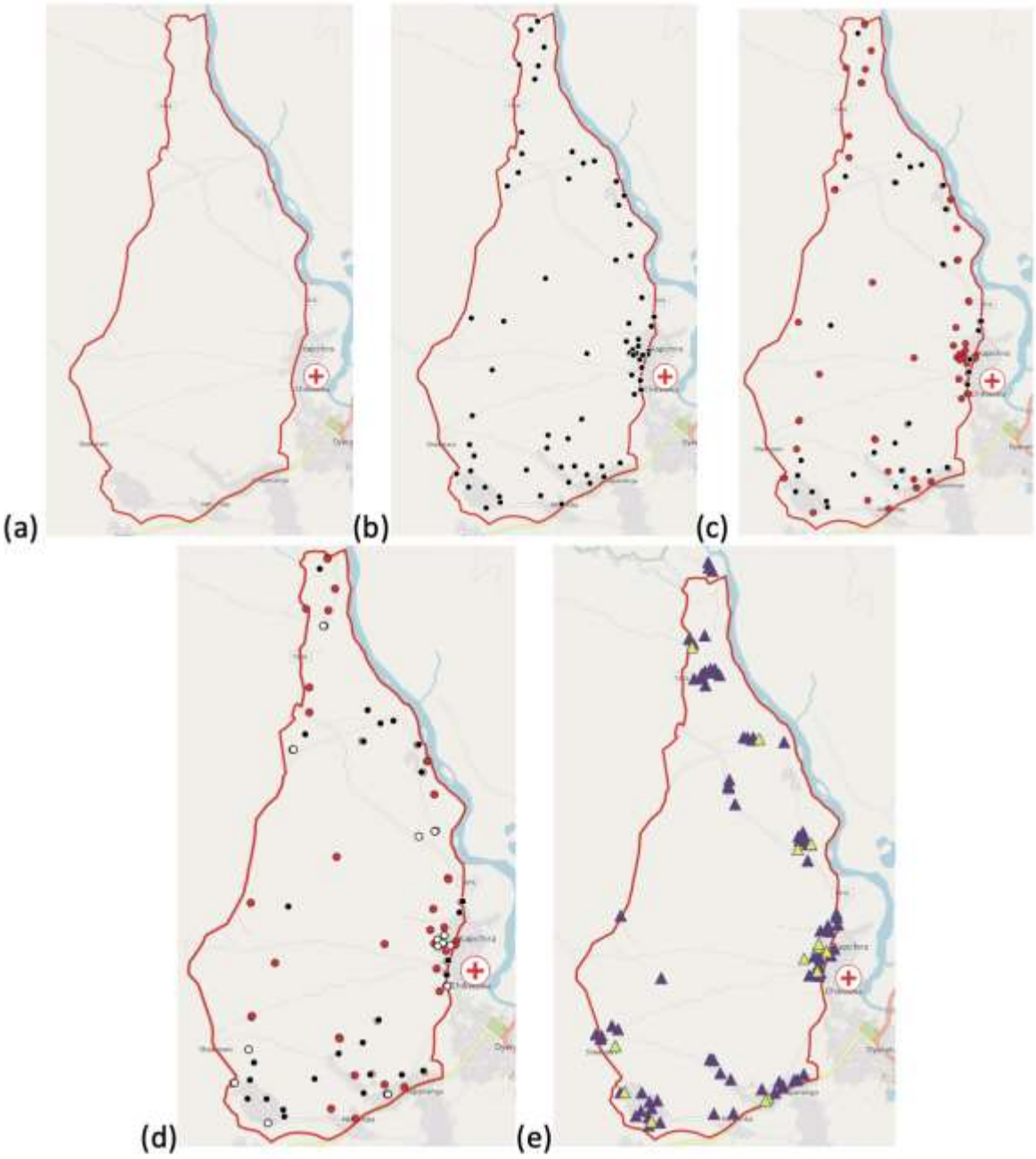


Figure 3.7. Map of rural polygon detailing the GPS selection process and allocation of sparse or intensive households. (a) Boundaries of urban polygon. (b) Geolocation of 100 GPS points [black] for household identification, using pairwise design within the urban polygon. (c) Random selection of 65 GPS points [red] chosen within the initial 100 allocated to have longitudinal follow-up (minimum of 4 visits). (d) Random selection of 15 households [white] within the 65 households allocated to "intensive" households (where WASH observations will be performed). (d) Final GPS point selection for household recruitment, categorised into "intensive" (white) and "sparse" (red or black) households, with sparse households sub-categorised for priory to longitudinal follow-up (red), and longitudinal follow-up if possible (black). (e) The GPS locations of "actual" households recruited into the study, including 15 "intensive" (yellow) households and 85 "sparse" (purple) households.

3.3. Household and participant characteristics

Within the 300 households recruited, there was a total of 1351 household members, 71.4% (n=965/1351) of whom consented to individualised questionnaires at baseline and again at each follow-up. This represented 67.9% (n=312/459) of the available household members from the urban site, 82.0% (383/467) of available household members from the peri-urban site and 63.5% (270/425) of the available household members from the rural site answering individual-level questions. The low response rate in the rural region is consequent upon COVID interruptions to the 35 sparse households, whereby 134 consented participants did not provide individual-level data. All 300 households provided baseline and follow-up data. The following results are descriptive summaries obtained from these data, pertaining to household and participant demographics, inclusive of health status, ABU and health seeking behaviour from each of the study sites (urban, peri-urban and rural).

3.4. Household demographics

The mean (SD) number of residents per household across the study was 4.5 (1.9), with the urban, peri-urban and rural sites having a 4.6 (1.9), 4.6 (1.9) and 4.2 (1.5) members per household respectively, illustrating limited variations in household density between the regions (**Table 3.1**). Households had between 2-13 household members, with little variation in the ranges seen between the regions (range = 2-13 in the urban site, 2-11 in the peri-urban site, and 2-12 in the rural site). Most households comprised of a mix of adults and children, with 2.5 (1.0) adults, 1.4 (1.2) adolescents, 0.4 (0.6) children and 0.3 (0.5) infants per household, and other than a predominance of adults at urban/peri-urban sites there was a limited difference between the regional composition of household age groupings (**Table 3.1**).

School attendance was high within the total study population, with 70.0% (n=70), 63.0% (n=63) and 69.0% (n=69) of households reporting 1 or more children attending primary school in the urban peri-urban and rural sites respectively, and 22.0% (n=22), 24.0% (n=24) and 20.0% (n=20) of households reporting 1 or more children attending a secondary school in the urban, peri-urban and rural sites (**Table 3.1).**

It was common for households in all three regions to live in “absolute poverty” as defined by the United Nations (293) and World Bank (294), with participants in the rural site more frequently earning less, having food insecurity, and conceptualising themselves as poor. Using the World bank metric of

the international poverty line, each *individual* would need to earn ≥45,600 MK /month (equivalent to $1.90/day at the time of the study) to be considered above the threshold for absolute poverty (294,295). Household income was evaluated as a crude estimate of poverty in this study, and here I found that in the urban, peri-urban and rural regions, 74.0% (n=74), 68.0% (n=68) and 95.0% (n=95) of *households* had an average total income of <50,000 MK /per month for the *entire household*. When adjusting monthly household income for the number of household members, only 2.3% (n=7) of households [1 urban, 4 peri-urban and 2 rural] therefore lie above the absolute poverty threshold per *individual*. Using food supply metrics as a proxy measure for poverty, 39.0% (n=39) of urban households, 50.0% (n=50) of peri-urban households and 75.0% (n=75) of rural households reported food shortages on a monthly basis, and 31.0% (n=31) of urban households, 45.0% (n=45) of peri-urban households and 59.0% (n=59) of rural households reported food shortages on a weekly basis. Lastly, when household members were approached directly to conceptualise how poor they felt using a poverty scale, they consistently placed themselves living on the lower steps (1-3) in all three study areas, with those living in the rural site describing themselves as on the poorer steps (1-2) more often than in the urban and peri-urban regions (**Figure 3.7**).

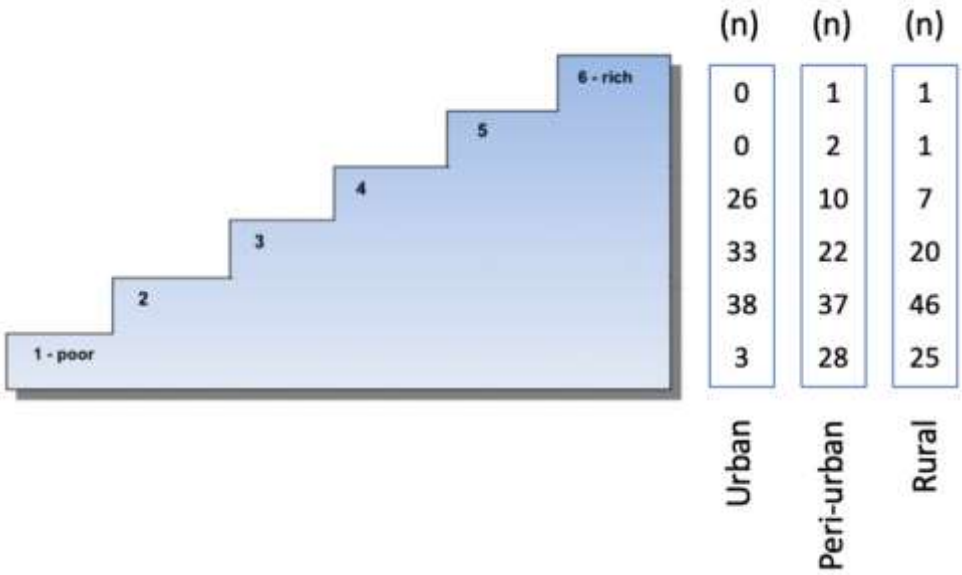


Figure 3.8. Poverty step response amongst households in urban, peri-urban and rural sites. The step scale is from 1-6, with 1 being poor and 6 being rich. Column n represents the number of households that responded to where they felt they sat on the scale.

Despite the low household income, mobile phone ownership was common, with 64.7% (n=194) of households, 56.1% (n=270) of adults, and 40.0% (n=386) of total participants in the study owning a

phone. There was a regional difference though, as we found less access or ownership of a phone in the rural site comparted to the urban and peri-urban site. (**Table. 3.2**).

Table 3.1. Characteristics of households in the urban, peri-urban and rural sites.

Household characteristic	n (%) unless otherwise indicated				
	Total	**Urban**	**Peri-urban**	**Rural**	***p^***
Average number of household members	mean =4.5 (SD=1.8) range 2-13	mean = 4.6 (SD=1.9) range 2-13	mean = 4.6 (SD=1.9) range 2-11	mean = 4.2 (SD=1.5) range 2-12	*.281*
Number of people living in each household					
1-2	n=27 (9.0%)	n=7 (7.0%)	n=14 (14.0%)	n=6 (6.0%)	*.127*
3-4	n=151 (50.3%)	n=44 (44.0%)	n=45 (45.0%)	n=62 (62.0%)	***.017***
≥5	n=122 (40.7%)	n=49 (49.0%)	n=41 (41.0%)	n=32 (32.0%)	*.053*
Age ranges of household members					
Number of Adults (>18yrs)	mean =2.5 (SD=1.0)	mean = 2.5 (SD=1.0)	mean = 2.7 (SD=1.2)	mean = 2.2 (SD=0.8)	***.012***
Number of Adolescents (14-17yrs)	mean =1.4 (SD=1.2)	mean = 1.4 (SD=1.2)	mean = 1.4 (SD=1.2)	mean = 1.4 (SD=1.2)	*.998*
Number of Children (1-14yrs)	mean =0.4 (SD=0.6)	mean = 0.4 (SD=0.6)	mean = 0.4 (SD=0.6)	mean = 0.4 (SD=0.5)	*.953*
Number of Infant/Neonate (>1yr)	mean =0.3 (SD=0.5)	mean = 0.3 (SD=0.5)	mean = 0.2 (SD=0.4)	mean = 0.3 (SD=0.5)	*.081*
How many members of the household attend primary school?					
0	n=98 (32.7%)	n=30 (30.0%)	n=37 (37.0%)	n=31 (31.0%)	*.549*
1-2	n=166 (55.3%)	n=58 (58.0%)	n=53 (53.0%)	n=53 (53.0%)	*.765*
3 or more	n=38 (12.7%)	n=12 (12.0%)	n=10 (10.0%)	n=16 (16.0%)	*.477*
How many members of the household attend secondary school?					
0	n=229 (76.3%)	n=71 (71.0%)	n=76 (76.0%)	n=80 (80.0%)	*.537*
1-2	n=65 (21.7%)	n=22 (22.0%)	n=23 (23.0%)	n=20 (20.0%)	*.908*
3 or more	n=1 (0.3%)	n=0 (0.0%)	n=1 (1.0%)	n=0 (0.0%)	*1.00*
Average household Income (MK/month)	mean = 45,462 (SD=48,256)	mean = 50,818 SD (40,647)	mean = 56,111 SD (55,528)	mean = 29,615 SD (43,839)	***>.001***
Household Income (MK/month) groupings					
0-50,000	n=237 (79.0%)	n=74 (74.0%)	n=67 (67.0%)	n=94 (94.0%)	***>.001***
51,000-100,000	n=39 (13.0%)	n=19 (19.0%)	n=19 (19.0%)	n=1 (1.0%)	***>.001***
101,000-150,000	n=13 (4.3%)	n=4 (4.0%)	n=8 (8.0%)	n=1 (1.0%)	*.055*
151,000-300,000	n=9 (3.0%)	n=1 (1.0%)	n=4 (4.0%)	n=4 (4.0%)	*.405*
>301,000	n=4 (1.3%)	n=2 (2.0%)	n=2 (2.0%)	n=0 (0.0%)	*.551*
Job status of the head of the household					
Paid employee	n=80 (26.7%)	n=35 (35.0%)	n=35 (35.0%)	n=10 (10.0%)	***>.001***
Paid domestic worker	n=14 (4.7%)	n=0 (0.0%)	n=6 (6.0%)	n=8 (8.0%)	***.008***
Self employed	n=115 (38.3%)	n=48 (48.0%)	n=43 (43.0%)	n=24 (24.0%)	***>.001***
Unemployed	n=89 (29.7%)	n=16 (16.0%)	n=15 (15.0%)	n=58 (58.0%)	***>.001***
Other	n=5 (1.7%)	n=1 (1.0%)	n=3 (3.0%)	n=1 (1.0%)	*.625*
Occupation of the head of the household*					

Agriculture	n=7 (3.3%)	n=1 (1.2%)	n=4 (4.7%)	n= 2 (4.8%)	*.414*
Domestic service	n=6 (2.8%)	n=0 (0.0%)	n=3 (3.5%)	n=3 (7.1%)	***.042***
Unskilled manual	n=47 (22.1%)	n=11 (13.1%)	n=17 (19.8%)	n=19 (45.2%)	***>.001***
Skilled manual	n=81 (38.2%)	n=35 (41.07%)	n=30 (34.9%)	n=16 (38.1%)	*.667*
Sales and service	n=50 (23.6%)	n=30 (35.7%)	n=19 (22.1%)	n=1 (2.4%)	***>.001***
Clerical Technical / managerial	n=13 (6.1%)	n=4 (4.8%)	n=9 (10.5%)	n=0 (0.0%)	***.049***
Healthcare	n=2 (0.9%)	n=1 (1.2%)	n=1 (1.2%)	n=0 (0.0%)	*1.00*
Other	n=6 (2.8%)	n=2 (2.4%)	n=3 (3.5%)	n=1 (2.4%)	*1.00*

**Number of employed household head (urban = 84, peri-urban = 86 and rural = 42).*
^p-values obtained by fisher's exact test for categorical and Kruskal-Wallis for continuous data

3.5. Participant demographics

The median age of the study population was 18yrs (IQR 7-34); 15yrs (IQR 7-32) in the urban region, 20yrs (IQR 9-37) in the peri-urban region and 17yrs (IQR 7-32) in the rural region (**Table 3.1)**. This young average age is best illustrated by the population pyramids of each site showing a predominance of younger age groupings (**Figure 3.8**) and is consistent with the 2018 Malawi census. The age of participants ranged from 1 month to 102 years old (**Table 3.2**). There were more female respondents than men at households from all sites (190:122 in the urban region, 213:170 in the peri-urban and 142:128 in the rural region), with little regional difference, and this is likely to be related to women being more present at the households during the daytime to undertake questionnaires and perhaps more willing to take part in research.

Most participants reported to belong to the Christian faith, with 92.1% (n=889) of the total population reporting practicing Christianity, and the remaining respondents either practicing Islam or preferred not to say. Understandably, due to geographic reasons there were differences seen in the tribal makeup of the urban/peri-urban households compared with the rural households, with urban and peri-urban respondents predominately identifying themselves as either Lomwe (urban: 33.7%, peri-urban: 23.8%) or Ngoni (urban: 24.4%, peri-urban: 43.1%), whereas rural respondents identified themselves as either Nyanja (44.1%) or Sena (25.6%) (**Table 3.1**). Tribal affiliations may lead to cultural differences in WASH practices, although limited evidence for this exists within the Malawian context (296).

In terms of schooling, 38.5% (n= 372) of the total study participants were enrolled in some form of education with limited differences seen between the regions. 39.3% (n=379) of the total study population was unemployed, and paid employment for those above the working age (>15) was 43% (n=66/154) in the urban site, 51.4% (n=111/216) in the peri-urban site and 20.6% (n=29/141) in the rural site, further evidencing the differences in income and poverty between rural and urban settings

(**Table 3.2**). On direct questioning of the household head, 84.0% (n=84), 85.0% (n=85) and 42.0% (n=42) of them in the urban, peri-urban and rural regions reported having a job, and the type of employment differed between setting, with household heads employed in either skilled manual roles (urban: 42%, peri-urban: 35%) and sales sector (urban: 36%, peri-urban: 22%) in the urban sites, whereas they were predominantly employed in unskilled (45%) or skilled manual (38%) jobs in the rural site (**Table 3.1**).

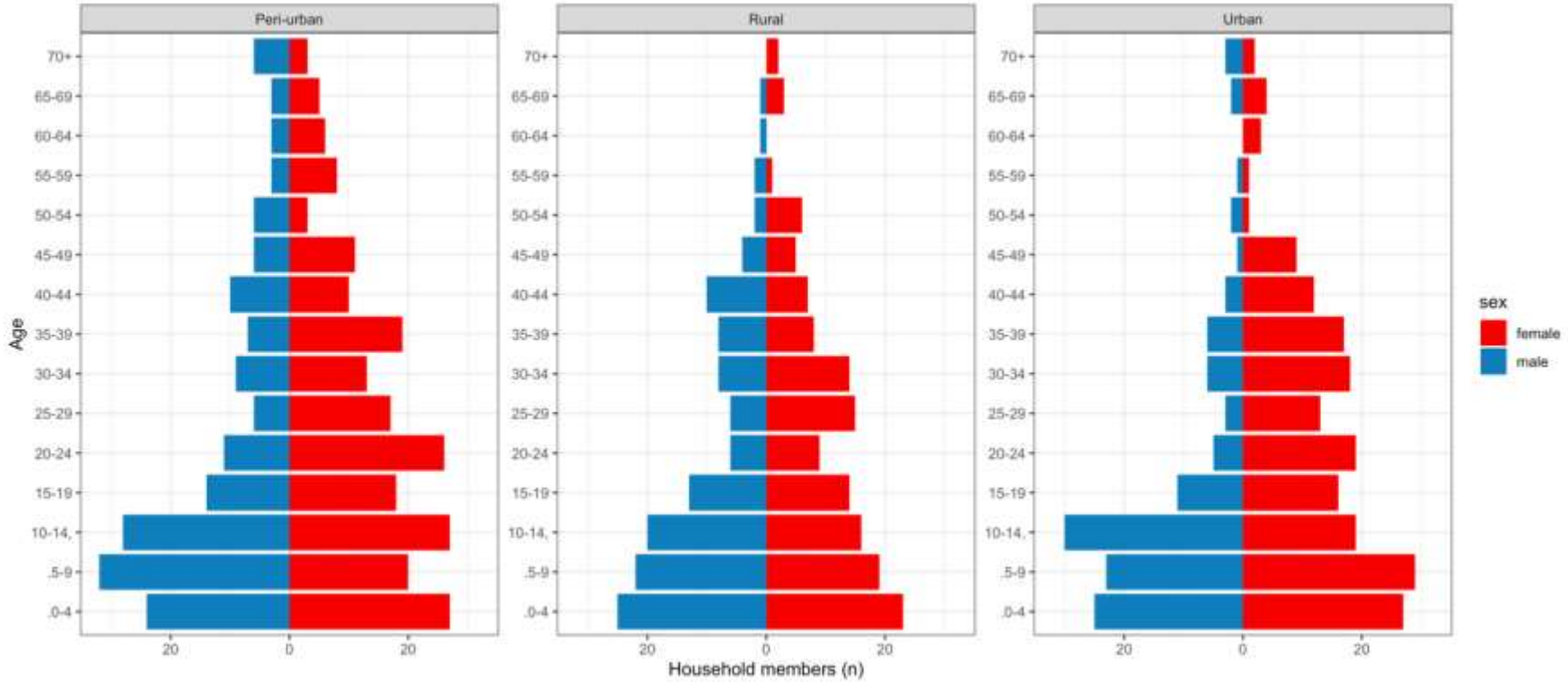


Figure 3.9. Population pyramid of study households in each region stratified into age grouping and sex.

Table 3.2. Household participant demographics of the urban, peri-urban and rural sites.

Characteristic	n (%) unless otherwise indicated				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Household members					
Number of household members	n=1348	n=459	n=467	n=422	
Number of participants recruited	n=965 (71.6%)	n=312 (68.0%)	n=383 (82.0%)	n=270* (64.0%)	***>.001***
Age	median = 18yrs (IQR = 7-34) range = 1 month to 102yrs	median = 15yrs (IQR = 7-32) range = 1 month to 87yrs	median = 20yrs (IQR = 9-37) range = 1 month to 102yrs	median = 17yrs (IQR = 7-32) range = 1 month to 88yrs	***.031***
Sex					
Male	n=420 (43.5%)	n=122 (39.1%)	n=170 (44.4%)	n=128 (47.4%)	*.121*
Religion					
Christianity	n=889 (92.1%)	n=285 (91%)	n=341 (89.0%)	n=263 (97.4%)	***>.001***
Islam	n=59 (6.1%)	n=22 (7%)	n=34 (8.9%)	n=3 (1.1%)	***>.001***
Other / prefer not to say	n=17 (1.8%)	n=5 (2%)	n=8 (2.1%)	n=4 (1.5%)	*.864*
Tribe					
Chewa	n=60 (6.2%)	n=32 (10.3%)	n=25 (6.5%)	n=3 (1.1%)	***>.001***
Lomwe	n=214 (22.2%)	n=105 (33.7%)	n=91 (23.8%)	n=18 (6.7%)	***>.001***
Ngoni	n=251 (26.0%)	n=76 (24.4%)	n=165 (43.1%)	n=10 (3.7%)	***>.001***
Nyanja	n=133 (13.8%)	n=12 (3.8%)	n=2 (0.5%)	n=119 (44.1%)	***>.001***
Sena	n=106 (11.0%)	n=19 (6.1%)	n=18 (4.7%)	n=69 (25.6%)	***>.001***
Tonga	n=7 (0.7%)	n=6 (1.9%)	n=1 (0.3%)	n=0 (0.0%)	***.010***
Tumbuka	n=33 (3.4%)	n=17 (5.4%)	n=16 (4.2%)	n=0 (0.0%)	***>.001***
Yao	n=106 (11.0%)	n=40 (12.8%)	n=64 (16.7%)	n=2 (0.7%)	***>.001***
Other / prefer not to say	n=55 (5.7%)	n=5 (1.6%)	n=1 (0.3%)	n=49 (18.1%)	***>.001***
Job Status					
Student	n=372 (38.5%)	n=137 (43.9%)	n=140 (36.6%)	n=95 (35.2%)	*.059*
Unemployed	n=379 (39.3%)	n=107 (34.3%)	n=129 (33.7%)	n=143 (53.0%)	***>.001***
Unpaid family forker	n=4 (0.4%)	n=0 (0%)	n=1 (0.3%)	n=3 (1.1%)	*.100*
Paid domestic worker	n=17 (1.8%)	n=0 (0%)	n=11 (2.9%)	n=6 (2.2%)	***.003***
Paid employee	n=68 (7.0%)	n=19 (6.1%)	n=40 (10.4%)	n=9 (3.3%)	***>.001***
Self employed	n=125 (13.0%)	n=49 (15.7%)	n=62 (16.2%)	n=14 (5.2%)	***>.001***
Occupational Exposure					
Number of participants who work in education (i.e. school)	n=9 (0.9%)	n=5 (1.6%)	n=4 (1.0%)	n=0 (0.0%)	***.029***
Number of participants who work in healthcare (i.e. hospital)	n=4 (0.4%)	n=2 (0.6%)	n=1 (0.3%)	n=1 (0.4%)	*.828*
Phone Ownership					
Yes (all ages)	n=386 (40.0%)	n=94 (30.1%)	n=243 (63.4%)	n=49 (18.1%)	***>.001***
Yes (adjusted for age >17)^	n=270 (56.1%)	n=89 (61.8%)	n=135 (65.5%)	n=46 (35.1%)	***>.001***

**Due to COVID interruptions individual CRF data collected on 65/100 rural households. 291 individuals at the first 65 households, representing 92.8% of participants recruited from within these households.*
^Regional numbers of adults: (n=144) urban, (n=206) peri-urban, and (n=131) rural
p values generated by Fishers exact (categorical variable), and Kruskal-Wallis (continuous variable) tests.

3.6. Participant health status

An overall HIV prevalence rate of 6.8% was seen in the study cohort [urban: 6.1%, peri-urban: 6.8%, rural: 7.8%. However, 51% (n=492) of the study population did not know their HIV status, with participants in the rural households less likely to report a positive or negative test result. This could represent an underreporting of true HIV prevelance. Therefore, we can also adjust the HIV prevelance rate to include only those participants who knew their HIV status (i.e. proportion of participants with a positive HIV test result from the total number of participants who had a test). This (adjusted) HIV prevalence rate for those who knew their status was 14.0% (n=66), and this is more consistent with national data **(Table 3.3)**. There were some differences seen in the (adjusted) HIV prevalence rate between study sites, with the highest HIV rate seen in the peri-urban [23.6% (n=26/110)] households compared to the urban [10.5% (n=19/177)] or rural 11.3% (n=21/186) households (*p=.026*). Anti-retroviral therapy (ARV) uptake was good amongst HIV positive participants, with 93.9% (n=66) of HIV individuals on treatment, and 90.9% (n=60) on CPT **(Table 3.3)**. There was no data within the survey to assess ARV adherence, and a poor knowledge and documentation of CD4 count and viral load to determine participant's level of immunosuppression or co-infection risk.

There were no cases of active TB diagnosed in the cohort, and only 12 participants had ever been treated for TB in the past, all of whom completed a full course of anti-tuberculous therapy. Non-infectious comorbidities were infrequently diagnosed, with 6.8% (n=66) of the cohort reporting conditions such as hypertension, peptic ulcer disease and COPD **(**for full list, see **Table 3.3)**, reflecting a broadly healthy population. However, it should be noted that there was a higher prevalence of comorbidities diagnosed within the rural participants compared to those in the urban or peri-urban settings (*p=.003*) **(Table 3.3)**.

Only 2.9% (n=28) of the total population took any form of regular medication other than for HIV, and there were no differences seen in medication use regionally (**Table 3.3**).

Table 3.3. Participant health status of the urban, peri-urban and rural sites.

Health Characteristic	n (%)				
	Total	**Urban**	**Peri-urban**	**Rural^**	***p***
HIV Status					
HIV reactive (adj) [$]	n=66 (14.0%)	n=19 (10.5%)	n=26 (23.6%)	n=21 (11.3%)	***.026***
HIV reactive	n=66 (6.8%)	n=19 (6.1%)	n=26 (6.8%)	n=21 (7.8%)	*.723*
HIV non-reactive	n=407 (42.2%)	n=158 (50.6%)	n=84 (21.9%)	n=165 (61.1%)	

HIV status unknown	n=492 (51.0%)	n=135 (43.3%)	n=273 (71.3%)	n=84 (31.1%)	
Co-morbidities *(Any non-communicable disease)*					
Yes	n=66 (6.8%)	n=12 (4.0%)	n=24 (6.3%)	n=30 (11.1%)	***.003***
Co-morbidities *(% of those reporting <u>any</u>)*					
Diabetes	n=4 (0.4%)	n=2 (16.7%)	n=2 (8.3%)	n=0 (0.0%)	*.568*
Chronic obstructive pulmonary disease/Asthma	n=15 (1.6%)	n=3 (25.0%)	n=5 (20.8%)	n=7 (23.3%)	*.308*
Malignancy	n=1 (0.1%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (3.3%)	*.280*
Chronic kidney disease	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	*1.00*
Stroke	n=3 (0.3%)	n=0 (0.0%)	n=3 (12.5%)	n=0 (0.0%)	*.118*
Hypertension	n=14 (1.5%)	n=2 (16.7%)	n=10 (41.7%)	n=2 (6.6%)	*.063*
Chronic anaemia	n=2 (0.2%)	n=0 (0.0%)	n=1 (4.2%)	n=1 (3.3%)	*.743*
Mental health	n=3 (0.3%)	n=0 (0.0%)	n=1 (4.2%)	n=2 (6.6%)	*.374*
Rheumatic disease	n=2 (0.2%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (6.6%)	*.078*
Ischemic heart disease	n=2 (0.2%)	n=0 (0.0%)	n=1 (4.2%)	n=1 (3.3%)	*.743*
Gastritis/Peptic ulcer	n=9 (0.9%)	n=2 (16.7%)	n=3 (12.5%)	n=4 (13.3%)	*.637*
Epilepsy or other primary central nervous disease	n=2 (0.2%)	n=1 (8.3%)	n=0 (0.0%)	n=1 (3.3%)	*.521*
Ear nose or throat disease	n=3 (0.3%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (10.0%)	***.022***
Other	n=8 (0.8%)	n=2 (16.7%)	n=0 (0.0%)	n=6 (20.0%)	***.005***
TB status					
Previous TB	n=12 (1.2%)	n=5 (2.0%)	n=4 (1.0%)	n=3 (1.1%)	*.765*
Active TB	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	*1.00*
Regular medications					
Non-communicable disease medications	n=28 (2.9%)	n=12 (3.8%)	n=6 (1.6%)	n=10 (3.7%)	*.120*
Antiretroviral therapy*	n=62 (93.9%)	n=17 (89.5%)	n=25 (96.2%)	n=20 (95.2%)	*.263*
Regimen 1p/2p	n=2 (3.2%)	n=1 (5.9%)	n=1 (4.0%)	n=0 (0.0%)	
Regimen 5	n=19 (30.6%)	n=12 (70.6%)	n=0 (0.0%)	n=7 (35.0%)	
Regimen 13A	n=39 (62.9%)	n=3 (17.6%)	n=24 (96.0%)	n=12 (60.0%)	
Another regimen	n=2 (3.2%)	n=1 (5.9%)	n=0 (0.0%)	n=1 (5.0%)	
Co-trimoxazole preventative therapy*	n=60 (90.9%)	n=16 (84.2%)	n=24 (92.3%)	n=20 (95.2%)	*.105*

[$] *Numbers of participants with known HIV result* = n=177 urban, 110 peri-urban and 186 in the rural site.

** Adjusted for HIV individuals in each site (urban (n=19), peri-urban (n=26) and rural (n=21))*

^ 35 rural households did not complete individual CRFs, so information available for 270 out of 422 participants.

p values generated by Fishers exact (categorical variable), and Kruskal-Wallis (continuous variable) test.

3.7. Recent health care exposure, health seeking behaviours and antibiotic usage (ABU)

At baseline, hospital attendance and healthcare exposure in the preceding 6 months was low across all sites, with 1.9% (n=6) of urban, 1.6% (n=10) of peri-urban and 3.3% (n=9) of rural participants reporting having stayed overnight at a healthcare facility, due to a range of antenatal, infectious and

non-infectious causes **(Table 3.4)**. 1.9% (n=6) of urban, 2.1% (n=8) of per-urban and 5.2% (n=14) of rural participants reported having attended healthcare as a guardian over the same period.

Periods of acute illness were separated into recent (with 1 month) and distant (1-3 months), with episodes of illness >3 months ago not captured other than through hospital admissions (up until 6 months). Episodes of illness not resulting in hospital admission were common, with 154 (16.0%) individuals recounting being unwell 4 weeks preceding recruitment, and 98 individuals (10.2%) reported being unwell 1-3-months prior to recruitment. Infection related symptoms such as fever, cough and malaria were more common than non-infectious diseases, and illness *of any cause* was more frequently reported amongst the rural population than the urban or peri-urban population (**Table 3.4)**. Antibiotic usage was frequently linked to periods of illness, irrespective of confirmed or presumed diagnosis, with 59.0% (n=36) of people reporting a preceding illness having received an antibiotic, broken down into 45.7% (n=16) of people who had been recently unwell (in the preceding month), and 76.9% (n=20/26) of people unwell in the preceding 1-3-month period.

Table 3.4. Participant illness metrics in urban, peri-urban and rural sites within the preceding 6 months

Reported illness	n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Healthcare exposure as patient*	n=25 (2.6%)	n=6 (1.9%)	n=10 (2.6%)	n=9 (3.3%)	*.541*
Reason for hospital admission					
Fever (unknown aetiology)	n=2 (8.0%)	n=1 (16.7%)	n=0 (0.0%)	n=1 (11.1%)	
Cough / CAP	n=5 (20.0%)	n=2 (33.3%)	n=0 (0.0%)	n=3 (33.3%)	
Skin infection	n=1 (4.0%)	n=0 (0.0%)	n=1 (10.0%)	n=0 (0.0%)	
Malaria (RDT confirmed)	n=1 (4.0%)	n=0 (0.0%)	n=1 (10.0%)	n=0 (0.0%)	
BSI	n=1 (4.0%)	n=0 (0.0%)	n=1 (10.0%)	n=0 (0.0%)	
Non-infectious cause	n=5 (20.0%)	n=0 (0.0%)	n=3 (30.0%)	n=2 (22.2%)	
Pregnancy / birth episode	n=10 (40.0%)	n=3 (50.0%)	n=4 (40.0%)	n=3 (33.3%)	
Healthcare exposure (guardian)*	n=28 (2.9%)	n=6 (1.9%)	n=8 (2.1%)	n=14 (5.2%)	***.046***
Recent illness reported					
Household participant unwell in last **0-1 month**	n=154 (16.0%)	n=35 (11.2%)	n=65 (17.0%)	n=54 (20.0%)	***.011***
Household participant unwell in last **1-3 months**	n=98 (10.2%)	n=26 (8.3%)	n=22 (5.7%)	n=50 (18.5%)	***>.001***
Illness (any in last 0-3 months)	n=252 (26.1%)	n=61 (19.5%)	n=87 (22.7%)	n=104 (38.5%)	***>.001***
Fever (unknown aetiology)	n=95 (37.7%)	n=12 (19.7%)	n=24 (27.6%)	n=59 (56.7%)	
Diarrhoea	n= 17(6.7%)	n=3 (4.9%)	n=4 (4.6%)	n=10 (9.6%)	
Cough / CAP	n=99 (39.3%)	n=27 (44.3%)	n=42 (48.3%)	n=30 (28.8%)	
Malaria (RDT confirmed)	n=41 (16.3%)	n=8 (13.1%)	n=16 (18.4%)	n=17 (16.3%)	
Skin infection	n=5 (2.0%)	n=3 (4.9%)	n=0 (0.0%)	n=2 (1.9%)	
UTI	n=1 (0.4%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (1.0%)	
ENT infection	n=10 (4.0%)	n=0 (0.0%)	n=3 (3.4%)	n=7 (6.7%)	
Non-infectious cause	n=42 (16.7%)	n=20 (32.8%)	n=8 (9.2%)	n=14 (13.5%)	

** Response time period = 6 months*

^p values generated by Fishers exact test

Overall antibiotic usage was 15.2% (n=147) amongst participants within the preceding 6 months, and there was higher antibiotic usage in rural residents compared to the urban or peri-urban residents **(Table 3.5).** This may relate to the frequency of illness episodes, overall health of the rural population, the type of illnesses encountered or local prescribing practices. In the urban population, 16.3% (n=51/312) of the total participants received an antibiotic in the last 6 months, with 13.1% (n=19/144) of adults, 12.9% (n=15/116) of children aged 5-17yrs and 32.7% (n=17/52) of children <5 receiving antibiotics. In the peri-urban site 9.1% (n=35/383) of the total participants received an antibiotic in the last 6 months, with 6.2% (n=13/210) of adults, 9.0% (n=11/122) of children aged 5-17yrs and 21.6% (n=11/51) of children <5 receiving antibiotics. In the rural site 22.6% (n=61/270) of total participants received an antibiotic in the last 6 months, with 20.6% (n=27/131) of adults, 14.3% (n=13/91) of children aged 5-17yrs and 43.8% (n=21/48) of children <5 receiving antibiotics. To note, across the study cohort, the age group of a participant was associated with antibiotic exposure at baseline, with children aged <5 more likely to receive an antibiotic than any other age group (**Table 3.5**).

Table 3.5. Participant antimicrobial usage metrics in urban, peri-urban and rural sites

Reported ABU	n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Antibiotic usage*	n=147 (15.2%)	n=51 (16.3%)	n=35 (9.1%)	n=61 (22.6%)	***>.001***
Antibiotics used in the last **4 weeks** *(due to presumed infection/ illness)*	n=64 (6.6%)	n=16 (5.1%)	n=21 (5.5%)	n=27 (10.0%)	***.038***
Antibiotics used in the last **3 months** *(due to presumed*	n=58 (6.0%)	n=20 (6.4%)	n=8 (2.1%)	n=30 (11.1%)	***>.001***
Antibiotics used in hospital in last **6 months**	n=31 (3.2%)	n=14 (4.5%)	n=3 (0.8%)	n=14 (5.2%)	***>.001***
On antibiotics **at baseline** recruitment *(other reasons)*	n=9 (0.9%)	n=3 (1.0%)	n=5 (1.3%)	n=1 (0.4%)	*0.491*
Antibiotic use by age group					
Child (<5)	n=49 (32.5%)	n=17 (32.7%)	n=11 (21.6%)	n=21 (43.8%)	
Adolescent (5-17)	n=39 (11.9%)	n=15 (12.9%)	n=11 (9.0%)	n=13 (14.3%)	
Adult (>17)	n=59 (12.2%)	n=19 (13.1%)	n=13 (6.2%)	n=27 (20.6%)	
Antibiotic use by age group$		**Child**	**Adolescent**	**Adult**	
Antibiotic usage (total all regions)	NA	n=49 (32.5%)	n=39 (11.9%)	n=59 (12.2%)	***>.001***

** A composite of antibiotic per participant at or prior to baseline visit (with multiple episodes of antibiotic used 1/3/6 months in the same participant counted as 1), thereby representing the number of participants receiving 1 or more antibiotics prior to recruitment,* ***not*** *during study period.*
^p values generated by Fishers exact test
$Total Adult (>17) = 485, Adolescents (5-17) = 329, and Children <5 = 151

The most frequent antibiotics used in all ages, were co-trimoxazole (35.9% n=65/181), amoxicillin (35.4% n=64/181) or metronidazole (12.7% n=23/181) accounting for 84% of the total antibiotics received, with the use of 3GC or fluoroquinolones uncommon (**Table 3.6)**. There were no differences in the antibiotic class received in hospital vs the community setting, and limited sample size precluded a more in-depth analysis of these data. There were regional differences in the choice of antibiotic prescription, but again, given the limited overall use of antibiotics within the cohort, wide variations in reason for antibiotic prescription, and absence of antibiotic availability data, I was unable to explore whether these regional differences were related to appropriateness or determined by access. Nevertheless, there was clearly a reliance on the three main antibiotics used in (HIV/TB) vertical campaigns in all three sites.

Table 3.6. Household (baseline) antibiotic choice from urban, peri-urban and rural sites.

Variable	Site		Antibiotic choice^										
		Total antibiotics	Amoxicillin	Benzylpenicillin	Cefuroxime	Cefixime	Ciprofloxacin	Co-trimoxazole	Doxycycline	Erythromycin	Gentamicin	Metronidazole	Other or Unknown
Antibiotic usage in household participants	Urban	n=63	n=24 (38.1%)	n=2 (3.2%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (3.2%)	n=20 (31.7%)	n=0 (0.0%)	n=2 (3.2%)	n=3 (4.8%)	n=7 (11.1%)	n=3 (4.8%)
	Peri-urban	n=40	n=9 (22.5%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (7.5%)	n=17 (42.5%)	n=0 (0.0%)	n=1 (2.5%)	n=1 (2.5%)	n=8 (20.0%)	n=1 (2.5%)
	Rural	n=78	n=31 (39.7%)	n=1 (1.3%)	n=1 (1.3%)	n=1 (1.3%)	n=2 (2.6%)	n=28 (35.9%)	n=2 (2.6%)	n=1 (1.3%)	n=2 (2.6%)	n=8 (10.3%)	n=1 (1.3%)
Antibiotic used in last 4 weeks	Urban	n=21	n=5 (23.8%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.8%)	n=10 (47.6%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.8%)	n=2 (9.5%)	n=2 (9.5%)
	Peri-urban	n=24	n=4 (16.7%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.2%)	n=12 (50.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.2%)	n=5 (20.8%)	n=1 (4.2%)
	Rural	n=29	n=12 (41.4%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (3.4%)	n=12 (41.4%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (10.3%)	n=1 (3.4%)
Antibiotic used in last 3 months	Urban	n=24	n=8 (33.3%)	n=1 (4.2%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.2%)	n=6 (25.0%)	n=0 (0.0%)	n=2 (8.3%)	n=1 (4.2%)	n=4 (16.7%)	n=1 (4.2%)
	Peri-urban	n=8	n=3 (32.5%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (12.5%)	n=2 (25.0%)	n=0 (0.0%)	n=1 (12.5%)	n=0 (0.0%)	n=1 (12.5%)	n=0 (0.0%)
	Rural	n=33	n=12 (36.4%)	n=1 (3.0%)	n=1 (3.0%)	n=0 (0.0%)	n=1 (3.0%)	n=11 (33.3%)	n=2 (6.0%)	n=0 (0.0%)	n=2 (6.0%)	n=3 (9.1%)	n=0 (0.0%)
Antibiotic used in last 6 months (healthcare)	Urban	n=15	n=9 (60.0%)	n=1 (6.7%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (20.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (6.7%)	n=1 (6.7%)	n=0 (0.0%)
	Peri-urban	n=3	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (33.3%)	n=1 (33.3%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (33.3%)	n=0 (0.0%)
	Rural	n=15	n=7 (46.7%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (6.7%)	n=0 (0.0%)	n=4 (26.7%)	n=0 (0.0%)	n=1 (6.7%)	n=0 (0.0%)	n=2 (13.3%)	n=0 (0.0%)
Antibiotic used at baseline	Urban	n=3	n=2 (66.7%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (33.3%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)
	Peri-urban	n=5	n=2 (40.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (40.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (20.0%)	n=0 (0.0%)
	Rural	n=1	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (100%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)

^Grey = Total usage, where antibiotics were selected by ≥1 households in region. Blue = cumulative total of antibiotics used. Yellow = antibiotic selected by ≥1 households in region. White = antibiotic not selected.

3.8. Human health seeking behaviour

A limited assessment of health care utilization was made based on response to self-reported symptoms and self-reported symptom severity in each region (**Table 3.7 [urban], Table 3.8 [per-urban], Table 3.9 [rural]**). Respondents from the urban region reported a heavy reliance on the local governmental health centres when they had a fever (82.7%), cough (84.6%), or diarrhoea (83.7%). Occasionally urban household members would choose to attend a governmental hospital (8.3-8.7%)

or self-treat with medications they owned (2.2-3.2%), but they would rarely attend a local pharmacy (0.3-1.9%). If urban participants felt “severely ill”, they would be more likely to visit a public or private hospital (26.6%), than if they reported a specific symptom or felt “ill”, but again there was a heavy reliance on the local health centre (72.7%) as a primary access point for healthcare in the urban setting. Akin to the urban site, participants from the peri-urban site also relied on the government health care centres when they had a fever (76.7%), cough (82.7%), or diarrhoea (76.7%), and if they were “severely ill”. They would be less likely to attend the governmental hospital than urban residents for all causes and instead visited private pharmacies, especially if they had a fever (11.5%) or diarrhoea (12.8%). When they felt “severely ill”, participants in the urban settings would rely on governmental health centres more than in the rural site but would also consider attending a public or private hospital facility **(Table 3.6)**. Reasons for facility choice were not captured in the survey, however, they could be influenced by cost, access, household location or personal preference. In the rural setting, there was a difference in heath seeking behaviour relating to the choice of facility used, as most participants in this setting would utilise the local governmental hospital (Chikwawa District hospital) if they had fever (84.4%), cough (85.9%) or diarrhoea (80.4%), rather than a health centre. This may be due to the close proximity of Chikwawa District hospital in comparison to other governmental health facilities, and the geographic sparsity of alternative health centres. Alternatively, rural participants would visit a range of places including governmental health centres, private pharmacies, traditional healers or consider self-treatment/nothing. To note, there was no reported use of traditional medicines or visiting traditional healers from respondents in the urban or peri-urban regions, and the reliance on the local hospital and heterogeneity of alternative choices set the rural site apart from the urban and peri-urban sites.

Table 3.7. Health seeking behaviour of urban households

Healthcare choice		**Reported symptom**			**Self-reported illness severity**	
		Fever	Cough	Diarrhoea	Felt ill?	Felt **severely** ill?
Health centre	*governmental*	n=258 (82.7%)	n=264 (84.6%)	n=261 (83.7%)	n=268 (85.9%)	n=215 (68.9%)
	private	n=13 (4.2%)	n=12 (3.8%)	n=13 (4.2%)	n=12 (3.8%)	n=12 (3.8%)
Hospital	*governmental*	n=27 (8.7%)	n=27 (8.7%)	n=26 (8.3%)	n=28 (9.0%)	n=69 (22.1%)
	private	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=14 (4.5%)
Pharmacy	*governmental*	n=1 (0.3%)	n=0 (0.0%)	n=1 (0.3%)	n=3 (1.0%)	n=0 (0.0%)
	private	n=6 (1.9%)	n=1 (0.3%)	n=1 (0.3%)	n=0 (0.0%)	n=1 (0.3%)
Self-treat or do nothing		n=7 (2.2%)	n=8 (2.6%)	n=10 (3.2%)	n=1 (0.3%)	n=1 (0.3%)

**Number of residents completing survey = 312*
^Yellow = Highest option selected, Blue = >5% of participants selected, Grey = <5% of participants selected, White = not selected

Table 3.8. Health seeking behaviour of peri-urban households

Healthcare choice		**Reported symptom**			**Reported perception**	
		Fever	Cough	Diarrhoea	Felt ill?	Felt **severely** ill?
Health centre	*governmental*	n=293 (76.7%)	n=316 (82.7%)	n=293 (76.7%)	n=318 (83.2%)	n=286 (74.9%)
	private	n=20 (5.2%)	n=19 (5.0%)	n=16 (4.2%)	n=20 (5.2%)	n=13 (3.4%)
Hospital	*governmental*	n=8 (2.1%)	n=14 (3.7%)	n=8 (2.1%)	n=12 (3.1%)	n=49 (12.8%)
	private	n=12 (3.1%)	n=10 (2.6%)	n=9 (2.4%)	n=11 (2.9%)	n=19 (5.0%)
CHAM facility		n=3 (0.8%)	n=4 (1.0%)	n=3 (0.8%)	n=3 (0.8%)	n=5 (1.3%)
Pharmacy	*governmental*	n=1 (0.3%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)
	private	n=44 (11.5%)	n=17 (4.5%)	n=49 (12.8%)	n=17 (4.5%)	n=10 (2.6%)
Self-treat or do nothing		n=1 (0.3%)	n=2 (0.5%)	n=4 (1.0%)	n=1 (0.3%)	n=0 (0.0%)

**Number of residents completing survey = 382*
^Yellow = Highest option selected, Blue = >5% of participants selected, Grey = <5% of participants selected, White = not selected

Table. 3.9. Health seeking behaviour of rural households

Healthcare choice		Reported symptom			Reported perception	
		Fever	Cough	Diarrhoea	Felt ill?	Felt **severely** ill?
Health centre	*governmental*	n=30 (11.1%)	n=29 (10.7%)	n=30 (11.1%)	n=31 (11.4%)	n=3 (1.1%)
	private	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)
Hospital	*governmental*	n=228 (84.4%)	n=232 (85.9%)	n=217 (80.4%)	n=235 (87.0%)	n=259 (95.9%)
	private	n=1 (0.4%)	n=1 (0.4%)	n=0 (0.0%)	n=1 (0.4%)	n=8 (3.0%)
Pharmacy	*governmental*	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)
	private	n=3 (1.1%)	n=0 (0.0%)	n=4 (1.5%)	n=1 (0.4%)	n=0 (0.0%)
Traditional healer		n=3 (1.1%)	n=0 (0.0%)	n=2 (0.7%)	n=2 (0.7%)	n=0 (0.0%)
Self-treat or do nothing		n=5 (1.9%)	n=8 (3.0%)	n=17 (6.3%)	n=0 (0.0%)	n=0 (0.0%)

**Number of residents completing survey = 270*
^Yellow = Highest option selected, Blue = >5% of participants selected, Grey = <5% of participants selected, White = not selected

3.9. Animal ownership and husbandry

58.7% (n=176) of households reported co-habitation with domestic or livestock animals, with 36% (n=36), 59% (n=59) and, 81% (n=81) of households in the urban, peri-urban and rural sites owning ≥1 animal respectively **(Table 3.10)**. A total of n=2169 animals were linked to a study household at baseline, and both the composition of species and number of animals present per households varied by region **(Table 3.10)**. Companion animals (i.e. cats and dogs) were located in low numbers per house and made up a large proportion of the animal species owned in urban (n=23/36) and peri-urban (n=25/59) households. Poultry (i.e. chickens, doves, ducks) was associated with low-level household farming practices, and chickens were both the most owned and numerous animals within the study; present at 18% (n=18), 39% (n=39) and, 59% (n=59) of households in the urban, peri-urban and rural sites respectively. Larger animals requiring bigger plots of land to sustain daily food requirements (i.e. pigs, goats, cattle) were seen at fewer households, primarily located in the rural or peri-urban setting **(Table 3.10)**. The reason for animal ownership was not fully captured in the study, but animals at several households were specifically reared for breeding and selling purposes, with 8.3% (n=3/36) of urban, 42.4% (n=25/59) of peri-urban, and 60.5% (n=49/81) of rural households reporting owning animals to sell or trade **(Table 3.10)**.

Table. 3.10. Domestic animal and livestock husbandry of urban, peri-urban and rural households.

Household ownership	n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Households with animal ownership	n=176 (58.7%)	n=36 (36%)	n=59 (59%)	n=81 (81%)	***>.001***
Total number of animals owned	n=2169	n=213	n=704	n=1252	
Species of animals owned^					
Households with chickens	n=116 (38.7%)	n=18 (18.0%)	n=39 (39.0%)	n=59 (59.0%)	***>.001***
Number of chickens owned	n=919	n=152	n=315	n=452	
Households with doves	n=13 (4.3%)	n=1 (1.0%)	n=5 (5.0%)	n=7 (7.0%)	*.092*
Number of doves owned	n=442	n=10	250	n=182	
Households with ducks	n=14 (4.7%)	n=2 (2.0%)	n=2 (2.0%)	n=10 (10.0%)	***.017***
Number of ducks owned	n=67	n=14	8	n=45	
Households with guinea fowl	n=3 (1.0%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (3.0%)	*.109*
Number of guinea fowl owned	n=34	n=0	n=0	n=34	
Households with turkeys	n=2 (0.7%)	n=0 (0.0%)	n=2 (2.0%)	n=0 (0.0%)	*.331*
Number of turkeys owned	n=8	n=0	8	n=0	
Households with dogs	n=43 (14.3%)	n=14 (14.0%)	n=19 (19.0%)	n=10 (10.0%)	*.212*
Number of dogs owned	n=100	n=27	44	n=29	
Households with cats	n=24 (8.0%)	n=9 (9.0%)	n=6 (6.0%)	n=7 (7.0%)	*.790*
Number of cats owned	n=31	n=10	11	n=10	
Households with cattle	n=23 (7.7%)	n=0 (0.0%)	n=0 (0.0%)	n=23 (23.0%)	*NA*
Number of cattle owned	n=23	n=0	n=0	n=74	
Households with pigs	n=17 (5.7%)	n=0 (0.0%)	n=5 (5.0%)	n=12 (12.0%)	***>.001***
Number of pigs owned	n=17	n=0	20	n=55	
Households with goats	n=49 (16.3%)	n=0 (0.0%)	n=12 (12.0%)	n=37 (37.0%)	***>.001***
Number of goats owned	n=419	n=0	48	n=371	

^ Total percentage (%) is taken from number of households in all regions (n=300), whereas regional percentage (%) is taken from number of households that owned per region (n=100).
**p values generated by Fishers exact test*

In terms of co-habitation and husbandry techniques, a high proportion of households with poultry or goats kept them inside the house, especially in the urban or peri-urban setting, posing a risk of environmental contamination **(Table 3.11)**. In rural households, while this practice was seen, they preferred to keep chickens or goats inside or outside the household compound instead of inside the house. Where owned, companion animals, were allowed to roam free, and this was consistent amongst settings, and cattle were kept within shelters / bomas (a term used in eastern Africa for an enclosure, especially for animals) inside or outside the household compound (rural site only) **(Table 3.11)**.

Table 3.11. Animal location and livestock production systems in urban, peri-urban and rural households.

Husbandry characteristic		n (%)				
		Total	**Urban**	**Peri-urban**	**Rural**	***p***
Where are animals kept?						
Chickens	In the house	n=70 (60.3%)	n=15 (83.3%)	n=25 (64.1%)	n=30 (50.8%)	***.041***
	Shelter /Boma within household compound	n=33 (28.4%)	n=2 (11.1%)	n=12 (30.8%)	n=19 (32.2%)	*.213*
	Shelter /Boma outside the household compound	n=9 (7.8%)	n=1 (5.6%)	n=1 (2.6%)	n=7 (11.9%)	*.190*
	Other	n=4 (3.4%)	n=0 (0.0%)	n=1 (2.6%)	n=3 (5.1%)	*1.00*
Dogs	Free roaming	n=34 (79.1%)	n=9 (64.3%)	n=15 (78.9%)	n=10 (100.0%)	*.110*
	Shelter /Boma outside the household compound	n=1 (2.3%)	n=1 (7.1%)	n=0 (0.0%)	n=0 (0.0%)	*.558*
	Shelter /Boma within household compound	n=8 (18.6%)	n=4 (28.6%)	n=4 (21.1%)	n=0 (0.0%)	*.242*
Cattle	Shelter /Boma within household compound	n=11 (47.8%)	NA	NA	n=11 (47.8%)	*NA*
	Shelter /Boma outside the household compound	n=11 (47.8%)	NA	NA	n=11 (47.8%)	*NA*
	Other	n=1 (4.4%)	NA	NA	n=1 (4.2%)	*NA*
Goats	In the house	n=8 (16.3%)	NA	n=5 (41.7%)	n=3 (8.1%)	***.015***
	Shelter /Boma within household compound	n=28 (57.1%)	NA	n=6 (50.0%)	n=22 (59.5%)	*.739*
	Shelter /Boma outside the household compound	n=12 (24.5%)	NA	n=1 (8.3%)	n=11 (29.7%)	*.247*
	Free roaming	n=1 (2.0%)	NA	n=0 (0.0%)	n=1 (2.7%)	*1.00*
Pigs	Shelter /Boma within household compound	n=10 (58.8%)	NA	n=5 (100.0%)	n=5 (41.7%)	***.044***
	Shelter /Boma outside the household compound	n=6 (35.3%)	NA	n=0 (0.0%)	n=6 (50.0%)	*.102*
	Free roaming	n=1 (5.9%)	NA	n=0 (0.0%)	n=1 (8.3%)	*1.00*
Livestock production system						
Beef cattle	Zero Grazing	n=2 (%)	NA	NA	n=2 (10.0%)	*NA*
	Communal Grazing	n=15 (%)	NA	NA	n=15 (75.0%)	*NA*
	Pastoral	n=3 (%)	NA	NA	n=3 (15.0%)	*NA*
Dairy cattle	Pastoral	n=4 (%)	NA	NA	n=4 (100.0%)	*NA*
Small ruminants	Zero Grazing	n=8 (%)	NA	n=6 (50.0%)	n=2 (5.4%)	***.001***
	Communal Grazing	n=26 (%)	NA	n=6 (50.0%)	n=20 (54.1%)	*1.00*
	Pastoral	n=15 (%)	NA	n=0 (0.0%)	n=15 (40.5%)	***.010***
Households that rear animals to sell?		n=77 (%)	n=3 (3.0%)	n=25 (25.0%)	n=49 (49.0%)	***>.001***

**p values generated by Fishers exact test*

3.10. Animal health metrics, access to veterinary services, ABU in animals and health seeking behaviour.

At baseline, most animals co-located at households were reported as being in good health, however, several households recounted episodes of animal illness or disease seen within the preceding year **(Table 3.12)**. Chickens were the species most likely to be ill, with 44.4% (n=8) of urban, 44.7% (n=17) of peri-urban, and 44.0% (n=26) of rural households reporting disease in chickens over the last 12 months. Chickens were not the only unwell animal, and disease symptoms were noted in pigs, cattle and goats **(Table 3.12)**. The types of symptoms differed by species, with the main problem being neurological symptoms (or confirmed Newcastle disease) in poultry and pigs, skin disease in cattle and digestive complaints in goats **(Table 3.12)**.

Table 3.12. Animal disease characteristics in urban, peri-urban and rural households

Animal disease characteristic		n (%)			
		Total	**Urban**	**Peri-urban**	**Rural**
Any disease noted in last 12 months?					
Cattle		n=8 (34.8%)	NA	NA	n=8 (34.8%)
Poultry		n=51 (44.3%)	n=8 (44.4%)	n=17 (44.7%)	n=26 (44.0%)
Goats		n=11(22.4%)	NA	n=1 (8.3%)	n=10 (27.0%)
Pigs		n=4 (23.5%)	NA	n=0 (0.0%)	n=4 (33.3%)
Main animal condition or symptom noted					
Cattle	Skin disease	n=3 (37.5%)	NA	NA	n=3 (37.5%)
	Digestive disease	n=2 (25.0%)	NA	NA	n=2 (25.0%)
	Respiratory disease	n=1 (12.5%)	NA	NA	n=1 (12.5%)
	Other / unknown	n=2 (25.0%)	NA	NA	n=2 (25.0%)
Poultry	Neurological	n=41 (80.4%)	n=7* (87.5%)	n=16 (94.1%)	n=18* (69.2%)
	Sudden death	n=4 (7.8%)	n=1 (12.5%)	n=0 (0.0%)	n=3 (11.5%)
	Digestive disease	n=3 (5.9%)	n=0 (0.0%)	n=0 (0.0%)	n=3 (11.5%)
	Respiratory disease	n=2 (3.9%)	n=0 (0.0%)	n=1 (5.9%)	n=1 (3.9%)
	Skin disease	n=1 (2.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (3.9%)
Goats	Digestive disease	n=4 (36.4%)	NA	n=0 (0.0%)	n=4 (40.0%)
	Respiratory disease	n=3 (27.3%)	NA	n=1 (100.0%)	n=2 (20.0%)
	Neurological	n=2 (18.2%)	NA	n=0 (0.0%)	n=2 (20.0%)
	Sudden death	n=2 (18.2%)	NA	n=0 (0.0%)	n=2 (20.0%)
Pigs	Neurological	n=3 (75.0%)	NA	n=0 (0.0%)	n=3 (75.0%)
	Skin disease	n=1 (25.0%)	NA	n=0 (0.0%)	n=1 (25.0%)

**Newcastle disease confirmed in 8 animals in rural setting, and 5 in urban setting.*
^Given low numbers of animals, no statistics were performed on regional differences in animal illness

If animals became unwell, only 26.9% (n=47) of households reported access to animal healthcare supported by local veterinarians, and this healthcare was more available to rural households than urban or peri-urban households **(Table 3.13)**. State funded animal healthcare was the predominant

service available, but there was variation seen between the regions, with urban or peri-urban households more likely to have the option of access to private practice.

Table 3.13. Access and usage of animal services in urban, peri-urban and rural households.

Access to animal health services	n (% of households that own animals)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Access to professional animal health services^	n=47 (26.9%)	n=7 (19.4%)	n=11 (19.0%)	n=29 (35.8%)	*0.054*
State or Government	n=38 (80.9%)	n=4 (57.1%)	n=8 (72.7%)	n=26 (89.7%)	
Private	n=6 (12.8%)	n=2 (28.6%)	n=2 (18.2%)	n=2 (6.9%)	
Both State/ Private	n=3 (6.6%)	n=1 (14.3%)	n=1 (9.1%)	n=1 (3.4%)	
Access to veterinarian as part of animal health service*	n=47 (100%)	n=7 (100%)	n=11 (100%)	n=29 (100%)	
Access to laboratory testing as part of animal health service	n=3 (6.4%)	n=1 (14.3%)	n=2 (18.2%)	n=0 (0.0%)	
Is the household involved in a regular animal health program (i.e. NGO rabies vaccination)?	n=7 (2.3%)	n=1 (1.0%)	n=4 (4.0%)	n=2 (2.0%)	

^Information from 36 urban, 58 peri-urban and 81 rural households with animals.
**In 3 rural households and 1 urban household the level of practitioner qualifications was unknown, so these have been classified as presumed veterinarian.*

When households were surveyed about what they would do in the advent of animal disease, the responses varied by species and setting (**Table 3.14**). Most frequently households would do nothing (35.8% n=57), or alternatively purchase medication (13.2% n=21) or use traditional remedies (13.8% n=22) instead of seeking a consultation from an animal healthcare specialist. Both the use of self-purchased medication and traditional remedies were more frequently used in chickens or poultry than in other species, and households rarely would use out of date medication. It was common for households to implement preventative measures to stop other animals from becoming unwell, including fencing, vaccination, or the separation of the sick animals from the rest of the herd rather than relying on treating sick animals (**Table 3.15**). Therefore, there was very little reported recent medication use in animals (within the preceding 2 months), and so medication usage data in animals was limited (**Table 3.16**). Where available, it showed that antibiotics including tetracyclines, penicillin and macrolides were used, alongside regular vaccinations, and feed supplements.

Table 3.14. Response to animal illness in urban, peri-urban and rural households, stratified by species.

		Response to sickness in household animals (n)							
Animal	**Site**	Consult a governmental veterinarian	Consult a private veterinarian	Use medication from a veterinarian drug store	Use left-over or vet applied drugs	Get medications from friends or family	Use traditional medication	Kill animal (+/- eaten)	Nothing
Cattle	Urban	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	NA	NA	NA	NA	NA	NA	NA	NA
	Rural	n=6	n=1	n=1	n=1	n=1	n=0	n=0	n=9
Goats	Urban	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	n=2	n=1	n=0	n=1	n=0	n=4	n=0	n=3
	Rural	n=9	n=2	n=4	n=2	n=0	n=1	n=0	n=9
Pigs	Urban	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	n=1	n=1	n=1	n=0	n=0	n=0	n=0	n=1
	Rural	n=2	n=0	n=1	n=0	n=0	n=1	n=0	n=6
Poultry	Urban	n=0	n=1	n=3	n=0	n=0	n=3	n=1	n=6
	Peri-urban	n=7	n=1	n=4	n=2	n=0	n=8	n=1	n=6
	Rural	n=7	n=2	n=7	n=2	n=0	n=5	n=5	n=17
All animals	**All regions**	n=34 (21.4%)	n=9 (5.6%)	n=21 (13.2%)	n=8 (5.0%)	n=1 (0.6%)	n=22 (13.8%)	n=7 (4.4%)	n=57 (35.8%)

^ Yellow = selected by ≥1 households in region. White = not selected. Grey = NA.

Table 3.15. Preventative measures and response to animal sickness in urban, peri-urban and rural households, stratified by species.

		Preventative measures used at households (n)					
Animal	**Site**	**Fencing**	**Not mixing with other animals/herd/ flock**	**Special feed / supplemental feed**	**Vet drugs (or vaccine)**	**Traditional medicine or local remedies**	**Do nothing**
Cows	Urban	NA	NA	NA	NA	NA	NA
	Peri-urban	NA	NA	NA	NA	NA	NA
	Rural	n=10	n=0	n=0	n=9	n=0	n=5
Goats	Urban	NA	NA	NA	NA	NA	NA
	Peri-urban	n=0	n=4	n=0	n=3	n=0	n=5
	Rural	n=11	n=2	n=2	n=6	n=0	n=17
Pigs	Urban	NA	NA	NA	NA	NA	NA
	Peri-urban	n=1	n=0	n=0	n=3	n=0	n=2
	Rural	n=5	n=0	n=0	n=1	n=0	n=6
Poultry	Urban	n=1	n=3	n=3	n=1	n=2	n=12
	Peri-urban	n=3	n=8	n=0	n=11	n=2	n=17
	Rural	n=12	n=0	n=0	n=9	n=3	n=36

^ Yellow = selected by ≥1 households in region. White = not selected. Grey = NA.

Table 3.16. Animal medication usage (within the 2 months preceding recruitment), stratified by species and region.

		Medication used by households (n households)									
Animal	**Site**	Vaccines	Antihelminth	Acaricide	Tetracycline	Sulphonamide	Penicillin	Fluoroquinolones	Macrolides	Aminoglycosides	Vitamins / suppliants
Cattle	Urban	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Rural	n=1	n=0	n=1	n=1	n=0	n=0	n=0	n=0	n=0	n=1
Goats	Urban	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0
	Rural	n=1	n=0	n=0	n=1	n=0	n=0	n=0	n=0	n=0	n=1
Pigs	Urban	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Peri-urban	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=1
	Rural	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0	n=0
Poultry	Urban	n=2	n=0	n=0	n=0	n=0	n=1	n=0	n=0	n=0	n=0
	Peri-urban	n=0	n=0	n=0	n=1	n=0	n=0	n=0	n=1	n=0	n=0
	Rural	n=2	n=0	n=1	n=0	n=0	n=0	n=0	n=0	n=0	n=1

^ Yellow = selected by ≥1 households in region. White = not selected. Grey = NA.

There were variations in how households would seek advice when selecting or using medication in animals, with 32.7% (n=98) of households relying on their own judgment for both the length of treatment and drug dose, and urban or peri-urban households likely to rely on their own judgement more so than rural households, whether through choice or necessity (i.e. cost or access to services) (**Table 3.17**).

Table 3.17. Attitudes to medication practices in urban, peri-urban and rural households.

Animal medication choice and disposal	n (%) of total households				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
If households were to use animal drugs, whose instructions would they follow for medication, dose, and length of treatment?					
Vet's	n=110 (36.7%)	n=28 (28.0%)	n=54 (54.0%)	n=28 (28.0%)	***>.001***
Animal health worker's (non-vet)	n=9 (3.0%)	n=7 (7.0%)	n=0 (0.0%)	n=2 (2.0%)	***.012***
Pharmacy's	n=1 (0.3%)	n=0 (0.0%)	n=1 (1.0%)	n=0 (0.0%)	*1.00*
Farmers or other households	n=3 (1.0%)	n=0 (0.0%)	n=2 (2.0%)	n=1 (1.0%)	*.776*
Their own judgement	n=98 (32.7%)	n=42 (42.0%)	n=42 (42.0%)	n=14 (14.0%)	***>.001***
Don't know / unknown	n=79 (26.3%)	n=23 (23.0%)	n=1 (1.0%)	n=55 (55.0%)	***>.001***
What would you do with animal drugs that have passed their expiry date? ^					
Dispose of them	n=82 (46.9%)	n=9 (30.8%)	n=29 (50.0%)	n=44 (54.3%)	
Return to pharmacy	n=6 (3.4%)	n=0 (0.0%)	n=3 (5.2%)	n=3 (3.7%)	
Give to another household or farmer	n=1 (0.6%)	n=0 (0.0%)	n=1 (1.7%)	n=0 (0.0%)	
Use for intended treatment	n=4 (2.3%)	n=0 (0.0%)	n=0 (0.0%)	n=4 (4.9%)	
Sell	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	
Nothing	n=83 (47.4%)	n=27 (69.2%)	n=25 (43.1%)	n=31 (38.3%)	

^Information from 36 urban, 58 peri-urban and 81 rural households with animals.
**p values generated by Fishers exact test*

Given the reliance on preventative measures and limited experience of animal medication, all households were asked what they thought the purpose of antibiotics and vaccinations were in animals. While there was uncertainty about their use within a large proportion of households, those that answered affirmatively thought that vaccinations were used to prevent sickness (45.3%), rather than treat sickness (14.3%), and antibiotics which were used to treat illness (45.7%), or both treat and prevent illness (5.0%) rather than purely used for prevention (4.0%).

Table 3.18. Knowledge of vaccine and antibiotic function in animals, stratified by region

Reason for use	n (%) of households				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
What do households think vaccines are used for in animals?					
Cure sick animals	n=43 (14.3%)	n=21 (21.0%)	n=7 (7.0%)	n=15 (15.0%)	***.016***
Prevent animals from becoming sick	n=136 (45.3%)	n=35 (35.0%)	n=56 (56.0%)	n=45 (45.0%)	***.012***
Cure sick animals and prevent them from becoming sick	n=11 (3.7%)	n=1 (1.0%)	n=8 (8.0%)	n=2 (2.0%)	***.048***
Fattening / increased growth	n=1 (0.3%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (1.0%)	*1.00*
Unsure	n=109 (36.3%)	n=43 (43.0%)	n=29 (29.0%)	n=37 (37.0%)	***.036***
What do households think antibiotics are used for in animals?					
Cure sick animals	n=137 (45.7%)	n=49 (49.0%)	n=55 (55.0%)	n=33 (33.0%)	
Prevent animals from becoming sick	n=12 (4.0%)	n=4 (4.0%)	n=4 (4.0%)	n=4 (4.0%)	
Cure sick animals and prevent sickness	n=15 (5.0%)	n=2 (2.0%)	n=9 (9.0%)	n=4 (4.0%)	
Fattening / increased growth	n=3 (1.7%)	n=0 (0.0%)	n=3 (3.0%)	n=0 (0.0%)	
Unsure	n=133 (44.3%)	n=45 (45.0%)	n=29 (29.0%)	n=59 (59.0%)	

**p values generated by Fishers exact test*

3.11. Discussion and limitations

Random-geolocation approaches were used to select 300 spatially diverse households within the study regions and where pre-existing data is available, these households had similar baseline metrics. An example of this was the household density for urban and rural households, whereby, at the urban and peri-urban sites the average number of household members was 4.6 and 4.2 respectively, and a previous large sero-surveillance study in Ndirande (STRATAA, unpublished) and community based malaria study in Chikwawa reported the average household density at 4.36 and 4.5 members per house respectively, indicating that urban and rural households in this study are likely to be representative of the typical household densities (275,297,298). In terms of age composition and tribal affiliation, these households tally with data from the 2018 Malawian population census (257). In this census Blantyre city and Chikwawa are broadly categorised, and both the age-grouped pyramids highlighting a predominance of a younger population and the common tribal affiliations (Lomwe and Ngoni in Blantyre, and Sena in Chikwawa) are reflected at study households. I should state that one

area that is not consistent with what we would expect to find, is the predominance of female respondents in the individual dataset. Data from the 2018 census illustrated a female: male ratio in Blantyre city of ~1:1, and due to the reduced consent from males within households the ratio of females: males was ~3:2 amongst household respondents. Therefore, females are overrepresented in the individual dataset, and this could bias some of the results in terms of underlying health status, attitudes to health seeking behaviour and antibiotic usage. However, health seeking behaviours can often be led by the household head, and the female:male ratio of household heads in Malawi is ~1:3, which replicates the same sex ratio found in household head respondents (257). So, while information was not always captured from *all* males at the households, it was adequately captured from male household heads.

In terms of poverty metrics, a high percentage of households lived in absolute poverty, with the rural site being the poorest, and this correlates to estimates for expected population income and comparable differences between urban and rural settings (257,295). The median age of household members was low (median 18yrs), and participants were frequently in full time education. Unemployment was high at 39.3%, with the rural region having more unemployment that the urban or peri-urban setting. The employment rate for Malawi is estimated to be 79.6%, and ~65% of people in Malawi are employed work in the agriculture, forestry and fisheries sectors (295). This was not reflected in this study, predominately because 82% of Malawians live in rural areas, and these figures represent the job types commonly found in the rural setting alone (258). Therefore, the unemployment rate and job roles (sales based or service sector) seen in household members from Ndirande and Chileka are likely to reflect the true nature of urbanised settings in Malawi. Overall, given the random geospatial selection process and the similarity between households in this study and those in previous local datasets, it is likely that there was no selection bias introduced, and recruited households broadly represent those found at urban, peri-urban and rural settings in Malawi.

The baseline findings from the study cohort and regional comparisons identified that household participants were predominantly in good health, but that underlying co-morbidities and episodes of illness were higher in the rural population than the peri-urban setting. Health status, including immunosuppression and episodes of recent illnesses can play a role in healthcare exposure and antibiotic use, which may in turn drive the selection pressure for gut colonisation with ESBL-producing bacteria (97,99,299–301). The adjusted HIV prevalence of 14.0% in the cohort was slightly lower than previous regional estimates and there were more HIV diagnosed participants in the peri-urban site, compared to the urban or rural site. Irrespective of these regional variances I did not find that

individuals from rural or peri-urban households had a greater chance of hospital admission but did see a difference in ABU between the regions, with the rural population more likely to have taken an antibiotic in the last 6 months compared to the other settings. The higher ABU in the rural setting correlated with a greater frequency of reported symptoms such as fever over the same timeframe, suggesting that either infectious conditions requiring antibiotics are more prevalent in the rural setting, or alternatively, that infectious symptoms and/or attitudes to ABU differ in this setting to the other regions, lead to a higher frequency of antibiotic consumption. Importantly, the age of participants correlated with the chance of having received an antibiotic with children under 5 having the highest antibiotic exposure. Children and immunosuppressed individuals (i.e. HIV) are likely to have different infection risks, healthcare seeking behaviours and rates of ABU compared to the adult population, so will be important group to consider separately.

The most commonly used antibiotics were co-trimoxazole, amoxicillin or metronidazole at all sites, which is consistent with previous descriptions from community-focussed ABU research undertaken in Chikwawa (116). These antibiotics are commonly available in our setting, and comparatively cheap compared to injectable medicines. They have a broad spectrum of activity which is likely to make them appropriate as first line therapy for several locally prevalent infectious diseases and have been integrated into international essential medicine lists for treatment of a number of conditions (302–304). In addition to the above, 90.9% (n=60) of HIV infected individuals were on CPT in line with international and local guidance (305). I did not assess antibiotic appropriateness or the reasons for antibiotic use, but community members rarely if ever relied on the use of 3GC or fluoroquinolones for treatment of infectious symptoms. It is therefore likely that exposure to these classes of antibiotic is limited to admissions to local hospitals, where they are highly utilised, and that sociocultural and economic factors shape the way antibiotics are accessed in the community (116)

Another limitation is that survey-based data can be subject to recall bias, and information on antibiotic usage and medications taken came directly from respondents in the study. This was mitigated where possible, using corroborative information from health passports, and implementing an adapted version of the drug-bag method to prompt participants to accurately recognise previously used antibiotics from a list of those that are locally available (306). If we assume that this data is accurate, and there is limited use of 3GCs or other agents, alongside low rates of hospital admissions or hospital exposures, this could point towards other factors other than human-ABU selection pressures driving colonisation of ESBL-producing bacteria in this cohort. Alternatively, distant 3GC usage or co-habitation with other household members or animals colonised with ESBL bacteria may be an

important factor in participants, as it is known that the persistence of ESBL gut colonisation is not always dependant on timing of antibiotic consumption, and co-habitation with other household members is associated with a risk of colonisation (300,307).

Where healthcare was sought, choices of healthcare facility accessed differed between settings, with the urban and peri-urban regions relying on health centres. It has been evidenced elsewhere that community healthcare facilities have limited microbiological support and knowledge of national action plans on IPC practices, and this is likely replicated in our setting (308). Therefore, there may be intrafacility or interfacility variations in the class or timing of antibiotic use leading to regional differences in antibiotic prescribing practices. As stated previously, however, this study constituted of primarily healthy participants, and as such there was low accounts of antibiotic usage and homogeneity of antibiotic classes prescribed, so further community-based health centre research will be needed to identify the role of physical and structural factors in these settings.

Animal co-habitation has previously been highlighted as an important factor in the acquisition, maintenance and transmission of ESBL-producing bacteria, often through contamination of the shared environment (69,307,309). Research undertaken in HIC has identified similar strains of ESBL *E. coli* or *K. pneumoniae* in pets and owners from the same household, inclusive of clonal lineages associated with infections (UTIs) in both humans and animals (307,310–313), with evidence in LMICs for shared clonal lineages and sequence types of ESBL-producing bacteria between the gut of subsistence farmers and their animals used in small-scale farming practices, such as chickens, goats and cattle (122,314). This is most critical in chickens, as the practice of household-level poultry farming is increasingly being reported in Malawi and other LMICs and is associated with high rates of AMR including ESBL *E. coli* (28–30). In terms of animal co-habitation, animal ownership was found to be commonplace across the study, with 58.7% of households owning an animal, highest in the rural site. The species present at households varied by setting, with larger livestock animals that required additional land (cattle, pigs, goats) more frequently seen in the rural settings, and domestic animals (cats, dogs) seen in the urban and peri-urban setting. Poultry was the most owned animal by households in all regions, present at 38.7% of the total households. Given the co-habitation rates of poultry, companion or livestock animals with humans in Malawian households, the role of animals should be further investigated to determine whether shared lineages exist between animals and household members in our setting, with a focus on whether there are any species specific, regional or husbandry associated factors.

Specific animal husbandry practices could play a role in ESBL transmission including the proximity and location of animal co-habitation, alongside household attitudes to animal waste management aimed at controlling contamination of the shared environment. Within the conceptual framework of ESBL transmission and acquisition the shared environment is important, and in this study poultry was the most commonly owned animal, and frequently kept inside urban households (36,315–318). Therefore, if waste management practices of these animals are inadequate or rarely employed this may drive the maintenance and transmission for ESBL-producing bacteria within the urban setting. Equally, the same applies to examining the role of livestock animal co-habitation at rural or peri-urban households.

ABU amongst animals is increasing globally, particularly within the livestock sector, and this has been highlighted by the many authorities as an important driver of ESBL-resistance in LMIC settings, that will require adaptive local solutions rather than universal standardisation (256,319). There is limited information available on antibiotic use in animals within our setting and low levels of ABU exposure in animals were identified within the cohort. However, ABU information was only recovered within the preceding 2 months, and animal sickness was commonplace over the preceding year, particularly in poultry. As stated previously, given the role of distant antibiotic use on ESBL colonisation, animals that were unwell over the preceding 12 months may have been exposed to antibiotics not captured in the questionnaires, and this could underrepresent the role of animal ABU in the dataset.

The choice of whether an animal receives an antibiotic, or the selection of which antibiotic is used are governed by human factors such as the attitudes to combating animal sickness, alongside the local availability, access and cost of medications and animal healthcare services. Within the households, if animals became unwell, often nothing would be done, instead preventative measures were preferentially employed to reduce the chance of animal illness. What was evident however, is where treatment was given, advice was rarely sought from veterinarians beforehand, and households would either purchase medication from a local drug store or use traditional remedies. It therefore will be important to better understand the attitudes to animal health and ABU through in-depth ethnographic studies in our settings.

Given the absence of ABU amongst animals in this study, I cannot ascribe a clear determination as to the antibiotics of first choice for animals at urban, peri-urban or rural households, nor can I state that recent ABU is likely to be a driver of local animal ESBL-colonisation, should ESBL-producing bacteria be identified. This last point is key, because if animals are found to have high levels of ESBL-colonisation it would indicate that this has been selected for, or acquired by a different means, such

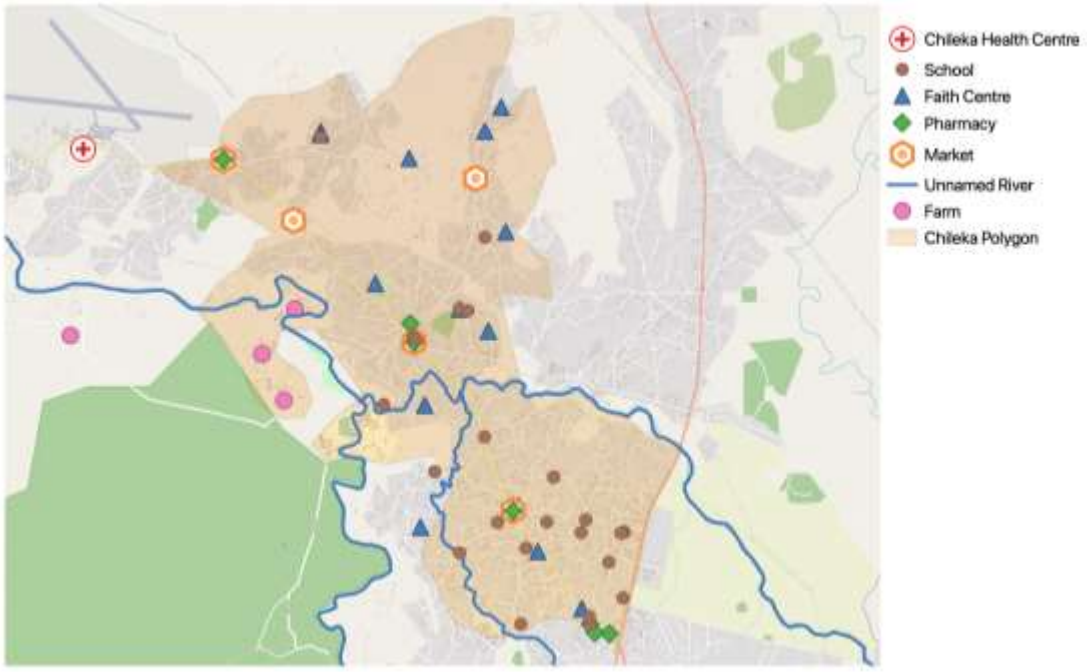
as interaction with a contaminated environment; driven by the absence of adequate hygiene and sanitation practices. Human and animal ABU may not be the leading driver of ESBL acquisition or maintenance in this community study, and the role of animal cohabitation and WASH factors are potentially one of the biggest features that differ by setting. If we are to develop putative intervention strategies, a One-Health approach should be considered with takes into consideration the role of animals and their shared environment. Therefore, in forthcoming chapters, I will identify the baseline WASH infrastructure and practices at households, including an exploration of regional differences.

3.12. Appendices

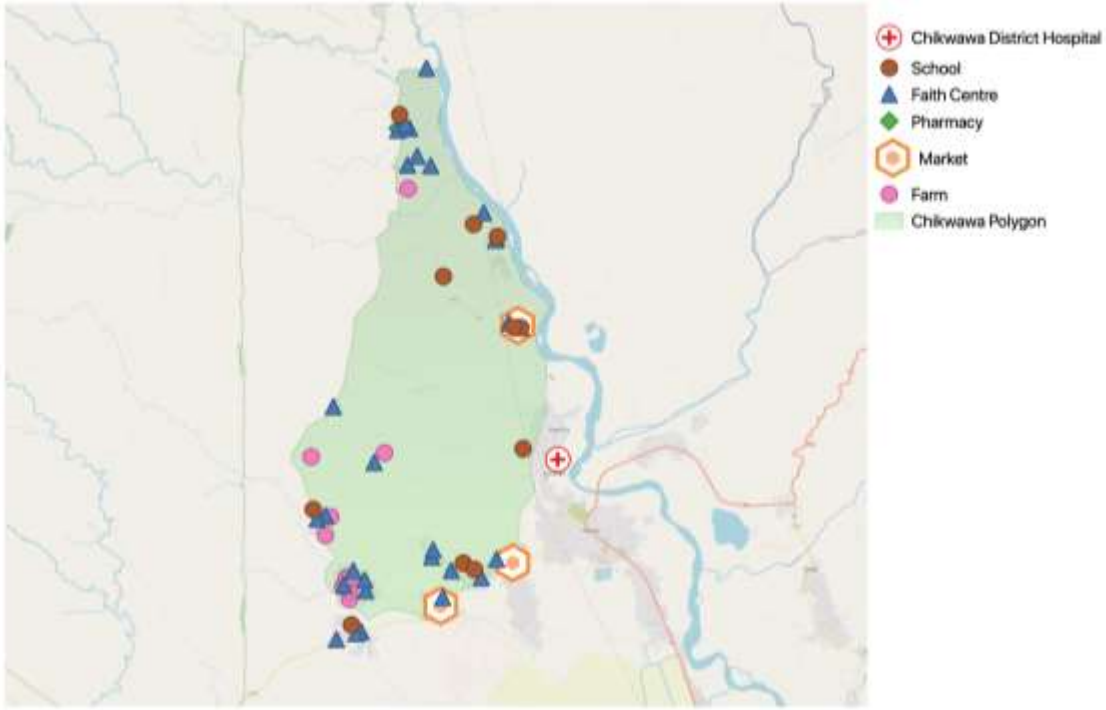
Appendix 3.i. Maps of the polygon surveys (May 2018) for (a) Ndirande, (b) Chileka and (c) Chikwawa. Images generated with QGIS (V3.22.5) software.

a) Ndirande

b) Chileka



c) Chikwawa



Chapter 4:

Comparison of Water, Sanitation and Hygiene infrastructure and practices between urban, peri-urban and rural households in Malawi.

4.0. Chapter summary

There is prior knowledge of household WASH infrastructure and human behaviours impacting on the likelihood of faecal contamination and subsequent acquisition of pathogens including protozoa and bacteria (209,320). However, detailed risk profiling on the contamination and acquisition of Enterobacteriaceae, in particular ESBL-producing Enterobacteriaceae, is often lacking, especially within LMICs (198). I hypothesise that the transmission of antimicrobial resistant AMR enteric bacteria in Malawi is dependent on numerous factors, including exposure to faecal waste, and that these factors are contextualised by key regional differences. Therefore, in Chapter 4, I have made a detailed comparison between WASH infrastructure and practices in urban, peri-urban and rural households in Malawi, and evaluated the prevalence of key WASH factors that may influence a household's ability to limit its exposure to faecal contamination both directly and from the environment. I have subsequently quantified the behavioural practices and attitudes to water storage, toileting, handwashing, food-hygiene and waste management at households, which may impact upon the risk of faecal-oral acquisition of Enterobacteriaceae leading to increased transmission of ESBL-producing bacteria. I have also compared differences in prevalences between settings and evaluated regional variances in human and animal interactions with the broader environment to describe how these interfaces may contribute to the ecological niches of AMR.

Overall, I found a paucity of household WASH infrastructure and access to materials that would enable safe toileting, adequate sanitation, effectual hand-hygiene or waste management across all sites. This was paralleled by behavioural factors that may increase the risk of bacterial transmission, such as household attitudes to water usage, food-hygiene, open defaecation, and handwashing. Finally, interactions were identified between household participants and key environmental sites likely to be contaminated with faecal material of human or animal origin, particularly within the urban setting. The frequency and nature of these interactions may be contributors to the acquisition, maintenance and transmission of ESBL bacteria in humans and animals within our setting.

My contributions to this chapter and those of others are included in Table 4.0.

Table 4.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted and analysed by the PhD candidate.
Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborators, Tracy Morse and Kondwani Chidziwisano. Statistical advice was sought from Chris Jewell. Data collection was aided by study staff, including: • Witness Mtambo, Gladys Namancha, Suzgo Mkandawire, Steria Chisesele, Dyson Rashid, Odetta Duwa, Lughano Ghambi, Chiyembekeso Palije, Fletcher Nangupeta and Taonga Mphasa

4.1 Regional household descriptions

All 300 households recruited into the study had baseline WASH infrastructure, food-hygiene and sanitation data obtained through a household enrolment CRF. WASH checklists which included a mixture of questions and observational data on toileting, handwashing, and environmental interactions were completed at baseline for 299 households (n=100 urban, n=100 peri-urban, n=99 rural) and at follow-up for pre-selected households. As stated in Chapter 3, COVID-associated interruptions reduced the number of households with longitudinal follow-up, and here I present the results of WASH data for 814 visits (n=263 urban, n=265 peri-urban, n=286 rural) at 300 households.

4.2. Household construction

The majority of houses in the study were constructed with baked bricks (n=224, 74.7%), had metal roofs (n=269, 89.7%) and cement flooring (n=189, 63.0%). Regional differences were seen in household construction, with unbaked bricks and metal roofs found in urban settings, and baked

bricks and thatched roofs seen in the rural setting (**Table 4.1**). Rural households were also more likely to have a floor made from soil or sand.

Table 4.1. Household construction in urban, peri-urban and rural sites.

Household characteristic	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Household wall construction					
Unbaked Bricks	n=67 (22.3%)	n=39 (39.0%)	n=23 (23.0%)	n=5 (5.0%)	***<.001***
Baked Bricks	n=224 (74.7%)	n=58 (58.0%)	n=75 (75.0%)	n=91 (91.0%)	***<.001***
Cement / Concrete / Other	n=6 (2.0%)	n=0 (0.0%)	n=2 (2.0%)	n=4 (4.0%)	*.172*
Other	n=3 (1.0%)	n=3 (3.0%)	n=0 (0.0%)	n=0 (0.0%)	*.109*
Household roof construction					
Metal	n=269 (89.7%)	n=99 (99.0%)	n=96 (96.0%)	n=74 (73.0%)	***<.001***
Thatch	n=30 (10.0%)	n=1 (1.0%)	n=4 (4.0%)	n=25 (25.0%)	***<.001***
Other	n=2 (0.7%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (2.0%)	*.331*
Household floor construction					
Cement	n=189 (63.0%)	n=85 (85.0%)	n=71 (70.0%)	n=33 (31.0%)	***<.001***
Tile	n=6 (2.0%)	n=4 (4.0%)	n=2 (2.0%)	n=0 (0.0%)	*.172*
Sand/soil	n=105 (35.0%)	n=11 (11.0%)	n=27 (28.0%)	n=67 (67.0%)	***<.001***

**99 households WASH baseline data in Chikwawa. p values obtained through fisher's exact test*

4.3. Water usage

The primary source for drinking water at households was from communal distribution points such as tube well / boreholes (48.7%, n=153) and water kiosks (25.2%, n=79), or from piped water, either inside (7.6%, n=24) or outside (16.9%, n=53) the household compound (**Table 4.2 & Figure 4.1**). The use of unprotected wells (0.6%, n=2) or surface waters such as rivers or ponds (0.3%, n=1) was reported in a low number of households (**Table 4.2**). There were regional differences in the primary water source used, with boreholes frequented by rural (84.3%, n=86) or peri-urban households (56.1%, n=60) and municipal kiosks utilized by urban households (61.0%, n=64). Water piped directly into the household compound was predominately seen at the urban sites, with 30.5% (n=32) of urban households and 29.9% (n=32) of peri-urban households receiving water supplied by Blantyre water board, versus 12.8% (n=13) of rural households supplied by Southern Region Water Board (SRWB).

Table 4.2. Drinking water sources utilised in urban, peri-urban and rural households.

Water source	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural***	***p***
Drinking water source^					
Bottled	n=1 (0.3%)	n=0 (0.0%)	n=1 (0.9%)	n=0 (0.0%)	*1.00*
Piped into dwelling	n=24 (7.6%)	n=10 (9.5%)	n=12 (11.2%)	n=2 (2.0%)	***.015***
Piped outside dwelling	n=53 (16.9%)	n=22 (21.0%)	n=20 (18.7%)	n=11 (10.8%)	***.036***
Public tap/ standpipe	n=79 (25.2%)	n=64 (61.0%)	n=12 (11.2%)	n=3 (2.9%)	***<.001***
Tube well/ Borehole	n=153 (48.7%)	n=7 (6.7%)	n=60 (56.1%)	n=86 (84.3%)	***<.001***
Tube well with powered pump	n=1 (0.3%)	n=0 (0.0%)	n=1 (0.9%)	n=0 (0.0%)	*1.00*
Unprotected well /spring	n=2 (0.6%)	n=1 (1.0%)	n=1 (0.9%)	n=0 (0.0%)	*1.00*
Surface water from river, lake or pond	n=1 (0.3%)	n=1 (1.0%)	n=0 (0.0%)	n=0 (0.0%)	*1.00*

**n=99/100 households had baseline WASH CRF completed in Chikwawa (rural). All 300 households completed a baseline household enrolment CRF. p values obtained through fisher's exact test*

Figure 4.1. Pictures of water sources used by households. a) water kiosk, b) household tap [in yard], c) public unprotected well, d) borehole, e) line of people waiting for urban kiosk water, showing standing water in the street, f) que for peri-urban borehole water and g) public tap [locked]. *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

There is a balance between need for water (quantity and quality), its cost, and the logistical constraints of accessing it. The weight of carrying it by hand and the distance from household to water sources can be deciding factors in which water source is chosen for drinking (235). In terms of water access, 100% (n=100) of urban households and 93% (n=93) of peri-urban households could access water within 30 mins of their house (inclusive of travel time, queuing and payment if required), whereas in the rural site, 22.0% (n=22) of households took more than 30 mins to obtain water (**Table 4.3**). Drinking water was treated prior to consumption by 8.3% (n=25) of households, with chlorination being the most frequently practiced method (80.0%, n=20). Chlorination of drinking water occurred more often at the rural site; however, water treatment of any kind was uncommonly undertaken, especially at the urban and peri-urban settings. When households were asked whether they believed that water was safe to drink, irrespective of treatment, 90.3% (n=271) of the total households responded that it was, with people in the rural setting most concerned about its safety profile (86.0%, n=86).

Overall, the majority of study households have access to basic drinking water (defined as improved water source available within 30 mins), with limited drinking water (defined as improved water source that takes longer than 30 mins to collect) seen in the rural and peri-urban regions (**Figure 4.2**).

Table 4.3. Household drinking water characteristics in urban, peri-urban and rural sites.

Drinking water characteristic	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural***	***p***
Drinking water treatment	n=25 (8.3%)	n=4 (4.0%)	n=4 (4.0%)	n=17 (17.0%)	***<.001***
Method of drinking water treatment (if applicable)?					
Boiled	n=5 (20.0%)	n=3 (75.0%)	n=1 (25.0%)	n=1 (5.9%)	
Chlorination	n=20 (80.0%)	n=1 (25.0%)	n=3 (75.0%)	n=16 (94.1%)	
Households that think their water is safe to drink, irrespective of treatment?	n=271 (90.3%)	n=96 (96.0%)	n=89 (89.0%)	n=86 (86.0%)	***.039***
How long does it take the household to access drinking water?					
On premises	n=56 (18.7%)	n=20 (20.0%)	n=28 (28.0%)	n=8 (8.0%)	
Less than 30mins	n=215 (71.7%)	n=80 (80.0%)	n=65 (65.0%)	n=70 (70.0%)	
More than 30mins	n=29 (9.7%)	n=0 (0.0%)	n=7 (7.0%)	n=22 (22.0%)	
What age do children in your house start drinking water?					
Less than 3 months	n=8 (2.7%)	n= 6 (6.0%)	n=2 (2.0%)	n=0 (0.0%)	
3 – 6 months	n=62 (20.7%)	n= 35 (35.0%)	n=13 (13.0%)	n=14 (14.0%)	
over 6 months	n=230 (76.7%)	n= 59 (59.0%)	n=85 (85.0%)	n=86 (86.0%)	

**n=99/100 households had baseline WASH CRF completed in Chikwawa (rural). All 300 households completed a baseline household enrolment CRF. P values obtained through fisher's exact test*
^ Households can acquire water from a variety of water sources, and there was at n=105 (urban), n=107 (peri-urban) and n=102 (rural) responses recorded at the 299 households.
$ Households can store water in various receptacles, and there was n=193 (urban), n=137 (peri-urban) and n=118 (rural) responses recorded at the 299 households.

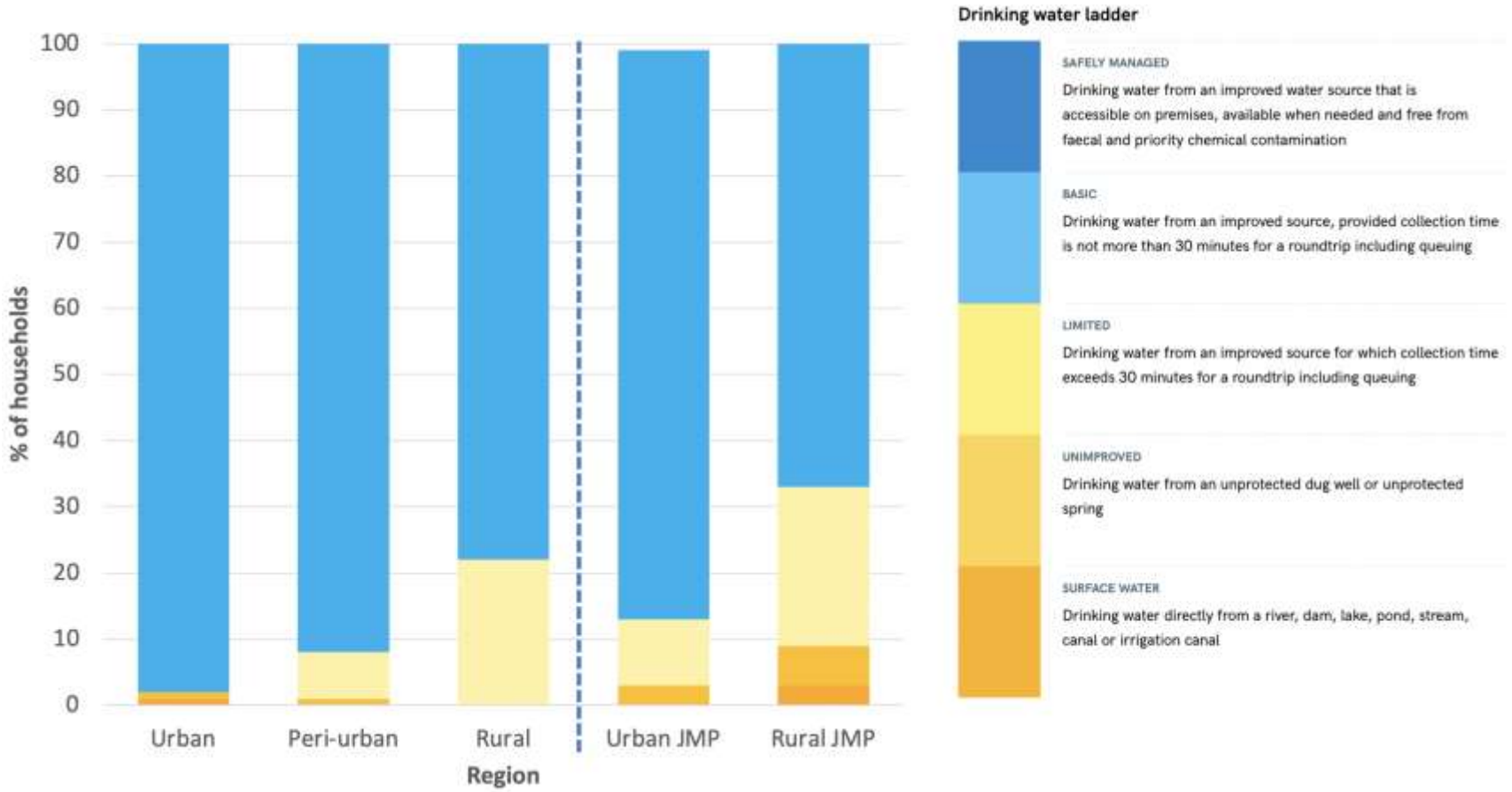


Figure 4.2. Proportion of urban, peri-urban and rural households with access to safely managed drinking water adopted form the JMP definitions, alongside 2020 JMP estimates for rural and urban Malawi (228).

4.3.1. Water storage

To combat water insecurities and reduce the need for repeated travel, households frequently store water on premises (235,239). 99.7% (n=298) of households in this study stored water inside the house and only 1 household in the peri-urban setting with piped mains water did not store any. The choice of receptacle, its placement and use of a cover impacts on the risk of bacterial contamination through animal or external environmental exposures in conjunction with inadequate hand-hygiene practices (239,321,322). Plastic buckets (with or without lids) were commonly used at all study sites, alongside (covered or uncovered) jerry cans (**Table 4.4**). 22.9% (n=27) of households in the rural setting stored their drinking water in (covered) clay pots, and there was occasional use of other receptacles such as plastic buckets with taps, uncovered metal buckets, drums or plastic bottles across the various settings (**Figure 4.3**). Drinking water was only covered 68.1% (n=305) of the time, and there were regional differences noted, with rural households more likely to protect their water than urban (62.2%, n=120) or peri-urban (62.0%, n=85) households. The reasons for household's choice of storage container, whether water was covered with a protective lid or why drinking water was not separated from other water were not explored in this study.

Table 4.4. Drinking water storage in urban, peri-urban and rural households.

Stored water characteristic	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural***	***p***
Households that store water inside the house	n=298 (99.7%)	n=100 (100.0%)	n=99 (99.0%)	n=99 (100.0%)	*1.00*
How is household drinking water stored? [$]					
Plastic bucket (no lid)	n=112 (25.0%)	n=56 (29.0%)	n=45 (32.8%)	n=11 (9.3%)	
Plastic bucket (lid)	n=217 (48.4%)	n=91 (47.2%)	n=71 (51.8%)	n=55 (46.6%)	
Plastic bucket with tap (no lid)	n=2 (0.4%)	n=1 (0.5%)	n=0 (0.0%)	n=1 (0.8%)	
Plastic bucket with tap (lid)	n=3 (0.7%)	n=1 (0.5%)	n=2 (1.4%)	n=0 (0.0%)	
Metal bucket (covered)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	
Metal bucket (uncovered)	n=3 (0.7%)	n=1 (0.5%)	n=2 (1.4%)	n=0 (0.0%)	
Clay pot (covered)	n=30 (6.7%)	n=1 (0.5%)	n=2 (1.4%)	n=27 (22.9%)	
Clay pot (uncovered)	n=1 (0.2%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (0.8%)	

Jerry can (covered)	n=33 (7.4%)	n=15 (7.8%)	n=3 (2.2%)	n=15 (12.7%)	
Jerry can (uncovered)	n=14 (3.1%)	n=6 (3.1%)	n=3 (2.2%)	n=5 (4.2%)	
Drum (covered)	n=16 (3.6%)	n=11 (5.7%)	n=2 (1.4%)	n=3 (2.5%)	
Drum (uncovered)	n=11 (2.5%)	n=9 (4.7%)	n=2 (1.4%)	n=0 (0.0%)	
Plastic bottles	n=6 (1.3%)	n=1 (0.5%)	n=5 (3.6%)	n=0 (0.0%)	
Is household drinking water covered?	n=305 (68.1%)	n=120 (62.2%)	n=85 (62.0%)	n=100 (84.7%)	***<.001***
Was visible separation seen in water used for drinking and water for household	n=225 (75.5%)	n=74 (74.0%)	n=70 (70.7%)	n=81 (81.8%)	*.072*

**n=99/100 households had baseline WASH CRF completed in Chikwawa (rural). All 300 households completed a baseline household enrolment CRF. P values obtained through fisher's exact test*
^ Households can acquire water from a variety of water sources, and there was at n=105 (urban), n=107 (peri-urban) and n=102 (rural) responses recorded at the 299 households.
$ Households can store water in various receptacles, and there was n=193 (urban), n=137 (peri-urban) and n=118 (rural) responses recorded at the 299 households.

Water is also needed for other purposes, such as cleaning or bathing, and households sometimes obtain water for these activities from a different source to their drinking water (**Figure 4.3**). In this study, 17.3% (n=52) of households used a different water source for the purpose of cleaning compared to drinking, and participants in the urban setting (29.0%, n=29) were more likely to choose an alternative water source than those in peri-urban or rural households (**Table 4.5**). In households where a different water source was used for washing items such as cooking utensils, they were less likely to use water kiosks which they had to pay for water. Instead, cheaper alternatives such as boreholes (38.5%, n=20) were used. Furthermore, there were examples of water being obtained from unprotected wells (3.8%, n=2) and surface waters (7.7%, n=4) which pose a risk of contamination with faecal material.

Figure 4.3. Pictures of household water storage and usage. a) household water stored in plastic buckets (without lids), for drinking purposes, b) household water used for cleaning of food utensils c) household water used for cleaning clothes, and d) use of river water for cleaning household clothes. *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

In relation to bathing, most households had an external (81.7%, n= 245) or internal (10.7%, n=32) bathroom. Those households that did not have a bathroom were primarily located in the rural area, and bathing options reported in these households occasionally included the use of local rivers. The water sources used in co-located bathrooms were similar to those used for cleaning water, and households relied on the use of boreholes (50.0%, n=150), or piped systems (47.3%, n=142) with the occasional use of surface water (2.3%, n=7).

Table 4.5. Water usage for bathing and cleaning in urban, peri-urban and rural households.

Alternative water usage and source	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Houses that use an alternative water source for cleaning than for drinking	n=52 (17.3%)	n=29 (29.0%)	n=12 (12.0%)	n=11 (11.9%)	***.001***
What is the water source used for cleaning cooking utensils?					
Piped into dwelling	n=5 (9.6%)	n=4 (13.8%)	n=1 (8.3%)	n=0 (0.0%)	
Piped outside dwelling	n=11 (21.2%)	n=10 (34.5%)	n=0 (0.0%)	n=1 (8.3%)	
Public tap / Standpipe	n=10 (19.2%)	n=9 (31.0%)	n=1 (8.3%)	n=0 (0.0%)	
Tube well / borehole	n=20 (38.5%)	n=4 (13.8%)	n=6 (50.0%)	n=10 (91.7%)	
Unprotected well / spring	n=2 (3.8%)	n=2 (6.9%)	n=0 (0.0%)	n=0 (0.0%)	
Surface water from river, lake or pond	n=4 (7.7%)	n=0 (0.0%)	n=4 (33.3%)	n=0 (0.0%)	
What is the water source used for bathing?					
Piped into dwelling	n=25 (8.3%)	n=12 (12.0%)	n=11 (11.0%)	n=2 (2.0%)	
Piped outside dwelling	n=54 (18.0%)	n=27 (27.0%)	n=17 (17.0%)	n=10 (10.0%)	
Public tap / Standpipe	n=63 (21.0%)	n=54 (54.0%)	n=6 (6.0%)	n=3 (3.0%)	
Tube well / borehole	n=150 (50.0%)	n=6 (6.0%)	n=59 (59.0%)	n=85 (85.0%)	
Unprotected well / spring	n=1 (0.3%)	n=0 (0.0%)	n=1 (1.0%)	n=0 (0.0%)	
Surface water from river, lake or pond	n=7 (2.3%)	n=1 (1.0%)	n=6 (6.0%)	n=0 (0.0%)	
Where do household members' bath?					
Bathing room (exterior)	n=245 (81.7%)	n=90 (90.0%)	n=76 (76.0%)	n=79 (79.2%)	
Bathing room (interior)	n=32 (10.7%)	n=8 (8.0%)	n=21 (21.0%)	n=3 (3.0%)	
No bathroom	n=21 (7.0%)	n=2 (2.0%)	n=3 (3.0%)	n=16 (15.8%)	
River or outside the house	n=2 (0.7%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (2.0%)	

p values obtained through fisher's exact test

4.4. Sanitation and waste management practices

Overall, there was a lack of access to key sanitation infrastructure and absence of effective waste management practices at households. Most notably these included the sharing of household toilets or the practice of open defecation, and inappropriate disposal of human and animal waste.

89.0% (n=267) of households in the study had a toilet present at the household or within the household compound; 88.8% (n=237) of which were pit latrines (**Table 4.6**). Toilets were more frequently seen at urban (n=95, 95.0%) or peri-urban (n=97, 97.0%) households compared to those at the rural setting (n=75, 75.8%), and the use of flush toilets, whether septic tank or mains integrated were only seen at the urban settings. Toilet construction broadly paralleled household construction with walls made from baked bricks and metal roofs, however there was often a soil floor and no roof found at peri-urban or rural toilets (**Table 4.6. & Figure 4.4**). Drophole covers which are employed to reduce flies and prevent disease transmission were present at 34.5% (n=92) of toilets and were more often found at rural toilets (48.0%, n=36) compared to those at peri-urban (35.4%, n=35) or urban (22.1%, n=21) households.

Table 4.6. Toilet presence and construction in urban, peri-urban and rural sites.

Toilet characteristic	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural***	***p***
Toilet present at household	n=267 (89.0%)	n=95 (95.0%)	n=97 (97.0%)	n=75 (75.8%)	***<.001***
Toilet type used by household					
Flush/pour flush toilet to mains	n=7 (2.6%)	n=1 (1.1%)	n=6 (6.2%)	n=0 (0.0%)	***.030***
Flush/pour flush toilet to septic tank	n=15 (5.6%)	n=4 (4.2%)	n=9 (9.3%)	n=0 (0.0%)	***.013***
Pit latrine	n=237 (88.8%)	n=90 (94.7%)	n=73 (75.3%)	n=74 (98.7%)	***<.001***
Shared toilet (as stated by household)	n=10 (3.7%)	n=0 (0.0%)	n=9 (9.3%)	n=1 (1.3%)	***<.001***
Toilet wall construction					
Concrete	n=1 (0.4%)	n=0 (0.0%)	n=1 (1.0%)	n=0 (0.0%)	
Baked bricks	n=172 (64.4%)	n=64 (67.4%)	n=55 (56.7%)	n=53 (70.7%)	
Unbaked bricks	n=74 (27.7%)	n=21 (22.1%)	n=33 (34.0%)	n=20 (26.7%)	
Metal sheets	n=6 (2.2%)	n=5 (5.3%)	n=0 (0.0%)	n=1 (1.3%)	
Plastic / maize sheets	n=9 (3.4%)	n=4 (4.2%)	n=5 (5.2%)	n=0 (0.0%)	
Reed mats	n=3 (1.1%)	n=1 (1.1%)	n=1 (1.0%)	n=1 (1.3%)	

No wall	n=2 (0.7%)	n=0 (0.0%)	n=2 (2.1%)	n=0 (0.0%)
Toilet Floor construction				
Concrete	n=114 (42.7%)	n=67 (70.5%)	n=40 (41.2%)	n=7 (9.3%)
Wood/tile	n=10 (3.7%)	n=6 (6.3%)	n=3 (3.1%)	n=1 (1.3%)
Soil	n=143 (53.6%)	n=22 (23.2%)	n=54 (55.7%)	n=67 (89.3%)
Toilet roof construction				
Metal sheets	n=130 (48.7%)	n=69 (72.6%)	n=48 (49.5%)	n=13 (17.3%)
Plastic sheets	n=12 (4.5%)	n=5 (5.3%)	n=2 (2.1%)	n=4 (5.3%)
Thatched	n=61 (22.8%)	n=5 (5.3%)	n=18 (18.6%)	n=38 (50.7%)
No roof	n=65 (24.3%)	n=16 (16.8%)	n=29 (29.9%)	n=20 (26.7%)

**n=99/100 households had baseline WASH CRF completed in Chikwawa (rural). All 300 households completed a baseline household enrolment CRF. p values obtained through fisher's exact test*

Figure 4.4. Pictures of typical pit latrines used by households. a) external structure of pit latrine in the [urban], b) internal structure of pit latrine [urban], without drophole cover in place, c) external structure of pit latrine [peri-urban] and d) internal structure of pit latrine [peri-urban], without drophole cover in place. *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

Toilets can be communal, and 41.9% (n=112) of total households reported sharing toileting facilities with other households within the compound or wider community (**Table 4.7**). Given the nature of high-density housing this practice was more often identified at the urban site, with 62.1% (n=59) of urban households sharing their toilet with a median of 3 (IQR=2-5) other households. Public toilets were not commonly in close proximity to households in this study but were more frequently available at the urban setting. 37.1% of households who had access to a public toilet nearby would consider using them for daily toileting activities. However, when participants were away from their house and needed to urinate/defecate they would invariably ask to use another household toilet nearby (52.0%, n=156) or wait until they returned home (27.3%, n=82) rather than use a public toilet or openly

defaecate. The examples where open defaecation in public were reported was limited to the rural setting and qualified by household members as when they were completing agricultural work in the fields and far away from the village.

Table 4.7. Household toileting practices at urban, peri-urban and rural sites

Toilet sharing practices	Region: n (%) unless otherwise indicated				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Households who share their toilet with non-household members?	n=112 (41.9%)	n=59 (62.1%)	n=30 (30.9%)	n=23 (30.7%)	***<.001***
Number of households the toilet is shared with?	median=3 (IQR=2-4)	median=3 (IQR=2-5)	median=2 (IQR=2-3)	median=2 (IQR=1-2)	
Public toilet available near house	n=35 (11.7%)	n=24 (24.0%)	n=7 (7.0%)	n=4 (4.0%)	
Public toilet ever used by households	n=13 (37.1%)	n=8 (33.3%)	n=3 (42.9%)	n=2 (50.0%)	
If away from home, where would household's toilet?					
Would wait until home	n=82 (27.3%)	n=44 (44.0%)	n=31 (31.0%)	n=7 (7.0%)	
Use public toilet	n=42 (14.0%)	n=13 (13.0%)	n=20 (20.0%)	n=9 (9.0%)	
Urinate / defecate in the open	n=20 (6.7%)	n=0 (0.0%)	n=0 (0.0%)	n=20 (20.0%)	
Borrow a household toilet nearby	n=156 (52.0%)	n=43 (43.0%)	n=49 (49.0%)	n=64 (64.0%)	
How do households dispose of rubbish[$]					
In a household bin collected from residence	n=6 (1.9%)	n=5 (4.9%)	n=1 (1.0%)	n=0 (0.0%)	
Deposited in communal bins and collected	n=18 (5.8%)	n=15 (14.6%)	n=2 (1.9%)	n=1 (1.0%)	
Placed in a rubbish pit next to house	n=63 (20.5%)	n=14 (13.6%)	n=21 (20.4%)	n=28 (27.5%)	
Placed in a communal rubbish pit	n=31 (10.1%)	n=16 (15.5%)	n=10 (9.7%)	n=5 (4.9%)	
Burned	n=2 (0.6%)	n=0 (0.0%)	n=2 (1.9%)	n=0 (0.0%)	
Thrown in a drain/open area	n=188 (61.0%)	n=53 (51.5%)	n=67 (65.0%)	n=68 (66.7%)	

$ Multiple ways of disposing of rubbish. Urban=103, peri-urban=103 and rural=102. P values obtained through fisher's exact test

In terms of toileting behaviours and human waste management at home, despite the availability of a toilet on site, 28.7% (n=86) of households reported open defaecation being practiced by one of more household members at some point each month, with no regional differences seen (**Table 4.8**). There were also examples of peri-urban and rural households that used human waste as "night soil" or crop fertiliser. When the study team looked for human faeces in the house or external compound they

were present 8.1% (n=66) of the time. Human faecal contamination of the household environment was identified more often at rural households than at urban settings.

Table 4.8. Household human waste management practices at urban, peri-urban and rural sites

Human waste management practice	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Do households have children who wear nappies/cloths?	n=83 (24.0%)	n=34 (41.0%)	n=27 (32.5%)	n=22 (26.5%)	
Where do households dispose of the cloths/nappies?					
Disposable nappies (collected)	n=7 (8.4%)	n=6 (17.6%)	n=1 (3.7%)	n=0 (0.0%)	
Disposable nappies (burned)	n=5 (6.0%)	n=2 (5.9%)	n=3 (11.1%)	n=0 (0.0%)	
Disposable nappies (thrown in pit or elsewhere)	n=6 (7.2%)	n=2 (5.9%)	n=2 (7.4%)	n=2 (9.1%)	
Washable nappies/cloth (faeces in toilet)	n=39 (47.0%)	n=17 (50.0%)	n=13 (48.1%)	n=9 (41.0%)	
Washable nappies/cloth (faeces washed off in bucket)	n=25 (30.1%)	n=7 (20.6%)	n=8 (29.6%)	n=10 (45.5%)	
Washable nappies/cloth (faeces washed in river)	n=1 (1.2%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (4.5%)	
Households where ≥1 member practices open defecation?	N=86 (28.7%)	n=25 (25.0%)	n=28 (28.0%)	n=33 (33.0%)	*.485*
Do households ever use human manure to fertilise their own crops?	N=8 (2.7%)	n=0 (0.0%)	n=6 (6.0%)	n=2 (2.0%)	***.031***
Human faeces present in / around the household compound? ^	n=66 (8.1%)	n=18 (6.8%)	n=6 (2.3%)	n=42 (14.7%)	***<.001***

^ Observational WASH data from a total of 814 longitudinal visits at 300 households. p values obtained through fisher's exact test

I collected observational data from household toilets including the presence of anal cleansing materials, flies, use of drophole covers and visible faecal contamination. At these household visits, anal cleansing materials were identified at 18.9% (n=133) of toilets, and these were more frequently seen at urban (10.7%, n=26) and peri-urban (33.4%, n=88) households compared to those in the rural (9.3%, n=19) setting. Paper, whether toilet paper or newspaper, was the only cleansing material found. As stated previously, drophole covers were present at 34.5% of households, but they were only observed to be in place at 73% (n=92) of toilet visits, with the rural region having the least chance of seeing correct placement (68.3%, n=71).

Flies were visible at 61.5% (n=432) of toilets and there was a strong or unbearable smell noted by field staff at 24.2% (n=170) of toilet visits (**Table 4.9**). When the study team looked for the presence of

faecal contamination on the floor or the walls, this was identified at 8.7% (n=62) of total visits; proportionately more at the urban toilets (18.9%, n=46) than at the other sites.

Table 4.9. Observed toileting materials and toilet hygiene at urban, peri-urban and rural households.

Toileting characteristic	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Signs of faecal contamination on the toilet walls or floor? ^	n=62 (8.8%)	n=46 (18.9%)	n=14 (5.5%)	n=2 (1.0%)	***<.001***
Anal cleansing materials present at toilet ^	n=133 (18.9%)	n=26 (10.7%)	n=88 (33.4%)	n=19 (9.3%)	***<.001***
Which cleansing materials present? ^					
Toilet paper	n=71 (53.4%)	n=12 (66.2%)	n=57 (64.8%)	n=2 (10.5%)	
Newspaper / other paper	n=34 (46.6%)	n=14 (53.8%)	n=31 (35.2%)	n=17 (89.5%)	
Flies visible in the toilet^	n=432 (61.5%)	n=160 (65.8%)	n=155 (60.5%)	n=117 (57.4%)	
Drophole cover present at toilet	n=92 (34.5%)	n=21 (22.1%)	n=35 (35.4%)	n=36 (48.0%)	***.002***
Drophole cover in place (where available) ^	n=184 (73.0%)	n=44/48 (91.7%)	n=69/100 (69.0%)	n=71/104 (68.3%)	***.003***
Toilet smell^					
No smell	n=110 (15.6%)	n=36 (14.8%)	n=42 (16.4%)	n=32 (15.7%)	
Slight smell	n=423 (60.2%)	n=166 (68.3%)	n=137 (53.5%)	n=120 (58.8%)	
Strong smell	n=166 (23.6%)	n=40 (16.5%)	n=77 (30.1%)	n=49 (24.0%)	
Unbearable smell	n=4 (0.6%)	n=1 (0.4%)	n=0 (0.0%)	n=3 (1.5%)	

^ observational WASH data from a total of 814 longitudinal visits at 300 households. A total of 703 visits where toilets are present: n=243 (urban), n=256 (peri-urban) and n=204 (rural) and 252 visits where toilet drophole covers are present n=48 (urban), n=100 (peri-urban) and n=104 (rural). P values obtained through fisher's exact test

Household rubbish was typically thrown into an open drain or placed into a rubbish pit near the house, with household collection or disposal in a collection bin only present at urban settings and infrequently utilised **(Table 4.10 & Figure 4.5)**. With regard to management of child faecal waste, 24% (n=72) of households used nappies, and these were most frequently washable nappies (for multiple uses) rather than disposal versions for single use. 47.0% (n=39) of households surveyed reported washing the nappies and disposing of the faeces in the toilet, with 30.1% (n=25) of households choosing to wash the nappies and dispose of the faeces in a bucket, and 1.2% (n=1) washing them in river water **(Table 4.8**). The rest of the nappies were single use, and either discarded into rubbish collection areas (8.4%, n=7), burned (6.0%, n=5) or thrown directly into the pit/toilet (7.2%, n=6).

Figure 4.5. Pictures of household waste management. a) sweeping away rubbish from household entrance, b) use of a pit next to the house for burning rubbish c) throwing rubbish into communal areas (peri-urban), and d) putting liquid and solid waste into the local rivers (urban). *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

Table 4.10. Household animal waste management practices at urban, peri-urban and rural sites

Waste management practices	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Households that have an adequate system to manage their animal waste?	n=13 (4.3%)	n=1 (1.0%)	n=8 (8.0%)	n=4 (4.0%)	*.055*
What would households do with animal waste?					
Nothing	n=181 (60.3%)	n=92 (92.0%)	n=50 (50.0%)	n=39 (39.0%)	
Use it as manure (by self or sold)	n=69 (23.0%)	n=2 (2.0%)	n=32 (32.0%)	n=35 (35.0%)	
Put it in the refuse pit	n=13 (4.3%)	n=1 (1.0%)	n=8 (8.0%)	n=4 (4.0%)	
Sweep it into the bush	n=37 (12.3%)	n=5 (5.0%)	n=10 (10.0%)	n=22 (22.0%)	
Do households use animal manure to fertilise their own crops?	N=82 (27.3%)	n=5 (5.0%)	n=36 (36.0%)	n=41 (41.0%)	***<.001***
Animal faeces present in / around the household compound? ^	n=433 (53.2%)	n=65 (24.7%)	n=135 (50.9%)	n=233 (81.5%)	***<.001***
Are animals near the household or inside the household complex? ^	n=372 (45.7%)	n=58 (22.1%)	n=150 (56.6%)	n=164 (57.3%)	***<.001***

^ Observational WASH data from a total of 814 longitudinal visits at 300 households. p values obtained through fisher's exact test

We classified animal waste (both domestic and companion) practices into common modalities and defined adequate waste management as its removal from the premises, and subsequent contained disposal away from human contact. By this standard, only 4.3% (n=13) of households reported an adequate system to manage animal waste, with 60.3% (n=181) of households doing nothing at all with animal faeces found on site; instead leaving them in situ. Alternativity households would manage animal waste by sweeping it into nearby bushes (12.3%, n=37, **Figure 4.5**) or selling/using as manure for crops (23.0%, n=69). In general, animal manure usage was low in the urban setting (2.0%, n=2), but higher in the peri-urban (36.0%, n=36) and rural (41.0%, n=41) settings, at households that grew their own produce.

When looking for the presence of animal faeces in or around the household compound, they were seen at a very high number of households (**Table 4.10**). In total, animal faeces were seen at 53.2% (n=433) of visits, with those in the peri-urban (50.9%, n=139) and rural (81.5%, n=233) settings having the highest environmental animal faecal-contamination. This tallied with the presence of animals seen in and around the households, with rural and peri-urban households having animals seen in the household complex more often than in the urban setting (**Table 4.10**).

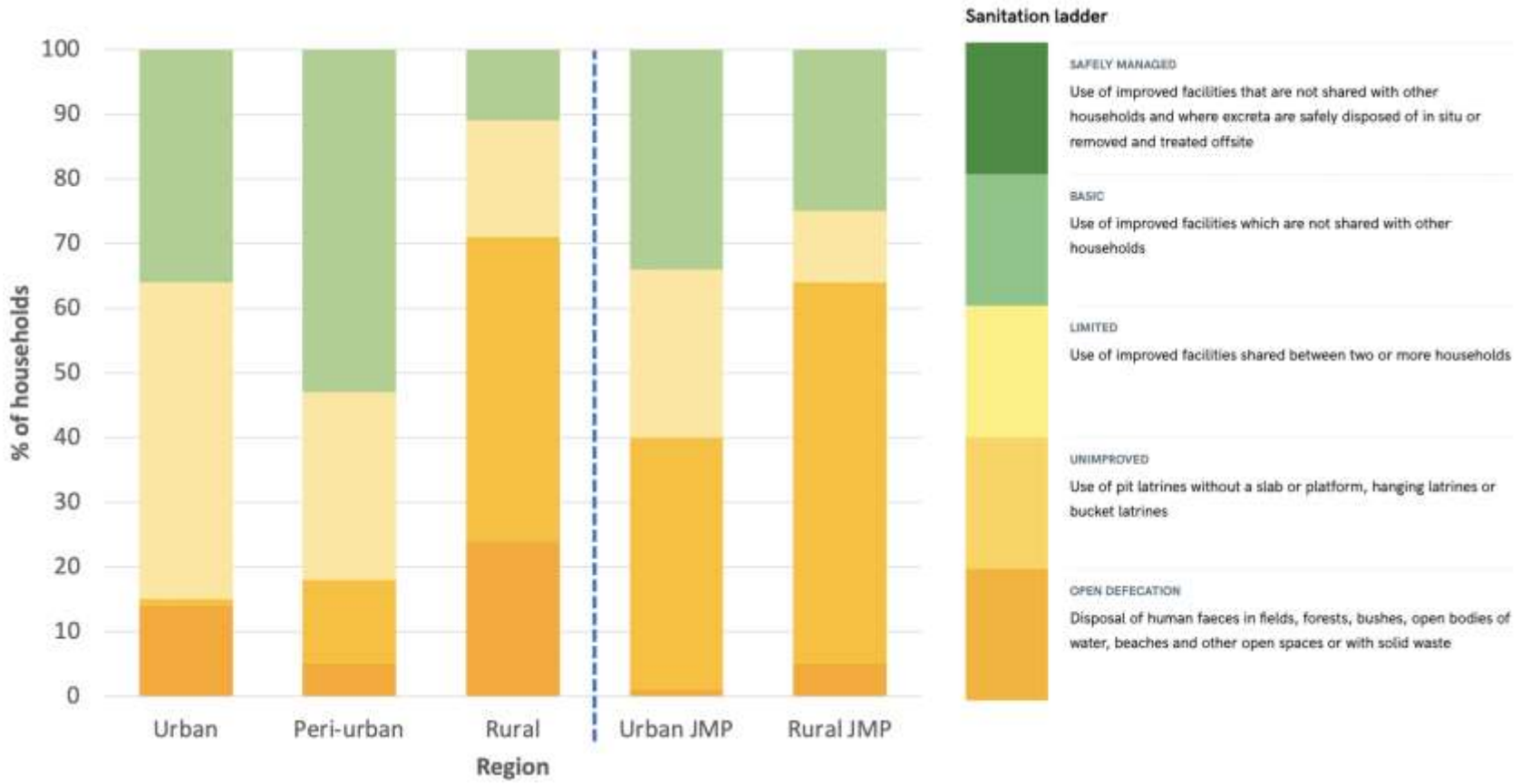


Figure 4.6. Proportion of urban, peri-urban and rural households with access to safe sanitation adopted form the JMP definitions, alongside historical 2020 JMP estimates for rural and urban Malawi (228).

4.5. Household access to handwashing facilities and attitudes to hand-hygiene practices

Access to handwashing facilities (HWFs) and adjunctive cleansing materials at key household locations was captured, illustrating deficits in infrastructure and availability of cleansing materials in all regions (**Table 4.11**). At baseline, HWFs were present at 41.0% (n=123) of total households, with the peri-urban site having ≥1 HWF at 63.0% (n=63) of recruited households. HWFs were present at low numbers per house, with toilets having dedicated HWFs at 12.4% (n=37) of households and food preparation areas having dedicated HWFs at 7.4% (n=22) of households. Instead, there was often a preference for generic HWFs either inside the house (15.4%, n=46) or in the yard (16.1%, n=48). Regionally, peri-urban households had the highest level of HWF access, with a notable exception of rural households' access to toilet HWFs (**Table 4.11**).

Water was present and visibly clear at most household HWFs, but soap was only identified at between 43.6-67.5% of HWFs, depending on their location and region. Soap and water were more frequently available at peri-urban households than in the other settings, and the lowest availability of soap was seen at the rural site.

Table 4.11. Hand washing facilities at urban, peri-urban and rural households.

Hand washing facility characteristic	n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Facilities for hand washing available at households (any)*	n=123 (41.0%)	n=37 (37.0%)	n=63 (63.0%)	n=23 (23.2%)	***<.001***
Specific hand washing facilities available					
Facilities for hand washing available at toilet	n=37 (12.4%)	n=6 (6.0%)	n=15 (15.0%)	n=16 (16.2%)	*.050*
Facilities for hand washing available at kitchen/food preparation area	n=22 (7.4%)	n=5 (5.0%)	n=14 (14.0%)	n=3 (3.0%)	***.009***
Facilities for hand washing available at household yard	n=48 (16.1%)	n=19 (19.0%)	n=25 (25.0%)	n=4 (4.0%)	***<.001***
Facilities for hand washing available inside the house	n=46 (15.4%)	n=14 (14.0%)	n=30 (30.0%)	n=2 (2.0%)	***<.001***
Soap (liquid/bar/powder) present at HWFs^	n=166 (49.0%)	n=28 (43.1%)	n=130 (58.8%)	n=8 (15.1%)	***<.001***
At baseline visit is soap (liquid/bar/powder) present at the HWF?					
Toilet	n=14 (50.0%)	n=3 (57.1%)	n=11 (72.2%)	n=0 (0.0%)	
Kitchen/food preparation area	n=13 (61.9%)	n=2 (33.3%)	n=10 (84.6%)	n=1 (33.3%)	
Household yard	n=17 (43.6%)	n=5 (43.7%)	n=11 (67.6%)	n=1 (25.0%)	
Inside house	n=27 (67.5%)	n=11 (86.7%)	n=16 (60.9%)	n=0 (0.0%)	

Water present at HWFs^	n=349 (85.5%)	n=65 (71.4%)	n=231 (94.3%)	n=53 (73.6%)	***<.001***
At baseline visit is water present at the HWF?					
Toilet	n=29 (78.4%)	n=5 (87.5%)	n=14 (90.0%)	n=10 (62.5%)	
Kitchen/food preparation area	n=21 (95.5%)	n=5 (100.0%)	n=13 (100.0%)	n=3 (100.0%)	
Household yard	n=39 (81.3%)	n=11 (66.7%)	n=24 (97.1%)	n=4 (100.0%)	
Inside house	n=40 (87.0%)	n=12 (78.9%)	n=28 (95.8%)	n=0/2 (0.0%)	
Is the water visibly dirty at the HWF?					
Toilet	n=2 (6.9%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (20.0%)	
Kitchen/food preparation area	n=1 (4.8%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (33.3%)	
Household yard	n=5 (12.8%)	n=1 (9.1%)	n=3 (12.5%)	n=1 (25.0%)	
Inside house	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	NA	

** Combination of 1 or more HWFs seen at a household. p values obtained through fisher's exact test*
^ 339 HWF visits where soap was documented as present/absent (urban = 65, peri-urban = 221, rural = 53) and 408 HWFs where water was documented as present/absent (urban=91, peri-urban=245, rural=72)

The type of HWF utilised varied by region and household location (**Table 4.12**). Buckets were the most common HWFs, identified at 53.9% (n=82) of locations, and these rarely had an integrated tap. Tippy taps (**Figure 4.8c**) were used at HWFs next to rural toilets or at a selection of peri-urban household locations, and taps connected to piped water were seen at the few urban sites that had HWFs or within the peri-urban setting.

Table 4.12. Handwashing facilities available within key areas at urban, peri-urban and rural households.

Facility household location	Region	Hand Washing Facility: n (%)					
		Tippy Tap	Mug	Bucket	Bucket with tap	Jerry can	Tap with running water
Toilet	Urban	n=0 (0.0%)	n=0 (0.0%)	n=2 (33.3%)	n=0 (0.0%)	n=2 (33.3%)	n=2 (33.3%)
	Peri-urban	n=9 (60.0%)	n=0 (0.0%)	n=2 (13.3%)	n=0 (0.0%)	n=0 (0.0%)	n=4 (26.7%)
	Rural	n=14 (87.4%)	n=1 (6.3%)	n=0 (0.0%)	n=1 (6.3%)	n=0 (0.0%)	n=0 (0.0%)
Kitchen area	Urban	n=0 (0.0%)	n=1 (20.0%)	n=0 (0.0%)	n=1 (20.0%)	n=0 (0.0%)	n=3 (60.0%)
	Peri-urban	n=4 (28.6%)	n=0 (0.0%)	n=5 (35.7%)	n=0 (0.0%)	n=0 (0.0%)	n=5 (35.7%)
	Rural	n=0 (0.0%)	n=0 (0.0%)	n=2 (66.7%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (33.3%)
Yard	Urban	n=0 (0.0%)	n=0 (0.0%)	n=16 (88.9%)	n=0 (0.0%)	n=0 (0.0%)	n=2 (11.1%)
	Peri-urban	n=6 (24.0%)	n=0 (0.0%)	n=13 (52.0%)	n=0 (0.0%)	n=0 (0.0%)	n=6 (24.0%)
	Rural	n=0 (0.0%)	n=0 (0.0%)	n=3 (75.0%)	n=0 (0.0%)	n=1 (25.0%)	n=0 (0.0%)
Inside house	Urban	n=0 (0.0%)	n=0 (0.0%)	n=13 (92.9%)	n=1 (7.1%)	n=0 (0.0%)	n=0 (0.0%)
	Peri-urban	n=5 (16.7%)	n=0 (0.0%)	n=24 (80.0%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (3.3%)
	Rural	n=0 (0.0%)	n=0 (0.0%)	n=2 (100.0%)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)
All locations	**All regions**	**n=38 (25.0%)**	**n=2 (1.3%)**	**n=82 (53.9%)**	**n=3 (2.0%)**	**n=3 (2.0%)**	**n=24 (15.8%)**

Highlighted panels represent the most common selected option for urban (blue), peri-urban (grey) and rural (yellow) sites.
152 handwashing facilities (urban=43, peri-urban-84, rural=25) located at 123 households (urban=37, peri-urban-63, rural=23).

Overall, the availability of handwashing facilities across all sites was either limited or basic, as per the JMP guidance (**Figure 4.7**). Household members would typically report washing their hands before eating (89.7%, n=269) or after toileting (89.7%, n=269). Some members would also wash their hands after eating (45.7%, n=137), before preparing food (36.7%, n=110) when they looked dirty (46.3%, n=139) or after cleaning nappies (22.3%, n=67). There were no regional differences seen in the reported frequencies of hand washing practices related to toileting, food consumption or in child waste management (**Table 4.13**). Where handwashing occurred, participants reported that they primarily used either a hand-dipping technique in a basin of water (47.3%, n=142) or would pour a jug

of water over their hands (48.3%, n=145), and very few individuals would use tippy-taps, or run their hands under piped water (**Table 4.13 & Figure 4.8**).

Table 4.13. Household hand-hygiene practices of urban, peri-urban and rural sites.

Hand-hygiene practices	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
When do household members normally wash their hands?					
Before eating	n=269 (89.7%)	n=90 (90.0%)	n=88 (88.0%)	n=91 (91.0%)	*.840*
Before feeding child[$]	n=52 (17.3%)	n=27 (27.0%)	n=12 (12.0%)	n=13 (13.0%)	***<.001***
Before preparing food	n=110 (36.7%)	n=52 (52.0%)	n=24 (24.0%)	n=34 (33.0%)	***<.001***
After toilet	n=269 (89.7%)	n=89 (89.0%)	n=89 (89.0%)	n=91 (91.0%)	*.916*
After cleaning child nappy	n=67 (22.3%)	n=28 (28.0%)	n=18 (18.0%)	n=21 (21.0%)	*.219*
After eating	n=137 (45.7%)	n=74 (74.0%)	n=39 (39.0%)	n=24 (24.0%)	***<.001***
After working outside	n=62 (20.7%)	n=24 (24.0%)	n=12 (12.0%)	n=26 (26.0%)	***.027***
When they look dirty	n=139 (46.3%)	n=39 (39.0%)	n=64 (64.0%)	n=36 (36.0%)	***<.001***
Where do they wash their hands at these times?					
At tap inside house with piped water	n=17 (5.7%)	n=5 (5.0%)	n=11 (11.0%)	n=1 (1.0%)	
At tap outside house with piped water	n=17 (5.7%)	n=6 (6.0%)	n=8 (8.0%)	n=3 (3.0%)	
Bottle/tippy tap next to toilet	n=15 (5.0%)	n=4 (4.0%)	n=2 (2.0%)	n=9 (9.0%)	
Bottle/tippy tap next to kitchen/cooking area	n=2 (0.7%)	n=0 (0.0%)	n=1 (1.0%)	n=1 (1.0%)	
Basin with water (hand dipping) at house	n=142 (47.3%)	n=46 (46.0%)	n=46 (46.0%)	n=50 (50.0%)	
Jug with water (pouring over hands) at house	n=145 (48.3%)	n=53 (53.0%)	n=43 (43.0%)	n=49 (49.0%)	

p values obtained through fisher's exact test

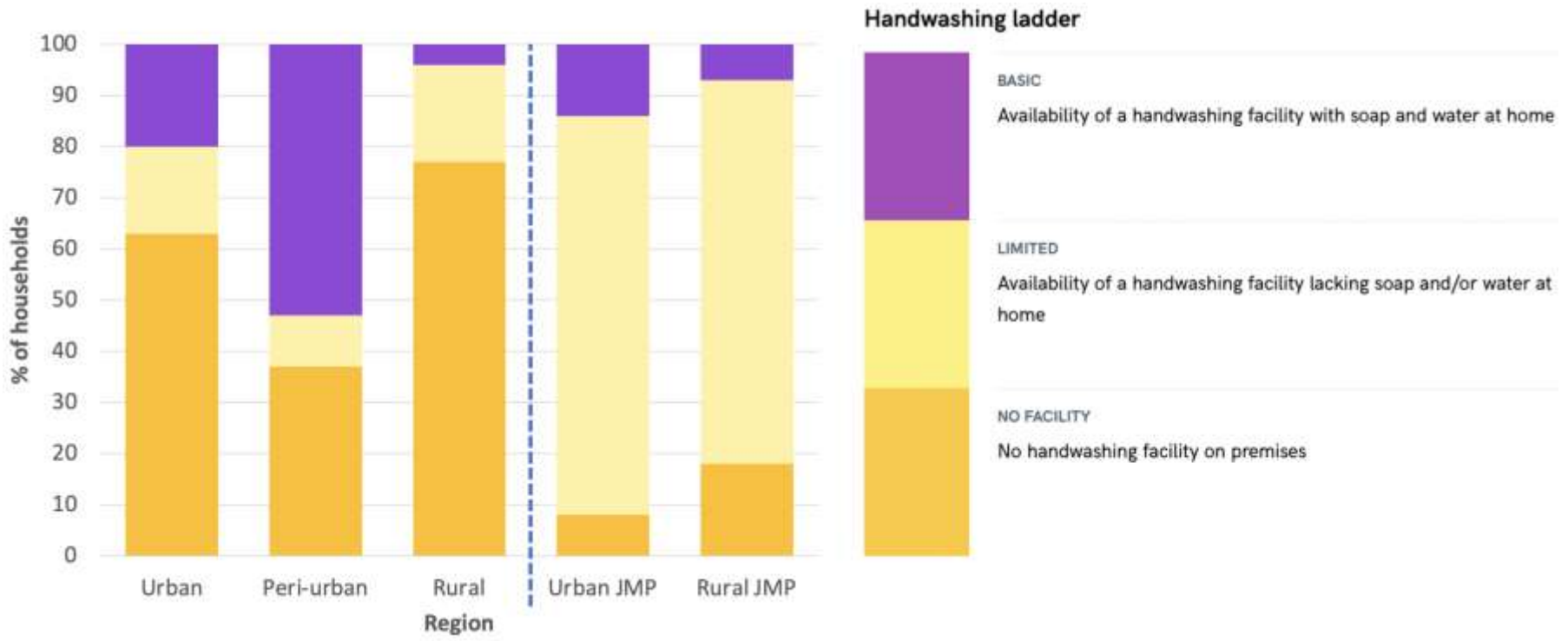


Figure 4.7. Proportion of urban, peri-urban and rural households with access to hand-hygiene infrastructure and materials, adopted form the JMP definitions, alongside 2020 JMP estimates for rural and urban Malawi (228).

Figure 4.8. Pictures of typical methods of hand washing by households. a) poured water and use of soap (urban), b) dip method into a bowl, c) tippy tap outside pit latrine, with no soap (rural) d) use of water for mixed purposes (including animal) with no soap (rural). *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

4.6. Food hygiene

Food consumed within study households commonly consisted of nsima (porridge made out of maize flour and water) and vegetables, supplemented with occasional meat. This was not designed as an in-depth study of dietary intake; however, there were regional differences seen in choice and availability of meat, with beef more often eaten in the urban and peri-urban setting and pork more often eaten by rural households (**Table 4.14**). Chicken and beef were the most consumed meats overall, and ~47% of households consumed milk or dairy products (**Table 4.14**).

Table 4.14. Household food consumption, stratified by region

Food product	Region	Frequency of product consumption by households (%)				
		Never	Less than once a week	About once a week	Several times a week	Daily
Beef	Urban	11.0%	44.0%	29.0%	16.0%	0.0%
	Peri-urban	4.0%	66.0%	19.0%	11.0%	0.0%
	Rural	36.0%	51.0%	13.0%	0.0%	0.0%
Pork	Urban	81.0%	14.0%	4.0%	1.0%	0.0%
	Peri-urban	72.0%	21.0%	6.0%	1.0%	0.0%
	Rural	54.0%	31.0%	11.0%	3.0%	1.0%
Chicken	Urban	2.0%	43.0%	44.0%	11.0%	0.0%
	Peri-urban	1.0%	62.0%	21.0%	16.0%	0.0%
	Rural	9.0%	68.0%	20.0%	3.0%	0.0%
Other meat *(inc. dried fish)*	Urban	33.0%	41.0%	23.0%	3.0%	0.0%
	Peri-urban	7.0%	77.0%	9.0%	6.0%	1.0%
	Rural	25.0%	52.0%	20.0%	3.0%	0.0%
Salad /raw vegetables *(Garden)*	Urban	89.0%	5.0%	1.0%	5.0%	0.0%
	Peri-urban	67.0%	9.0%	5.0%	7.0%	12.0%
	Rural	75.0%	8.0%	7.0%	8.0%	2.0%
Salad /raw vegetables *(Local market)*	Urban	2.0%	34.0%	16.0%	46.0%	2.0%
	Peri-urban	8.0%	39.0%	8.0%	20.0%	25.0%
	Rural	30.0%	32.0%	8.0%	30.0%	0.0%
fruit *(Garden)*	Urban	87.0%	6.0%	4.0%	3.0%	0.0%
	Peri-urban	42.0%	30.0%	5.0%	20.0%	3.0%
	Rural	72.0%	13.0%	6.0%	6.0%	0.0%
fruit *(Local market)*	Urban	5.0%	25.0%	22.0%	45.0%	3.0%
	Peri-urban	4.0%	40.0%	17.0%	29.0%	10.0%
	Rural	7.0%	42.0%	43.0%	8.0%	0.0%
fresh milk from cow/sheep/goat	Urban	54.0%	21.0%	11.0%	14.0%	0.0%
	Peri-urban	52.0%	37.0%	3.0%	5.0%	3.0%
	Rural	54.0%	31.0%	12.0%	3.0%	0.0%
Street food	Urban	7.0%	10.0%	4.0%	71.0%	8.0%
	Peri-urban	11.0%	18.0%	14.0%	49.0%	8.0%
	Rural	15.0%	31.0%	33.0%	21.0%	0.0%

Highlighted panels represent the most common selected option for urban (blue), peri-urban (grey) and rural (yellow) sites.

There was a reliance on purchasing fruit (94.7%, n=284) and vegetables (86.7%, n=260) from local markets, especially in the urban or peri-urban settings, with most households not able to grow their own produce (**Table 4.15**). However, even households that grew their own produce still used the markets, and 70.0% (n=70) of rural households relied on local markets for either supplementing their own vegetables or as the primary source of vegetables. Meat, like fruit and vegetables were most frequently obtained from local markets. Lastly, in study households, 89.0% (n=267) of families reported eating street food at least once a week, with 71% (n=71) of urban households eating street food several times a week (**Table 4.15**).

Most people washed raw food obtained from the market in drinking water prior to eating (75.0%, n=225) (**Table 4.15**). Other households would prefer to wash their hands first (15.7% n=47), peel away the outside surface (7.3%, n=22) or in some cases do nothing (2.0%, n=6). Cooked food was not part of the microbiological sampling frame, given the propensity for bacteria to be destroyed in the heating process, but contamination of cooked food left out to eat later in the day is a possible route of household transmission. Uneaten cooked food was identified at 38.1% (n=310) of the household visits, however, this was frequently covered (92.3%, n=286) to protect it from flies and other animals.

Given that food in Malawi is often eaten by hand, the acquisition of faecal-oral pathogens can be related to poor hand-hygiene and the sharing of food. In households, 43.0% (n=129) reported eating from shared plates (**Figure 4.9**), with the use of shared plates being more common at rural households than at urban or peri-urban sites.

Table 4.15. Household food-hygiene practices at urban, peri-urban and rural sites

Food hygiene practices	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Method of food preparation					
Eat straight from the market	n=6 (2.0%)	n=1 (1.0%)	n=0 (0.0%)	n=5 (5.0%)	
Wash with drinking water before eating	n=225 (75.0%)	n=76 (76.0%)	n=65 (65.0%)	n=84 (84.0%)	
Peel skin before eating	n=22 (7.3%)	n=13 (13.0%)	n=6 (6.0%)	n=3 (3.0%)	
Wash hands before eating	n=47 (15.7%)	n=10 (10.0%)	n=29 (29.0%)	n=8 (8.0%)	
Use of shared plates	n=129 (43.0%)	n=28 (28.0%)	n=36 (36.0%)	n=65 (65.0%)	***<.001***
Consumption of street food	n=267 (89.0%)	n=93 (93.0%)	n=89 (89.0%)	n=85 (85.0%)	*.213*
Consumption of market produce (vegetable)	n=260 (86.7%)	n=98 (98.0%)	n=92 (92.0%)	n=70 (70.0%)	***<.001***
Consumption of market produce (fruit)	n=284 (94.7%)	n=95 (95.0%)	n=96 (96.0%)	n=93 (93.0%)	*.730*
Cooked food seen at the house	n=310 (38.1%)	n=130 (47.1%)	n=80 (30.2%)	n=100 (35.0%)	***<.001***
Was the cooked food covered	n=286 (92.3%)	n=124 (95.4%)	n=77 (96.3%)	n=85 (85.0%)	***.007***
Animals in the cooking area	n=196 (24.1%)	n=30 (10.9%)	n=70 (26.4%)	n=96 (33.6%)	***<.001***
Animals in contact with food[$]	n=123 (62.8%)	n=24 (80.0%)	n=32 (45.7%)	n=67 (69.8%)	***<.001***

**Total of 827 observed fruit or vegetable storage at 814 visits. Urban=272/263, peri-urban=268/265, rural=287/286. P values obtained through fisher's exact test*

Figure 4.9. Pictures of household food-hygiene and storage of utensils. a) preparation of maise and storage of cooking utensils, b) clean (stored) and dirty utensils with animal exposures c) shared plates and d) cooking utensils clean and stored utensils with visible animal interactions. *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

4.6.1. Direct and indirect interactions of food with animals

Interactions with animals has the potential to play an important role in the transmission of bacteria within the household environment, especially in areas that food is stored or prepared. In the study, animals were observed to be present in and around the food preparation areas at 24.1% (n=196) of household visits (**Figure 4.9**), with a greater likelihood of observing this in the rural than the peri-urban or urban households. Furthermore, when located at food-preparation areas animals were observed to be in contact with food 62.8% (n=123) of the time.

Food storage methods and environmental hygiene in food-preparation equipment and areas can impact the likelihood of contamination, either through human or animal contact. Overall, rural sites were less likely to cover their utensils or food to protect them from animals (**Table 4.16**). Utensils used to cook food were found to be primarily kept in uncovered basins (51.8%, n=421) or on a shelf/rack

(30.0%, n=244) rather than stored in a cupboard or covered basin. Fresh fruit and vegetables are also stored in uncovered basins (54%, n=181), but are more frequently placed in covered basins (23.3%, n=77) or fridges (12.4%, n=41) and meat is often stored in covered basins (39.1%, n=70) or fridge/freezers (41.9%, n=75) away from animals.

Table 4.16. Household food storage at urban, peri-urban and rural sites

Food and utensil storage methods	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural^**	***p***
Utensils (covered)	n=129 (15.9%)	n=68 (25.9%)	n=53 (20.0%)	n=8 (2.8%)	***<.001***
Fresh fruit and vegetables (covered)	n=131 (38.1%)	n=86/171 (50.3%)	n=36/103 (35.0%)	n=9/70 (12.9%)	***<.001***
Meat (covered)	n=145 (84.3%)	n=79/108 (73.1%)	n=52/52 (100.0%)	n=8/12 (66.7%)	***<.001***
Where are clean utensils stored					
Basin (covered)	n=88 (10.8%)	n=63 (24.0%)	n=19 (7.2%)	n=6 (2.1%)	
Basin (uncovered)	n=421 (51.8%)	n=85 (32.3%)	n=137 (51.7%)	n=199 (70.1%)	
Shelf / rack	n=244 (30.0%)	n=110 (41.8%)	n=60 (22.6%)	n=74 (26.1%)	
In cupboard	n=41 (5.0%)	n=5 (1.9%)	n=34 (12.8%)	n=2 (0.7%)	
On floor	n=18 (2.2%)	n=0 (0.0%)	n=15 (5.7%)	n=3 (1.1%)	
Where are fresh fruit and vegetables stored*					
Basin (covered)	n=77 (23.3%)	n=61 (35.7%)	n=12 (11.7%)	n=4 (5.7%)	
Basin (uncovered)	n=181 (54.7%)	n=76 (44.4%)	n=55 (53.4%)	n=50 (71.4%)	
Shelf / rack	n=13 (3.9%)	n=4 (2.3%)	n=9 (8.7%)	n=0 (0.0%)	
In cupboard	n=13 (3.9%)	n=10 (5.8%)	n=0 (0.0%)	n=3 (4.3%)	
Fridge	n=41 (12.4%)	n=15 (8.8%)	n=24 (23.3%)	n=2 (2.9%)	
On floor	n=8 (2.4%)	n=0 (0.0%)	n=1 (1.0%)	n=7 (10.0%)	
On table surface or in a plastic bag	n=11 (3.3%)	n=5 (2.9%)	n=2 (1.9%)	n=4 (5.7%)	
No fruit or vegetables seen	*n=483*	*n=101*	*n=165*	*n=217*	
Where is meat stored$					
Basin (covered)	n=70 (39.1%)	n=54 (50.0%)	n=8 (15.4%)	n=1 (8.3%)	
Basin (uncovered)	n=32 (17.9%)	n=29 (26.9%)	n=0 (0.0%)	n=3 (25.0%)	
In cupboard	n=1 (0.6%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (8.3%)	

Fridge	n=28 (15.6%)	n=7 (6.5%)	n=18 (34.6%)	n=3 (25.0%)
In freezer	n=47 (26.3%)	n=18 (16.7%)	n=26 (50.0%)	n=3 (25.0%)
On floor	n=1 (0.6%)	n=0 (0.0%)	n=0 (0.0%)	n=1 (8.3%)
No meat seen	*n=648*	*n=159*	*n=214*	*n=275*

^ household data for n=99 in rural location

**Total of 827 observed fruit or vegetable storage at 814 visits. Urban=272/263, peri-urban=268/265, rural=287/286. P values obtained through fisher's exact test*

4.7. Household interactions with the broader environment

Standing water and rivers are potential reservoirs for ESBL-producing bacteria, and household interactions with these key sites were evaluated (**Figure 4.10**). Initially, I looked at the frequency of flooding events and presence of standing water surrounding the households (**Table 4.17**). Here, 7.0% (n=21) of families recounted widespread flooding of the compound; standing water was present at 8.7% (n=26) of households. There was a greater likelihood of standing water being reported at the urban site (18.0%, n=18) compared to the peri-urban or rural site, and this was corroborated through observational analysis. 50.0% of households that had the presence of standing water reported that their children interacted with it, and 42.3% reported that they had seen animals interacting with it, providing a potential conduit for AMR transmission between animals and humans (**Table 4.17**). When the regions flood, the drains fill with water, are at these events 38.1% (n=8) of households reported their children would interact with the drains and 7.3% (n=22) reported their adults would. It is important to state here that there was a very low response rate in returning the questions pertaining to flooded drains, so these results are hampered by limited data capture.

To improve the understanding of the frequency of these interactions the study team performed brief observations at the 814 household visits. Standing water and open drains were seen surrounding 8.0% (n=65) and 16.8% (n=137) of households, respectively, with more chance of them being present in the urban areas. Where standing water existed, the study team observed children interacting with it 24.6% (n=16) of the time, and animals interacting with it 50.8% (n=33) of the time. No variations in the frequency of interactions between children or animals and standing water were identified between the regions. Children and animals were also observed interacting with open drains, at 20.4% (n=28), and 43.8% (n=60), respectively. Again, there were no differences in the frequency of interactions between children or animals and the drainage systems of the three regions. Limited temporal data precluded a detailed seasonal analysis of drain exposures; however this may be an important contributor to the risk profile of interactions with these environments.

Figure 4.10. Pictures of urban waterways and key interactions. a) standing water in communal settings, b) animals interacting with standing water surrounding households, c) typical river in urban site d) children interacting with river in urban site. *Photo credit = Thoko Chikondi, collected as part of Wellcome Trust funded DRUM photojournalism project, June 2021.*

Table 4.17. Broader environmental interactions at urban, peri-urban and rural households

Standing water and drain interactions	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Does the area around the household ever flood	n=21 (7.0%)	n=4 (4.0%)	n=7 (7.0%)	n=10 (10.0%)	*.273*
If so, how often does it flood?					
Once a year	n=7 (33.3%)	n=3 (75.0%)	n=0 (0.0%)	n=4 (40.0%)	
Every time it rains	n=8 (38.1%)	n=1 (25.0%)	n=5 (71.4%)	n=2 (20.0%)	
Only after heavy rains	n=6 (28.6%)	n=0 (0.0%)	n=2 (28.6%)	n=4 (40.0%)	
Households reporting their children interact with the flooded drains around the	n=8 (38.1%)	n=2 (50%)	n=2 (28.6%)	n=4 (40.0%)	
Households reporting their adults interact with the drains around the household area?	n=22 (7.3%)	n=10 (10.0%)	n=7 (7.0%)	n=5 (5.0%)	
Is standing water present around the household?	n=26 (8.7%)	n=18 (18.0%)	n=7 (7.0%)	n=1 (1.0%)	***<.001***
Do children interact with the standing water? *	n=13 (50.0%)	n=10 (55.6%)	n=2 (28.6%)	n=1 (100.0%)	*.378*
Do animals interact with the standing water? *	n=11 (42.3%)	n=9 (50.0%)	n=1 (14.3%)	n=1 (100.0%)	*.128*
Observed behaviours^					
Standing water seen near the household	n=65 (8.0%)	n=36 (13.7%)	n=26 (9.8%)	n=3 (1.0%)	***<.001***
Children observed interacting with standing water	n=16 (24.6%)	n=11 (30.6%)	n=4 (15.4%)	n=1 (33.3%)	*.350*
Animals observed interacting with standing water	n=33 (50.8%)	n=18 (50.0%)	n=14 (53.8%)	n=1 (33.3%)	*.847*
Open drains seen near the household	n=137 (16.8%)	n=41 (15.6%)	n=89 (33.6%)	n=7 (2.4%)	***<.001***
Children observed interacting with drains	n=28 (20.4%)	n=11 (26.8%)	n=17 (19.1%)	n=0 (0.0%)	*.304*
Animals observed interacting with drains	n=60 (43.8%)	n=20 (48.8%)	n=35 (39.3%)	n=5 (71.4%)	*.205*

**Percentages obtained from 26 households where standing water was found (urban=18, peri-urban=7, rural=1). p values obtained through fisher's exact test*

$21 responses (urban=4, peri-urban-7, rural=10)

^ observational WASH data from a total of 814 longitudinal visits at 300 households. 263,265,286

Next, we asked whether there were interactions with the riverine environments external to the house. From this approach, 33.0% (n=99) of households reported their adults would interact with the rivers and 22.0% (n=66) of households would report their children would interact with the rivers. The reason for the riverine interactions in adults included washing clothes (51.4%, n=73), commuting to work

(19.0%, n=27) or bathing (12.7%, n=18), and washing clothes (33.0%, n=36), playing (32.1%, n=35), bathing (21.1%, n=23) or commuting to school (13.8%, n=15) in the case of children (**Table 4.18**).

Table 4.18. River interactions at urban, peri-urban and rural households

River water interactions	Region: n (%)				
	Total	**Urban**	**Peri-urban**	**Rural**	***p***
Households reporting their children interacting with river water?	n=66 (22.0%)	n=18 (18.0%)	n=34 (34.0%)	n=14 (14.0%)	***.001***
Households reporting their adults interacting with river water?	n=99 (33.0%)	n=26 (26.0%)	n=56 (56.0%)	n=17 (17.0%)	***<.001***
Reasons stated for children interacting with river water^					
Walking to school	n=15 (13.8%)	n=5 (20.0%)	n=6 (9.7%)	n=4 (9.1%)	
Playing with friends	n=35 (32.1%)	n=11 (44.0%)	n=14 (22.6%)	n=10 (45.5%)	
Bathing	n=23 (21.1%)	n=2 (8.0%)	n=15 (24.2%)	n=6 (27.3%)	
Washing clothes or housework	n=36 (33.0%)	n=7 (28.0%)	n=27 (43.5%)	n=2 (9.1%)	
Reasons stated for adults interacting with river water^					
Walking	n=27 (19.0%)	n=9 (25.0%)	n=13 (15.7%)	n=5 (21.7%)	
Bathing	n=18 (12.7%)	n=1 (2.8%)	n=11 (13.3%)	n=6 (26.1%)	
Washing clothes or utensils	n=73 (51.4%)	n=20 (55.6%)	n=46 (55.4%)	n=7 (30.4%)	
Water for household usage	n=10 (7.0%)	n=3 (8.3%)	n=7 (8.4%)	n=0 (0.0%)	
Irrigation for crops	n=9 (6.3%)	n=0 (0.0%)	n=4 (4.8%)	n=5 (21.7%)	
Use of sand/soil for business or other purposes	n=5 (3.5%)	n=3 (8.3%)	n=2 (2.4%)	n=0 (0.0%)	

^Multiple responses possible for river interactions per households. Total of 251 responses; with n=109 for children (urban=25, peri-urban=62, rural=22), and n=142 for adults (urban=36, peri-urban=83, rural=23). p values obtained through fisher's exact test

4.8. Discussion

There are many routes for the faecal-oral acquisition of AMR bacteria, as seen in the adapted F-diagram in Chapter 1, including fluids, fields, flies, fingers, fomites and food. Each of these pose barriers or risks for bacterial transmission, and within this chapter I have systematically evaluated them in urban, peri-urban and rural households. Broadly I identify widespread deficiencies across all WASH proxies, with regional variations in the specific WASH factors sites were more or less deficient. Households in all regions frequently lacked the infrastructure to enable safe toileting, adequate sanitation (faeces disposal and containment), effectual hand-hygiene or waste management and there were self-reported practices identified that may increase the risk of bacterial transmission, such as water usage, food-hygiene, open defaecation, and handwashing. Furthermore, there was a high frequency of interactions seen between household participants and environmental sites likely to be contaminated with faecal material of human or animal origin, potentially supporting the maintenance, acquisition and transmission of AMR in our setting, and contributing to key ecological niches for AMR.

Households recruited were typically constructed with baked or unbaked bricks, metal roofs, and concrete or soil floors. Within the household, concrete floors are easier to clean effectively compared to other permeable surface types such as soil or wood, and thereby reduce the risk of bacterial contamination (323). This may put households in the rural or peri-urban areas at a greater risk, as the floors in their homes and toilets are more frequently constructed with materials such as soil. Other construction differences included the absence of a roof in 24.3% of household toilets which can lead to rainwater entry causing flooding of latrines, and the ingress of flies which in turn spread AMR faecal material (324,325).

The presence of a toilet on-site is important to both enable household privacy and provide access to sanitation close by the home (326,327). Toilet access has been recognised as a factor that reduces the chance of protozoal or bacterial enteric infections at households in low-income settings; within this study 89.0% of households owned a toilet (320,328). The rural area once again had reduced availability, with only 75.8% of households possessing a toilet. In relation to the type of toilet available, 88.8% of households had access to a pit latrine, with the rest of households having access to a flush pour toilet that deposited into the mains sewerage networks or household sewerage tanks. There is little known difference in the risk of ESBL contamination from various toilet types, and given that aerosolization can be a modality of transfer in either flush toilets or when emptying pit latrines, future studies may wish should consider whether having a pit latrine or flush toilet provide a protective

benefit to AMR bacterial transmission (221,329). This is compounded by inadequate siting and poor construction of pit latrines, leading to overflow in times of heavy rainfall and their breakdown; with subsequent dissemination of faecal matter into the surrounding environment (291,330). Furthermore, while the operational effectiveness of sewerage systems, and downstream sanitations systems in urban Malawi is not fully described in the literature, it is widely considered to be poor (291). Ineffectual sewerage management leads to the deposition of human, animal and solid waste into rivers, farmlands and groundwaters, especially in urban settings (175,209,331–333), and this widespread dispersal of AMR bacteria into the broader environment provides a key conduit for transmission risk, especially in the urban settings.

Toilet hygiene is important given the presence of faecal bacteria and pathogens including ESBL *E. coli* have long been known to contaminate and persist on uncleaned toilet surfaces including handles, seats, floors and walls (334–336). Access to HWFs with soap and water and anal cleaning materials at toilets promotes good toilet-hygiene (239,322,337); here, it was identified that only 18.9% of toilets had anal cleaning materials and 12.4% of toilets had dedicated handwashing facilities. Where they existed, 78.4% of toilet HWFs had water and 50.0% were noted to have the presence of soap. Visible faecal contamination was found on the walls or floor at 8.4% of toilet visits, and flies were seen at 61.5% of household toilets. Drophole covers can mitigate the quantity of flies and smells from the toilet that attract further flies (338), and these were present at 34.5% of households, more often in the rural site. However, at the visits, they were only in position 73.0% of the time, highlighting, as with hand-hygiene equipment, that access does not indicate usage (241). These data suggest that household members are at risk of faecal-oral acquisition of AMR bacteria via reduced access to appropriate materials and facilities associated with toileting. The reasons for the observed discrepancies in cleansing material and HWF access were not explored in this data, and these may be related to household preference, sociocultural, risk perception or economic factors.

The Millennium Development Goals (MDG) and the subsequent Sustainable Development Goal (SDG) section 6.2 recommends the use of “improved” pit latrines and does not allow for shared toilets. There are contrasting opinions as to whether the sharing of toilets provides a risk to faecal-oral exposure, with one Tanzanian study illustrating that sharing of toilets was actually protective against faecal exposure (336,339). Nevertheless, should appropriate toilet hygiene not be maintained, sharing of toilets could be a transmission point for mixing between households and thus transmission of ESBL bacteria. In this study, 41.9% of households shared their toilet with a median of 3 other households, with toilet sharing particularly common in the urban setting. Public toilets are often only available to

households in urban areas, and where they are present nearby, households would utilise them 37.1% of the time. When individuals needed to defaecate while away from the home, they would prefer to seek the use of another household's toilet rather than use a public one. Therefore, due to the practice of sharing household toileting facilities, we should consider the household toilet as a potential focal point for household-household transmission.

Waste management practices for human and animal faeces and household rubbish can influence the transmission of ESBL bacteria within the household and the broader community (321,340). Children would wear re-usable nappies, and child faeces were either washed off in buckets, thrown into the toilet or in the case of disposable nappies thrown into a pit nearby the house. It is estimated that 946 million people worldwide practice open defaecation, and 28.7% of the households reported one or more member openly defaecating on a monthly basis, with no regional differences identified. The motivations for this toileting choice were not explored, but previous local research has identified social vulnerabilities, including educational and economic aspects that play a role in the decision to openly defaecate (341). When the team visited households, they identified human waste visible on the ground surrounding the house 8.1% of the time. This was compounded by seeing animal waste at 53.2% of visits, more frequently at the rural site. 2.7% of households would consider using human stool as nightsoil and 27.3% of households would use animal manure on their gardens or crops. When asked how animal waste was managed, households rarely had an effective system and would often either do nothing or sweep it into nearby bushes. Reports of contaminated drinking water, through improper waste management of animal faeces has been reported in comparable urban settings (321). Household rubbish was thrown into open drains or pits at 61.0% of households, and this can contaminate the broader environment. Poor waste management and the presence of environmental contamination with human and animal waste poses a clear risk for transmission of bacteria between household members and between humans and animals.

AMR bacteria can also be transmitted via ingestion of water or food contaminated prior to entry into the household, or via subsequent contamination of these items through improper storage or handling within the household (235,342). Therefore, I evaluated WASH factors pertaining to these areas at the households. There were clear regional differences in the sources that households used to obtain their drinking water, with water kiosks utilised in the urban households, boreholes almost exclusively used in the rural settings and a combination of various sources used at peri-urban households. Drinking water was sometimes acquired from unsafe sources such as unprotected wells, springs and surface waters, although this was uncommon. Water was rarely treated prior to consumption, despite

households' perception of its safety, and all except one house stored drinking water for future use. Contamination of stored drinking water with *E. coli* has been well documented, and international guidance (WHO) on water protection states that drinking water should be stored in a clean container with a cover, away from water used for domestic purposes (235,343,344) Water in the households was stored in a variety of receptacles, but only 68.1% had a cover to protect them from animal or environmental contamination and 75.5% had a visible separation from water used in other domestic activities. Here, urban and peri-urban households had less overall water protection and separation than rural households, providing a potentially important regional difference.

Water is integral for other sanitation and hygiene activities, including cleaning of household equipment, particularly food preparation equipment, and bathing. These activities are often considered as less of a risk for faecal oral acquisition, and so households will consider an alternative, cheaper water source (200), with previous research having demonstrated that multiple water sources were used by a single household for varying tasks (345). We identified that 17.3% of households would use an alternative water source for cleaning or bathing, and this was more frequently the case in the urban setting. Where households obtained water from a secondary source, they would frequently choose a cheaper form of water such as borehole or tube-well water, and 11.5% of households would consider utilising water obtained from unprotected sites such as wells, springs, or rivers. 51% of adults and 33% of children who reported interactions with river water did so for the purpose of washing clothes. These types of water sites have frequently been found in other studies to be contaminated with gut enterics (such as *E. coli*) and are not recommended for household use (177,343,344,346).

There was a wide range of diets seen, with regional variations in household meat intake. Households acquire food produce from a number of sources including local markets and street vendors alongside their own gardens. Given the cost and availability of physical space, rural households had access to personal gardens and a more regular source of household produce than urban settings. However, in the survey, a total of 94.7% of households would obtain fruit, and 86.7% of households would obtain vegetables from the market on a regular basis. Given the variations in food-handling practices and environmental health measures seen at marketplaces in LMICs the reliance on purchasing goods from local markets could serve as a possible entry point for acquisition of AMR bacteria (20). Furthermore, 89.0% of households would supplement home-cooked meals with street food, especially in the urban settings. Akin to marketplaces, street food is widely reported as a high risk for acquisition with enteric bacteria and AMR pathogens, so this entry point into the household warrants further detailed evaluation in future studies (21,22).

Food handling and storage practices were assessed to determine whether there were key behaviours that could promote the contamination of food between purchase and ingestion. Some ready to eat raw foods do not undergo a cooking process, and inadequate cooking of contaminated food can enable bacteria to be ingested (347–350). It was found that stored fruit and vegetables, and meat were covered for protection from animals and environmental exposures 38.1% and 84.3% of the time, respectively. 15.9% of cooking utensils were stored in a secure space, with alternative water sources often used to clean them, as previously mentioned. Cooked high risk food was commonly seen, and regularly covered, but nevertheless, animals were present in and around food preparation areas at 24.1% of visits and were frequently witnessed to be in contact with the food at these times. These examples indicate that household practices pertaining to food and utensil storage is often inadequate and enables animal and environmental contamination that could allow for the transmission of ESBL bacteria.

Households reported washing ready to eat raw food prior to cooking and/or would wash their hands first, but the availability of HWFs at food preparation areas was limited to only 7.4% of households, and soap and water were not always present at these HWFs. Food is eaten by hand, and sharing of prepared food was common, especially in the rural site. 43.0% of total households consumed food from shared plates; 65.0% in rural households. 89.7% of participants from all regions stated they washed their hands prior to eating; however, the most common choice of handwashing technique was hand dipping in a communal basin or pouring water over hands using a jug of water. Given the absence of soap at households, these methods may be ineffectual to reduce bacterial contamination from dirty hands. While reported hand hygiene was high among participants, it is well documented that stated practice and observed practice differ, with actual handwashing frequently substantially lower than that reported (240). In this regard, some of the WASH behavioural factors described in this chapter should be considered as proxies for true behaviour, indicating what we think may happen or not alongside self-reported actions.

Hand hygiene and cleaning of contaminated surfaces is an important factor in reducing bacterial transmission between members of the same household (16–18,23,24). In this study HWFs were only present at 41.0% of households, located primarily at either the yard or inside the house, and that only 49.0% of HWFs had soap available. The absence of access to suitable, convenient and functional HWF to facilitate hand washing with soap in our settings may drive contamination of the home environment and promote faecal oral acquisition of AMR pathogens. The finding of increased HWFs at toilets in the

rural site could be influenced by community-led total sanitation (CLTS) programs that exist in the rural areas aimed at eliminating open defaecation (351). Notably however these programs do not necessarily relate to a behaviour change and their long-term sustainability has been questioned (352–354).

There are many locations in the broader environment that have been shown to harbour ESBL bacteria; notably the riverine network, comprising of surface waters and rivers, alongside the sanitation network, particularly open drains (158,162,177,210,292,355–358). It was identified that study participants of all ages had regular interactions with these sites. These exposures were more prevalent in the urban settings, with children and animals frequently found to interact with drains and standing water. Household members also frequently came into contact with the river, and there were age-dependant reasons for these exchanges. Typical activities that led to river interactions included washing clothes, commuting, bathing or in the case of children, playing. Animals also encountered these environments and were frequently observed by the study team interacting with drains, rivers and standing water, again most commonly in the urban setting. Interactions between urban residents, animals and the broader environment are potentially important in the acquisition and transmission of ESBL bacteria (36,342,359). These broader environmental exposures depend on water availability and are subject to seasonal changes in rainfall. There are previous reports of potential contamination events from pit latrines flooding in response to rainfall with the potential to contaminate surface and groundwater (333,360). There were no differences identified in the regional frequency of flooding events seen in this study; however, it was not possible to fully assess the level of rainfall, nor the ability of household water and sanitation infrastructure to cope with rains (203,208,333,360,361). This highlights a clear limitation with this study, in that we did not incorporate seasonal effects into the analysis of responses on household WASH practices or interactions with the broader environment.

Other limitations of this descriptive analysis include the possibility of participant recall bias, and discrepancies between reported and actual behaviours that could impact the validity of the survey findings. However, as part of ongoing work by the DRUM consortium, a Risks, Attitudes, Norms, Abilities and Self-regulation (RANAS) survey (not included in this thesis) will be able to identify these divergences and allow us to adjust for them. Furthermore, financial and time restrictions constrained the numbers of households recruited and ability to undertake WASH checklist observations at follow-ups, so we should be mindful that the sample size may not provide the accuracy necessary to make definitive assumptions on regional WASH practices. Also, activities such as handwashing and food-preparation involve complex behaviours and motivations, and it will be important to assess these

aspects through other methodologies. It will be important to contextualise the findings in relation to the detailed observations and hand-hygiene audits undertaken at households in each region. These hand-hygiene audits and unstructured observations have been completed and will be evaluated to assess missed opportunities for practice, however they are included within the observational dataset and therefore are not included in this thesis.

This chapter has reported the key regional and household differences in access and availability to WASH infrastructure, alongside a basic understanding of the common household WASH practices surrounding water usage, toileting, waste management, food-preparation and hand-hygiene. I have incorporated a One-Health approach into the descriptive analysis, presenting results on human and animal interactions and the critical environments that form a nexus at which humans and animals interact. In forthcoming chapters I will continue to assess whether ESBL bacteria are present within the guts of humans and animals, the food and water that they drink, and the household and broader environment in which they inhabit. Together, these data will inform models to determine whether there are WASH-specific risk factors for human ESBL colonisation using a selection of key variables identified from this data.

Chapter 5:

A comparison of ESBL-colonisation of humans and animals and ESBL-contamination of their households and broader environment

5.0. Chapter summary

To understand the transmission dynamics of antimicrobial resistant enteric bacteria between humans, animals and the environment in Malawi I have made a longitudinal description of ESBL-colonisation of humans and co-located animals and their surroundings. Specifically, in Chapter 5, I make a detailed microbiological summary of the urban, peri-urban and rural landscape of ESBL *E. coli* and ESBL *K. pneumoniae* at both a household and broader environmental level. These have been stratified into ESBL presence and absence, and *E. coli* and *K. pneumoniae* specific.

The phenotypic results illustrated a very high level of ESBL colonisation in humans, animals and the environments of southern Malawi; especially those with inadequate WASH infrastructure or poorly governed waste management systems (i.e. dumping of waste in rivers, open drains). A higher rate of ESBL colonisation was identified in the urban setting compared to the other regions, for both human and animal stool, and this was compounded by a high prevalence in food, household surfaces, floors and the external environment. Other than urbanisation, seasonality was identified as a key factor pertaining to ESBL colonisation and environmental contamination, with the highest rates seen in the wet season.

Here, I have undertaken genuine One-Health approach to the surveillance of ESBL *E. coli* and ESBL *K. pneumoniae* addressing key knowledge gaps. This will enable us to build a more detailed understanding of the drivers and ecological niches for ESBL *E. coli* and ESBL *K. pneumoniae* AMR within this setting through the broader DRUM consortium.

My contributions to this chapter and those of others are included in Table 5.0.

Table 5.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted and analysed by the PhD candidate
Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborator, Tracy Morse. Statistical advice was sought from Chris Jewell and Marc Henrion. Laboratory analysis was aided by study staff, including: • Madalitso Mphasa, Mary Charles, Tamandani Mandula, Winnie Bakali, Rachel Banda, Chifundo Salife, Allan Zuza and Victor Maiden.

5.1. Phenotypic ESBL results from the household study

All households underwent a microbiological survey at baseline and again at each follow-up visit. The following results are descriptive summaries obtained from these household visits, stratified by sample type, bacterial species, region and season. Regional comparisons were analysed using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables, and differences in ESBL presence between sample types and animal species were evaluated using Chi-squared testing.

As previously stated, only 195/300 households had longitudinal visits and for the 105 remaining households a baseline visit was undertaken where only human stool samples were collected.

5.2. Overview of human colonisation, animal colonisation, household and broader environment contamination with ESBL *E. coli* or *K. pneumoniae*

Between May 2018 and October 2020, a total of 11,975 samples were screened for the presence of ESBL bacteria, inclusive of 2845 (23.8%) human stool, 973 (8.1%) animal stool and 8157 (68.1%) household or broader environmental samples (**Table 5.1**). Sample numbers were similar between

urban (n=3675, 30.7%), peri-urban (n=4018, 33.6%) and rural (n=4282, 35.8%) sites. 43 samples were collected but not processed, due to inaccurate labelling (urban; n=13, peri-urban; n=15, rural; n=15).

Table 5.1. Numbers of samples screened for ESBL *E. coli* and ESBL *K. pneumoniae*, stratified by sample type and region.

Broad sample type		Sample number n (%)			
		Total	**Urban**	**Peri-urban**	**Rural**
Human stool		n=2845 (23.8%)	n=821 (22.3%)	n=982 (24.4%)	n=1042 (24.3%)
Animal stool		n=973 (8.1%)	n=118 (3.2%)	n=229 (5.7%)	n=626 (14.6%)
Environment		n=8157 (68.1%)	n=2736 (74.5%)	n=2807 (69.9%)	n=2614 (60.1%)
	Food	n=1168 (9.8%)	n=333 (9.1%)	n=440 (11.0%)	n=395 (9.2%)
	Drinking water	n=1254 (10.5%)	n=532 (14.5%)	n=449 (11.2%)	n=273 (6.4%)
	Source water	n=527 (4.4%)	n=79 (2.1%)	n=216 (5.4%)	n=232 (5.4%)
	Household surfaces	n=2458 (20.5%)	n=766 (20.8%)	n=744 (18.5%)	n=948 (22.1%)
	Household floor	n=745 (6.2%)	n=247 (6.7%)	n=244 (6.1%)	n=254 (5.9%)
	Clothing	n=742 (6.2%)	n=245 (6.7%)	n=242 (6.0%)	n=255 (5.9%)
	Hand-contact samples	n=451 (3.8%)	n=129 (3.5%)	n=69 (1.7%)	n=253 (5.9%)
	Household drains	n=300 (2.5%)	n=151 (4.1%)	n=149 (3.7%)	n=0 (0.0%)
	River water	n=512 (4.3%)	n=254 (6.9%)	n=254 (6.3%)	n=4 (0.1%)
TOTAL		**11975**	**3675**	**4018**	**4282**

Samples were pre-enriched in buffered peptone water and visible growth was present in 94.1% (n=2581) of human stool, 92.3% (n=898) of animal stool and 92.5% (n=7548) of environmental samples (**appendix 5i)**. Subsequent growth on chromogenic agar identified pink (ESBL *E. coli*), white (possible ESBL *E. coli*) and blue (possible ESBL *K. pneumoniae*) colonies. White colonies were tested with indole, and indole-positive were re-classified as ESBL *E. coli*. Blue colonies underwent HRM PCR testing to identify *K. pneumoniae* genes, and PCR-positive isolates were classified as ESBL *K. pneumoniae* (**appendix 5i**). Non- *K. pneumoniae* colonies are likely to represent *Citrobacter spp*, *Enterobacter spp* or other *Klebsiella* species (i.e. *K. oxytoca*). No further speciation or antimicrobial sensitivity testing was undertaken.

A total of 2293 ESBL-E isolates and 1091 ESBL-K isolates were identified from the study samples via growth on ESBL ChromAgar in conjunction with indole and HRM PCR testing (**Figure 5.2**). 1063 non-ESBL *K. pneumoniae* blue colonies were also recovered, are these are likely to represent other species that have intrinsic or acquired 3GC resistance, as mentioned above.

Table 5.2. Overview of bacterial isolates recovered from ESBL ChromAgar, stratified by sample type.

Broad sample type	ESBL ChromAgar growth				
	Total isolates	ESBL bacteria n (% total isolates)		Other bacterial colonies n (% total isolates)	
		ESBL *E. coli*	ESBL *K. pneumoniae*	White *(non- E. coli)*	Blue *(non- K. pneumoniae)*
Human stool	n=1674	n=1065 (63.6%)	n=341 (20.4%)	n=92 (5.5%)	n=176 (10.5%)
Animal stool	n=394	n=274 69.5%)	n=53 15.9%)	n=63 (19.0%)	n=52 (15.7%)
Environment	n=5176	n=954 (18.4%)	n=697 (13.4%)	n=2690 (51.9%)	n=835 (16.1%)
Food	n=937	n=80 (8.5%)	n=108 (11.5%)	n=649 (69.3%)	n=100 (10.7%)
Drinking water	n=730	n=155 (21.2%)	n=160 (21.9%)	n=225 (30.8%)	n=190 (26.0%)
Source water	n=159	n=25 (15.7%)	n=19 (11.9%)	n=80 (50.3%)	n=35 (22.0%)
Household surfaces	n=1199	n=106 (8.8%)	n=93 (7.8%)	n=874 (72.9%)	n=126 (10.5%)
Household floor	n=384	n=57 (14.8%)	n=25 (6.5%)	n=236 (61.5%)	n=66 (17.2%)
Clothing	n=465	n=33 (7.1%)	n=27 (5.8%)	n=363 78.1%)	n=42 (9.0%)
Hand-contact samples	n=276	n=65 (23.6%)	n=47 (17.0%)	n=101 (36.6%)	n=63 (22.8%)
Household drains	n=311	n=123 (39.5%)	n=58 (18.6%)	n=59 (19.0%)	n=71 (22.8%)
River water	n=721	n=310 (43.0%)	n=160 (22.2%)	n=103 (14.3%)	n=148 (20.5%)

The findings from the microbiological analysis indicated a high rate of gut colonisation with ESBL-E or ESBL-K across the cohort, with 41.8% (n=1190) of human stool samples positive for ESBL (**Figure 5.1**). There was high prevelance of ESBL-E or ESBL ESBL-K colonisation in animal stool (29.8%) and high levels of ESBL-E or ESBL-K contamination of household environments (11.5%), food (13.4%), and external environmental samples (38.5%) (**Figure 5.1**). Within the household environment the sample types with the highest return of ESBL positivity were stored water and rinse water (~21.3%). In study households, 5.5% of the source water (i.e. borehole or kiosk) was contaminated with ESBL-E or ESBL-K prior to consumption. Within the external environment the highest proportion of ESBL was identified in river water samples (66.2%), particularly at the urban setting (74.0%).

Household hand-hygiene samples (rinse water) were often contaminated with ESBL-E or ESBL-K (21.3%), alongside contamination of clothing (7.4%), household floors (9.1%), and household surfaces (6.8%) (**Figure 5.1**). The differences in ESBL prevelance seen between sample types was significant (X^2, p = <.001).

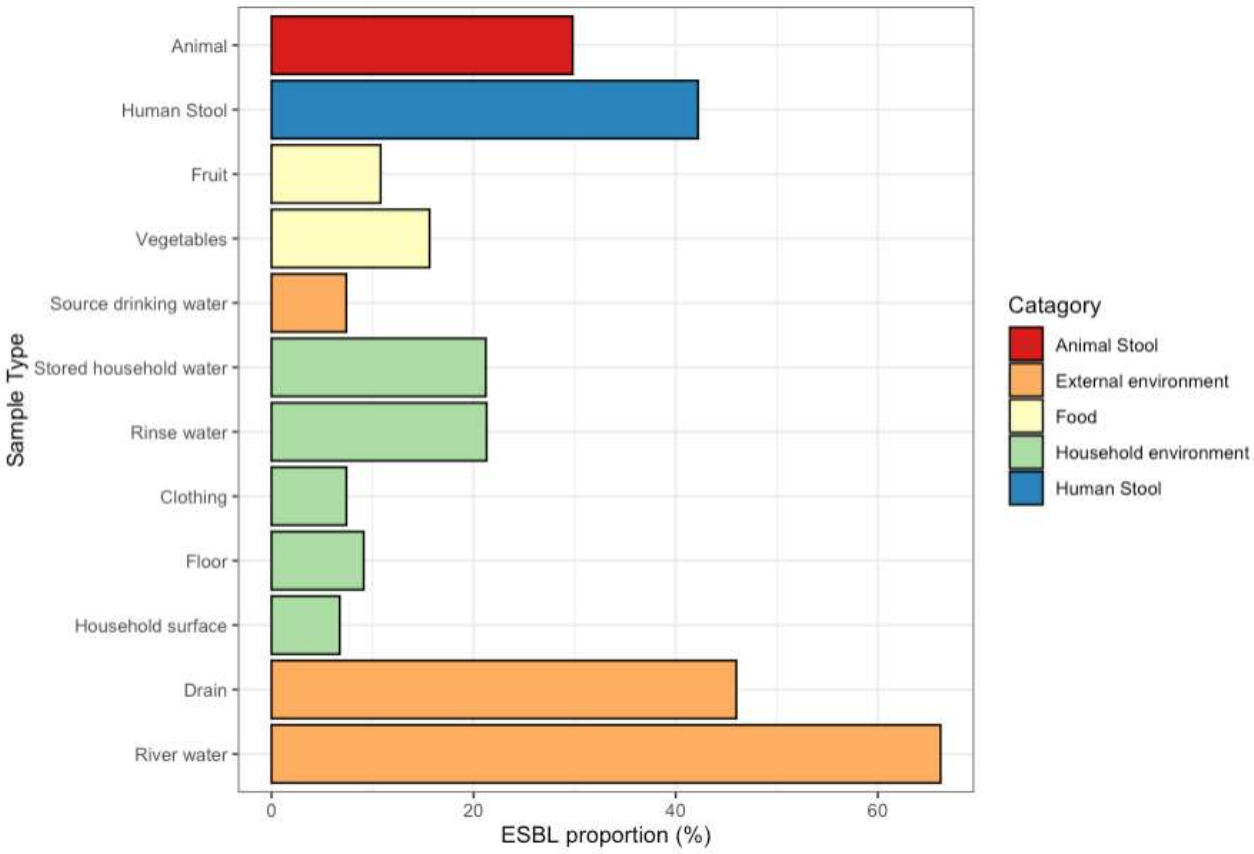


Figure 5.1. Proportion of total household samples that were positive for ESBL *E. coli* **or** ESBL *K. pneumoniae*, inclusive of human stool, animal stool, household and broader environmental samples; coloured by category and stratified by sample type

5.3. ESBL *E. coli* and ESBL *K. pneumoniae* composition within human, animal, household and broader environment samples

In total, 61.6% (n=1733) of positive samples yielded ESBL-E isolates alone, 20.4% (n=573) yielded ESBL-K isolates alone, and both were isolated from 18.0% (n=508) (**Figure 5.2**). ESBL *E. coli* was more common from stool samples (human or animal), whereas ESBL-K was more common in food (**Figure 5.3**). Drinking water, source water, household surfaces, clothes and hand-hygiene samples had similar

proportions of *E. coli* or *K. pneumoniae*, and river water had the highest frequency of detection of both species (**Figure 5.3**).

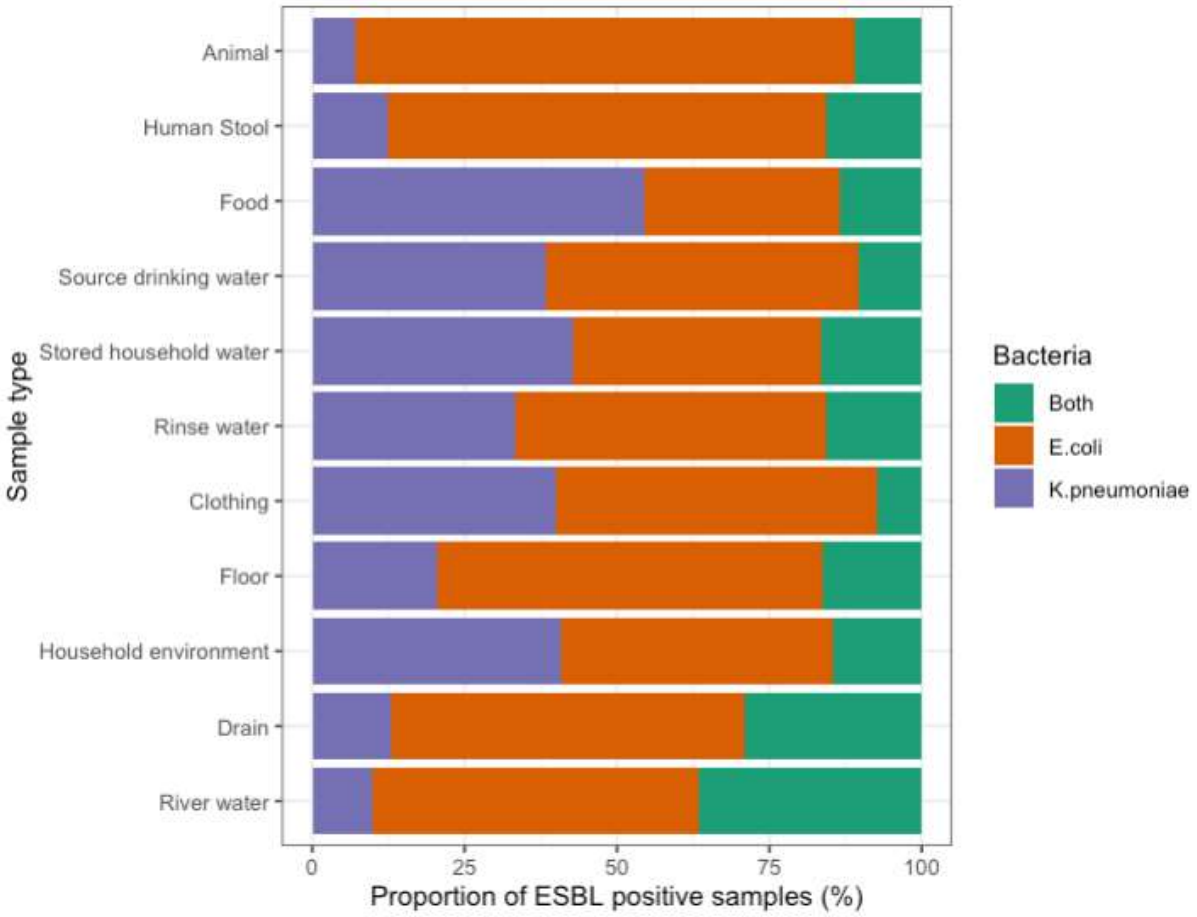


Figure 5.2. Proportion of the total household human stool, animal stool and environmental samples that were positive for ESBL *E. coli*, ESBL *K. pneumoniae* or both ESBL *E. coli* & ESBL *K. pneumoniae*, stratified by sample type and coloured by bacterial species.

5.4. Regional differences in human colonisation, animal colonisation, household contamination and broader environment contamination with ESBL *E. coli* or *K. pneumoniae*

There were regional differences seen in the ESBL colonisation in household members (**Figure 5.3**), with those in the urban setting having the highest overall ESBL colonisation rate (47.1%), compared to those in the peri-urban (34.5%) or rural (35.4%) region (p = <.001, Fishers exact).

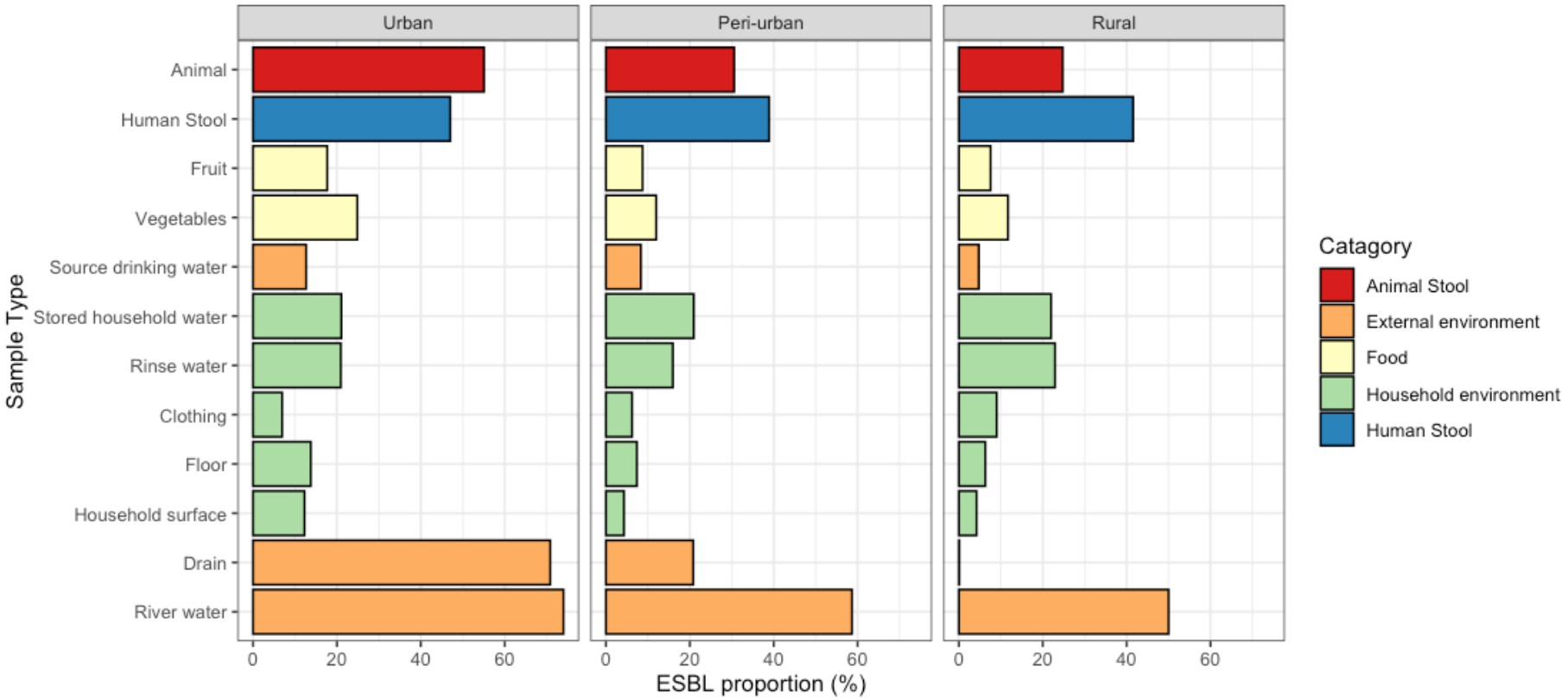


Figure 5.3. Proportion of samples positive for ESBL *E. coli* ***or*** ESBL *K. pneumoniae*, at urban, peri-urban and rural households. Samples have been coloured by category and stratified by sample type.

The urban region returned the highest prevelance of ESBL bacteria in animal stool (55.1%), and this may be related in part to the species present at households (Section 5.5). Regional differences in ESBL prevelance were also noted in food, household floors and surfaces, alongside river and drains, with no statistical difference seen in the presence of ESBL in source water, drinking water, clothing, or hand-hygiene samples (**Figure 5.3** & **Table 5.3**). Of note, no drain samples were returned from the rural region, but differences were seen in the presence of ESBL between the urban and peri-urban setting (X^2, p = <.001).

Table 5.3. Household ESBL (*E. coli* ***or*** ESBL *K. pneumoniae*) results, stratified by sample type and region.

Broad sample type		Urban ESBL n (%)	Peri-urban ESBL n (%)	Rural ESBL n (%)	*p*
Human stool		n=384 (47.1%)	n=377 (38.6%)	n=429 (41.5%)	***.002***
Animal stool		n=65 (55.1%)	n=70 (30.6%)	n=155 (24.8%)	***<.001***
Environment					
	Food	n=71 (21.3%)	n=46 (10.5%)	n=39 (9.9%)	***<.001***
	Drinking water	n=112 (21.1%)	n=94 (20.9%)	n=59 (21.7%)	*.967*
	Source water	n=10 (12.7%)	n=18 (8.3%)	n=12 (5.2%)	*.076*
	Household surfaces	n=94 (12.3%)	n=32 (4.3%)	n=40 (4.2%)	***<.001***
	Household floor	n=34 (13.8%)	n=18 (7.4%)	n=16 (6.3%)	***.010***
	Clothing	n=17 (6.9%)	n=15 (6.2%)	n=23 (9.0%)	*.501*
	Hand-contact samples	n=27 (20.9%)	n=11 (15.9%)	n=58 (22.9%)	*.467*
	Household drains	n=107 (70.9%)	n=31 (20.8%)	n=0 (0.0%)	***<.001***
	River water	n=188 (74.0%)	n=149 (58.7%)	n=2 (50.0%)	***<.001***

^p values generated by Fishers exact test

There were differences in the proportions of ESBL-E (X^2, p = <.001) and ESBL-K (X^2, p = <.001) seen between sample types, and regional differences in the presence of ESBL-E and ESBL-K (**Figure 5.4 & Table 5.4**). The urban region had the highest prevelance of ESBL-E (52.5%) and ESBL-K (11.9%) in animal stool. When I assessed for the regional differences in ESBL-E by sample type, I found that there were variations in the presence of *E. coli* in human stool, animal stool, food, floor, drains and river

samples (**Table 5.4**). The total number of ESBL-K was lower than *E. coli*, which precluded a detailed analysis (**Table 5.5**).

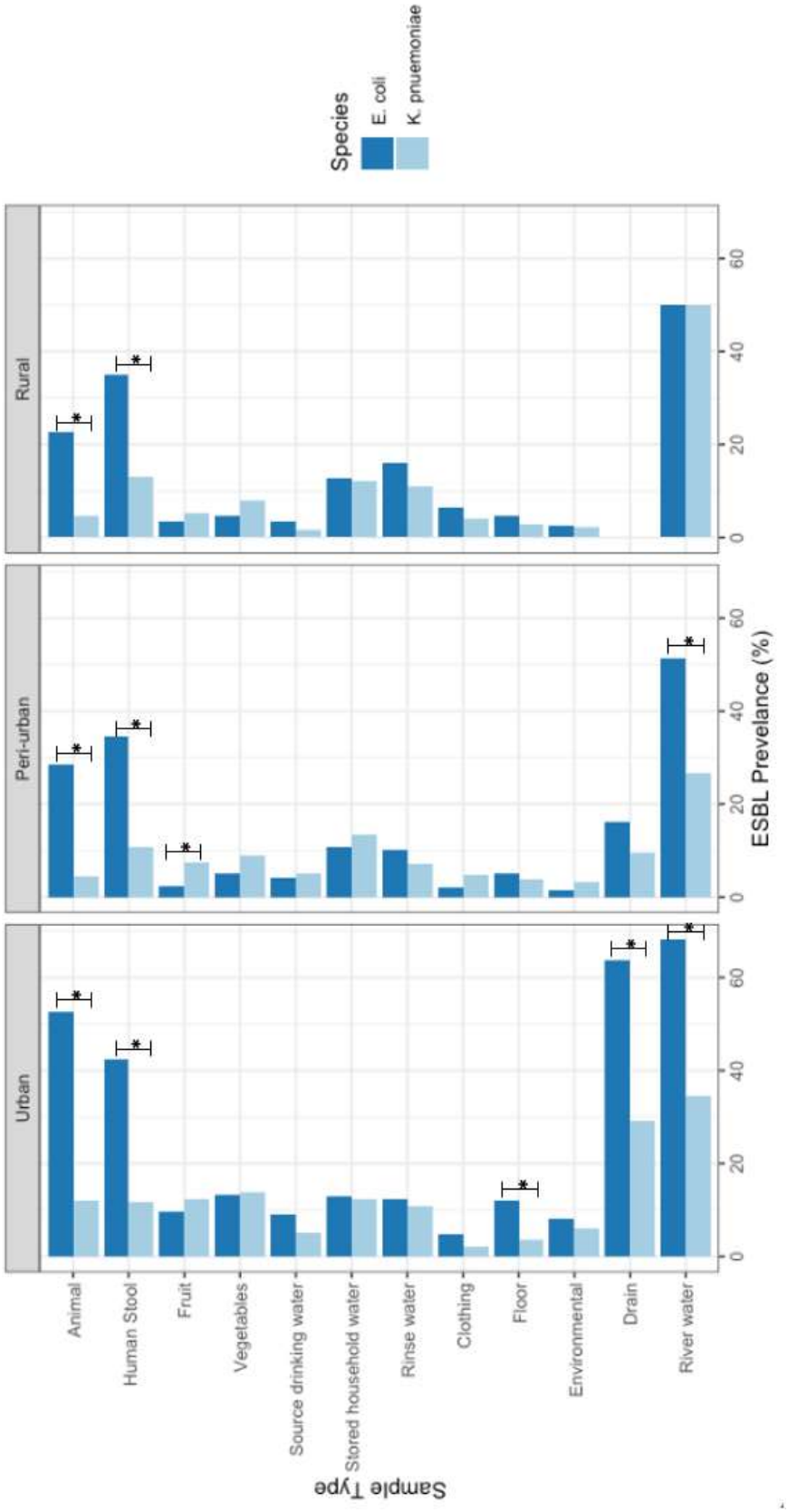


Figure 5.4. Proportion of the household human stool, animal stool and environmental samples that were positive for ESBL *E. coli* or ESBL *K. pneumoniae*, stratified by sample type, bacterial species and region. *Variations in the proportion of ESBL-E vs ESBL-K by sample type are highlighted by *(X^2, p = <.001)*

Table 5.4. ESBL *E. coli* results, stratified by sample type and region.

Broad sample type		Urban ESBL *E. coli* n (%)	Peri-urban ESBL *E. coli* n (%)	Rural ESBL *E. coli* n (%)	*p*
Human stool		n=347 (42.3%)	n=339 (34.5%)	n=365 (35.4%)	***<.001***
Animal stool		n=62 (52.5%)	n=65 (28.4%)	n=142 (22.7%)	***<.001***
Environment					
	Food	n=38 (11.4%)	n=17 (3.9%)	n=16 (4.1%)	***<.001***
	Drinking water	n=69 (12.3%)	n=48 (10.7%)	n=35 (12.8%)	*.510*
	Source water	n=7 (8.9%)	n=9 (4.2%)	n=8 (3.4%)	*.145*
	Household surfaces	n=63 (8.2%)	n=11 (1.5%)	n=24 (2.5%)	***<.001***
	Household floor	n=30 (12.1%)	n=12 (4.9%)	n=12 (4.7%)	***.002***
	Clothing	n=12 (4.9%)	n=5 (2.1%)	n=16 (6.3%)	*.058*
	Hand-contact samples	n=16 (12.4%)	n=7 (10.1%)	n=41 (16.2%)	*.406*
	Household drains	n=96 (63.6%)	n=24 (16.1%)	n=0 (0.0%)	***<.001***
	River water	n=173 (68.1%)	n=130 (51.2%)	n=2 (50.0%)	***<.001***

^p values generated by Fishers exact test

Table 5.5. ESBL *K. pneumoniae* results, stratified by sample type and region.

Broad sample type		Urban ESBL KPN n (%)	Peri-urban ESBL KPN n (%)	Rural ESBL KPN n (%)	*p*
Human stool		n=96 (11.7%)	n=106 (10.8%)	n=137 (13.1%)	*.261*
Animal stool		n=14 (11.9%)	n=10 (4.4%)	n=29 (4.6%)	***.011***
Environment					
	Food	n=43 (12.9%)	n=36 (8.2%)	n=27 (6.8%)	***.015***
	Drinking water	n=65 (11.5%)	n=60 (13.4%)	n=33 (12.1%)	*.838*
	Source water	n=4 (5.1%)	n=11 (5.1%)	n=4 (1.7%)	*.088*
	Household surfaces	n=47 (6.1%)	n=23 (3.1%)	n=22 (2.3%)	***<.001***
	Household floor	n=9 (3.6%)	n=9 (3.7%)	n=7 (2.8%)	*.819*
	Clothing	n=5 (2.0%)	n=11 (4.6%)	n=10 (3.9%)	*.279*
	Hand-contact samples	n=14 (10.9%)	n=5 (7.2%)	n=28 (11.1%)	*.701*
	Household drains	n=44 (29.1%)	n=14 (9.4%)	n=0 (0.0%)	***<.001***
	River water	n=88 (34.6%)	n=68 (26.8%)	n=2 (50.0%)	*.077*

^p values generated by Fishers exact test

5.5. Flux in human ESBL colonisation

Only 4 households in the longitudinal cohort (i.e. 195/300 households, 65 per region) did not return at least one ESBL-E or ESBL-K human stool result over the total study period, with the other 191 households having ≥1 ESBL colonised individual at some point. There were no ESBL-free households in the urban setting (**Figure 5.5**), 3 ESBL-free households in the peri-urban setting (**Figure 5.6**) and 1 ESBL-free household in the rural setting (**Figure 5.7**). It was evident that amongst household participants the ESBL colonisation status regularly fluctuated and 78.9% (n=585/741) of participants returned at least 1 ESBL result over the ~6-month timeframe. This was particularly high in the urban setting, where 84.1% (n=175/208) of participants returned ≥1 ESBL result, compared with 76.3% (n=206/270) in the peri-urban setting and 77.6% (n=204/263) in the rural setting. However, there were no statistical differences between the flux in ESBL status depending on the region (*p = .086*, Fishers exact).

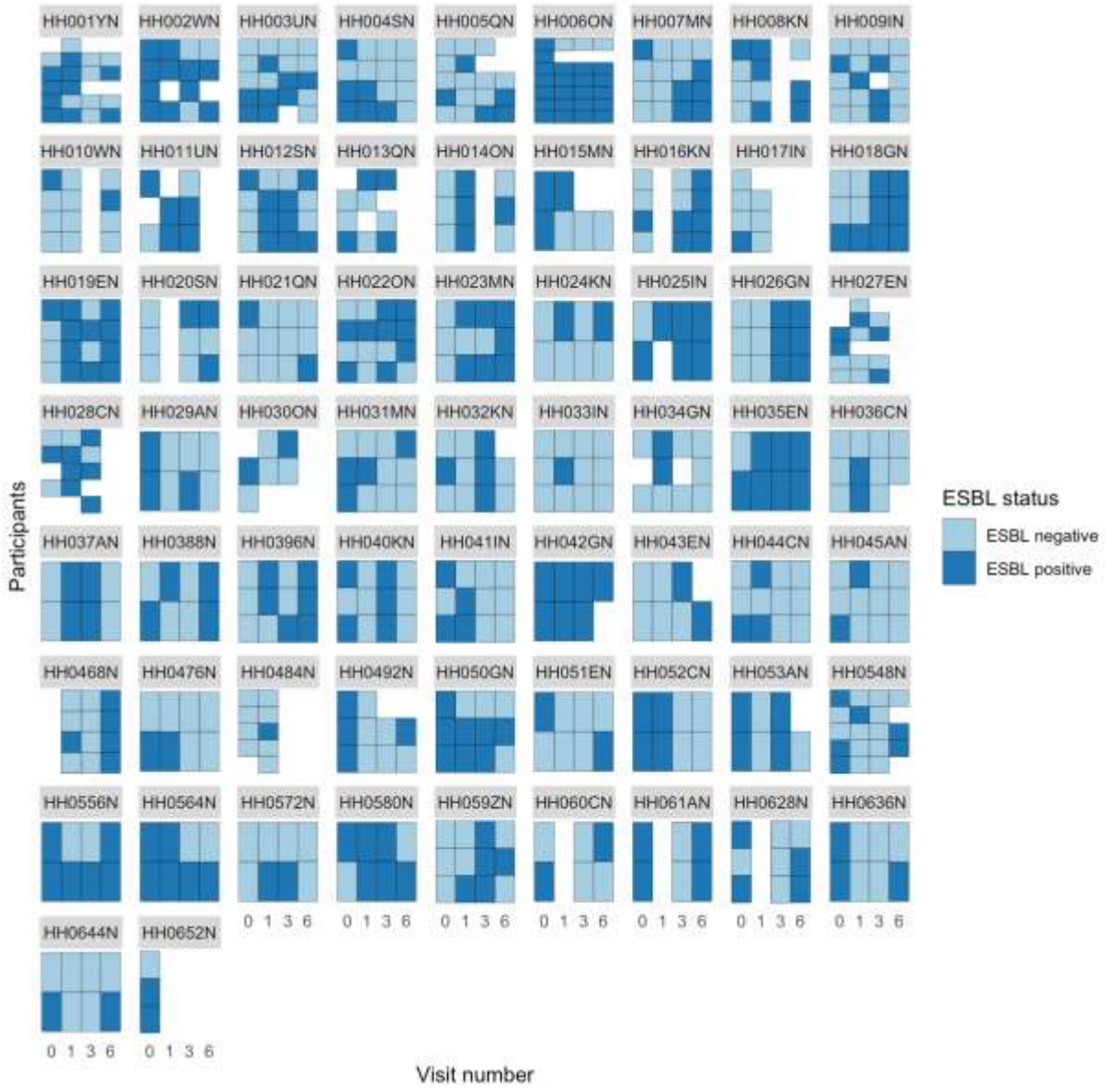


Figure 5.5. Facet Plot showing flux of human ESBL (*E. coli* or *K. pneumoniae*) colonisation amongst **urban** household members over time, grouped by the 65 households recruited. Each row represents a participant, each column represents a visit, and each small square is a sample coloured by EBSL status (positive or negative). Where no sample was returned for an individual at a visit the square remains blank.

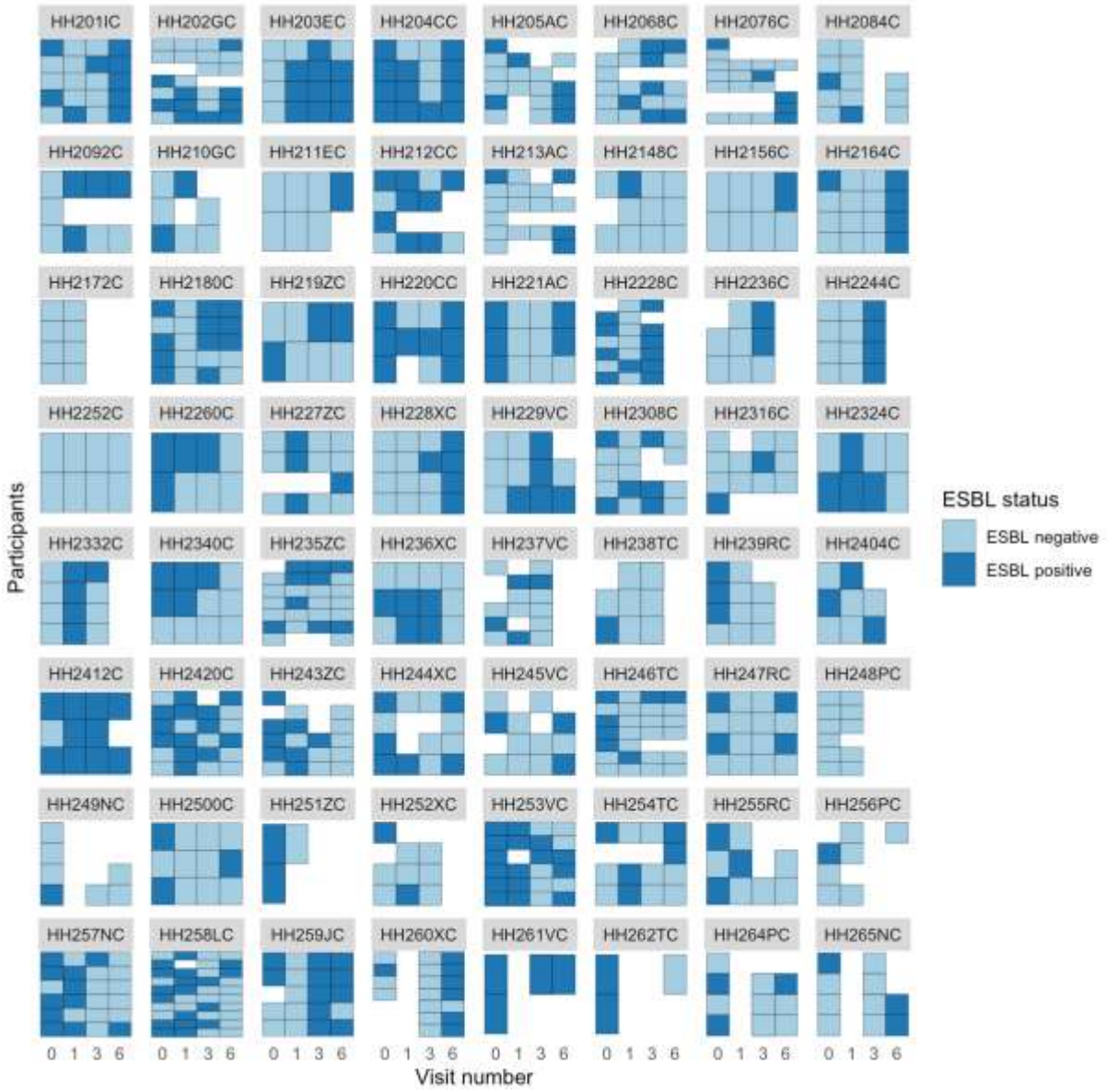


Figure 5.6. Facet Plot showing flux of human ESBL colonisation (*E. coli* or *K. pneumoniae*) amongst **peri-urban** household members over time, grouped by the 65 households recruited. Each row represents a participant, each column represents a visit, and each small square is a sample coloured by EBSL status (positive or negative). Where no sample was returned for an individual at a visit the square remains blank.

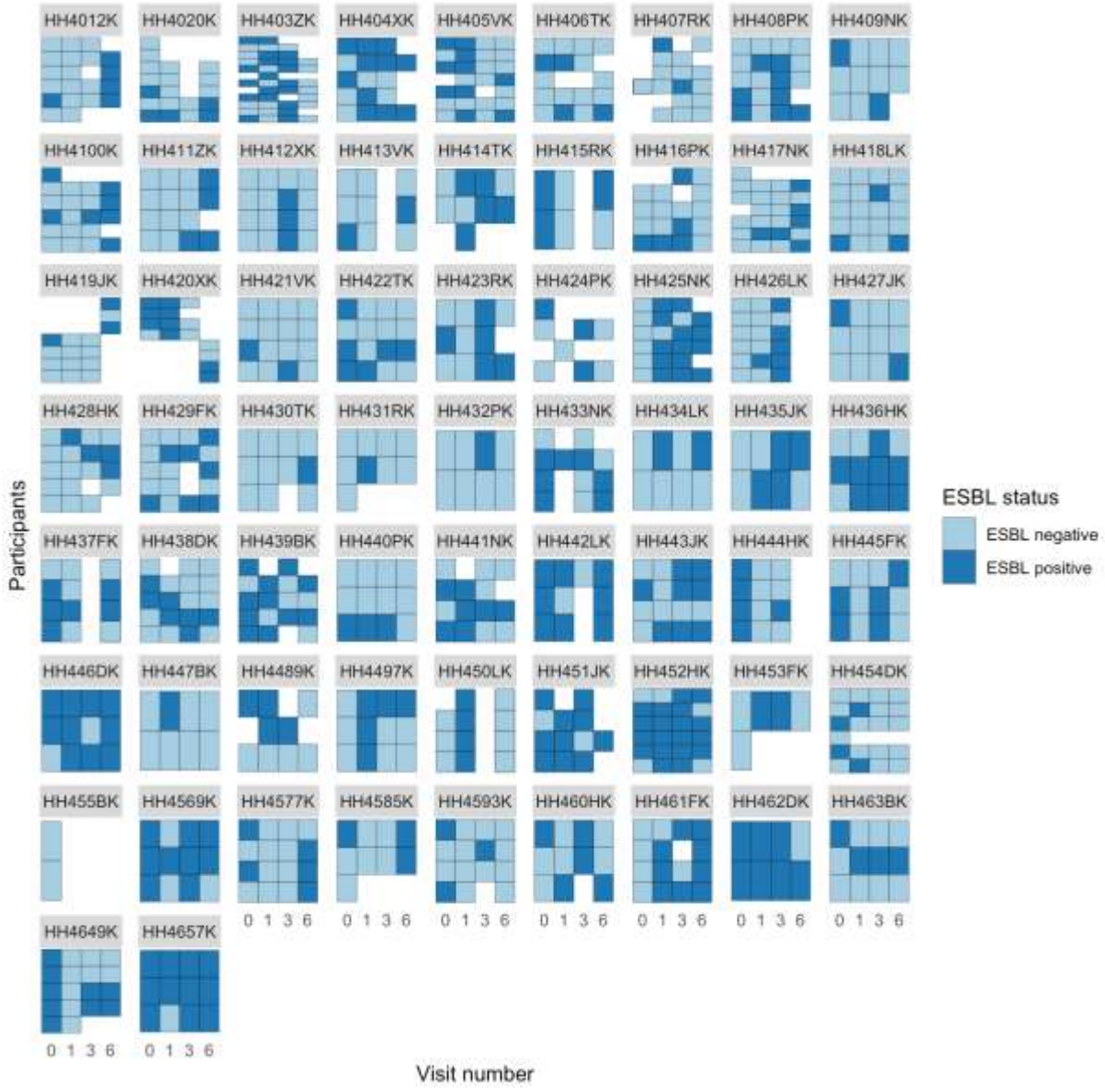


Figure 5.7. Facet Plot showing flux of human ESBL (*E. coli* or *K. pneumoniae*) colonisation amongst **rural** household members over time, grouped by the 65 households recruited. Each row represents a participant, each column represents a visit, and each small square is a sample coloured by EBSL status (positive or negative). Where no sample was returned for an individual at a visit the square remains blank.

78.9% of people were colonised at some point with either ESBL-E or ESBL-K, illustrating that ESBL status is likely to be both transient and the norm. 9.3% (n=69) of participants are always ESBL colonised, 21.1% (n=156) are uncolonised and 69.6% (n=516) are ESBL colonised "sometimes", equating to a ratio of 2.3: 7.5: 1.0 (never:sometimes:always) (**Table 5.6**). Little difference was identified between the regions (**Figure 5.8 & Table 5.6**). In relation to colonisation with ESBL-E, 6.9% (n=51) of participants are always colonised, and 66.5% (n=493) colonised only "sometimes" (**Figure**

5.9 & Table 5.6). ESBL-K colonisation is far less frequent, with 1.2% (n=9) of participants always colonised and 32.1% (n=238) colonised only "sometimes" (**Figure 5.10 & Table 5.6**).

Table 5.6. Flux of ESBL, ESBL-E and ESBL-K colonisation status expressed as ratios

Region	**Ratio ESBL-E colonisation** (never:sometimes:always)	**Ratio ESBL-K colonisation** (never:sometimes:always)	**Ratio ESBL colonisation** (never:sometimes:always)
Urban	2.3: 7.1: 1.0	47.7: 20.7: 1.0	1.2: 5.5: 1.0
Peri-urban	4.2: 9.8: 1.0	46.5: 20.0: 1.0	3.0: 8.8: 1.0
Rural	5.8: 13.5: 1.0	82.5: 46.0: 1.0	2.8: 8.7: 1.0
Total	3.9 : 9.7 : 1.0	54.9 : 26.4: 1.0	2.3: 7.5: 1.0

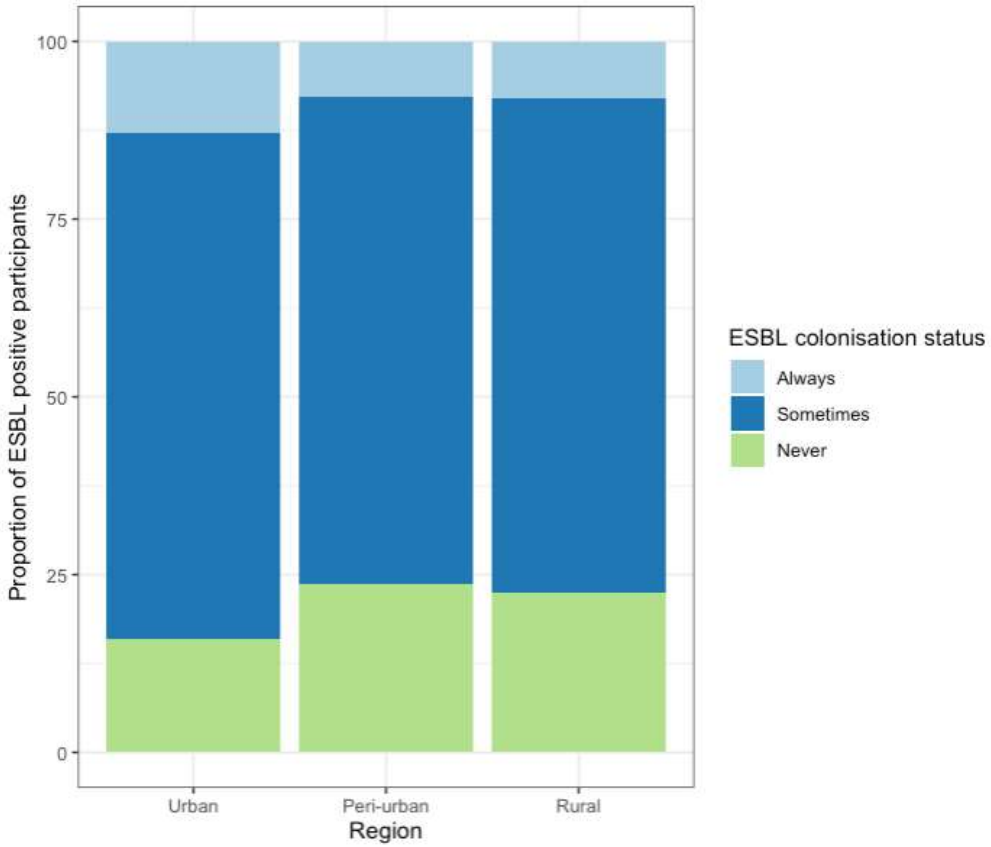


Figure 5.8. Plot of human **ESBL** (*E. coli* or *K. pneumoniae*) colonisation status, categorised into always (*all stool samples returned a positive result from the same individual*), never (*all stool samples returned a negative result from the same individual*) and sometimes (*stool samples returned both a positive and negative result from the same individual*), stratified by region.

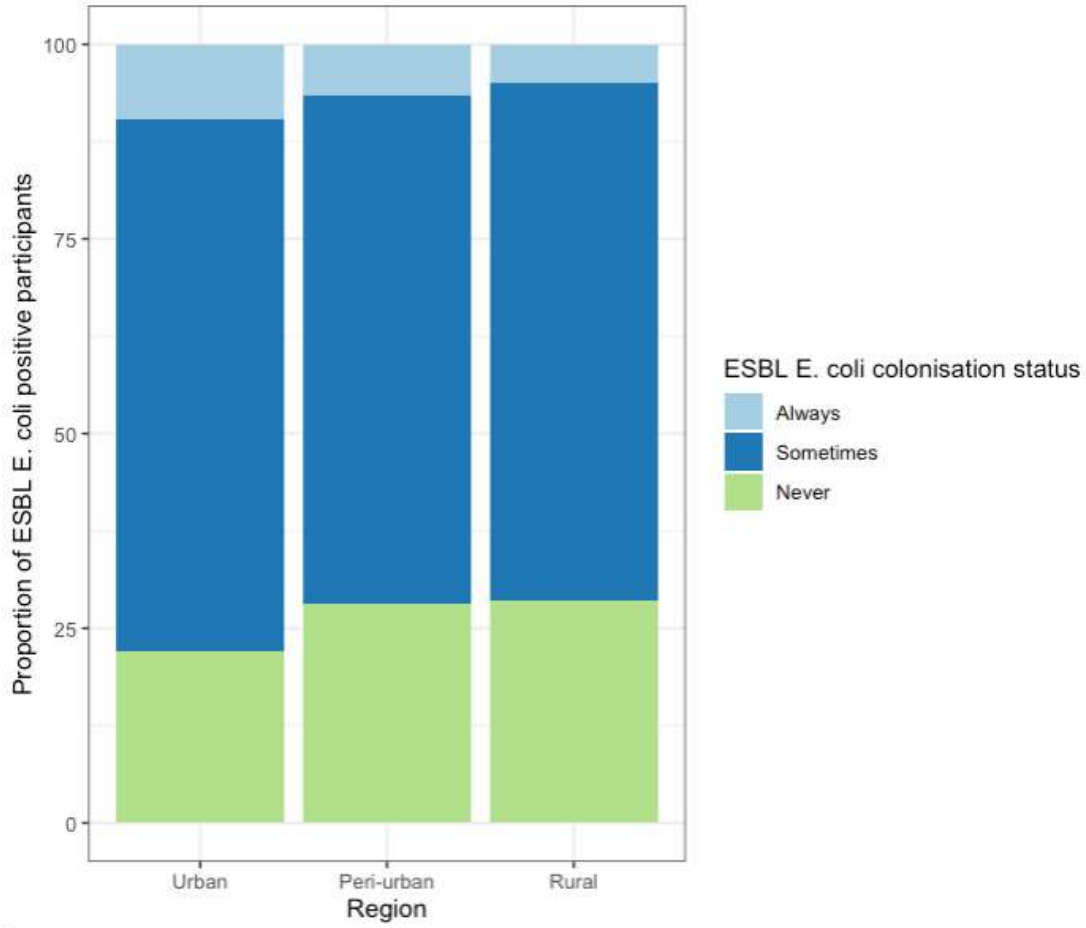


Figure 5.9. Plot of human **ESBL *E. coli*** colonisation status, categorised into always (*all stool samples returned a positive result from the same individual*), never (*all stool samples returned a negative result from the same individual*) and sometimes (*stool samples returned both a positive and negative result from the same individual*), stratified by region.

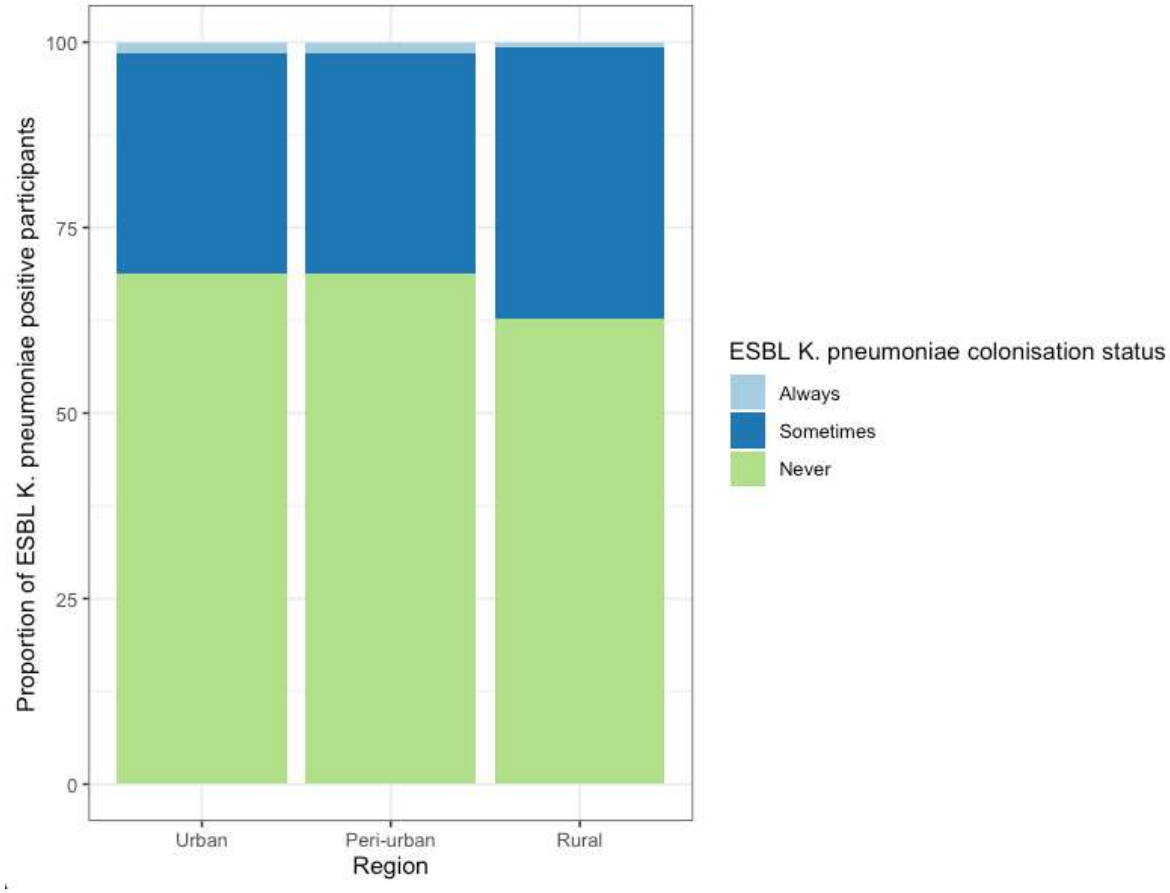


Figure 5.10. Plot of human **ESBL *K. pneumoniae*** colonisation status, categorised into always (*all stool samples returned a positive result from the same individual*), never (*all stool samples returned a negative result from the same individual*) and sometimes (*stool samples returned both a positive and negative result from the same individual*), stratified by region.

5.6. ESBL colonisation in animals

29.8% (n=290) of animal samples from 9/13 species were ESBL positive. Only geckos, rabbits, wild birds and dairy cattle were not identified as being ESBL colonised (**Figure 5.11**). There was a clear difference in the prevelance of ESBL colonisation between the animal species (X^2, p = <.001), with pigs most commonly colonised (56.8%), followed by companion animals (46.6%), poultry (32.5%), goats (27.1%), cattle (17.1%) and wild animals (3.8%) (**Table 5.7**). No pig or cattle samples were obtained from the urban region, and no cattle samples obtained from the peri-urban region for assessment. Poultry, consisting of chickens, doves and ducks, provided the greatest number of EBSL positive animal samples (n=148), due in part to the high numbers of these animals owned at households, and returned ESBL prevalence rates of 32.7%, 33.3%, and 29.2% respectively (**Figure 5.11**).

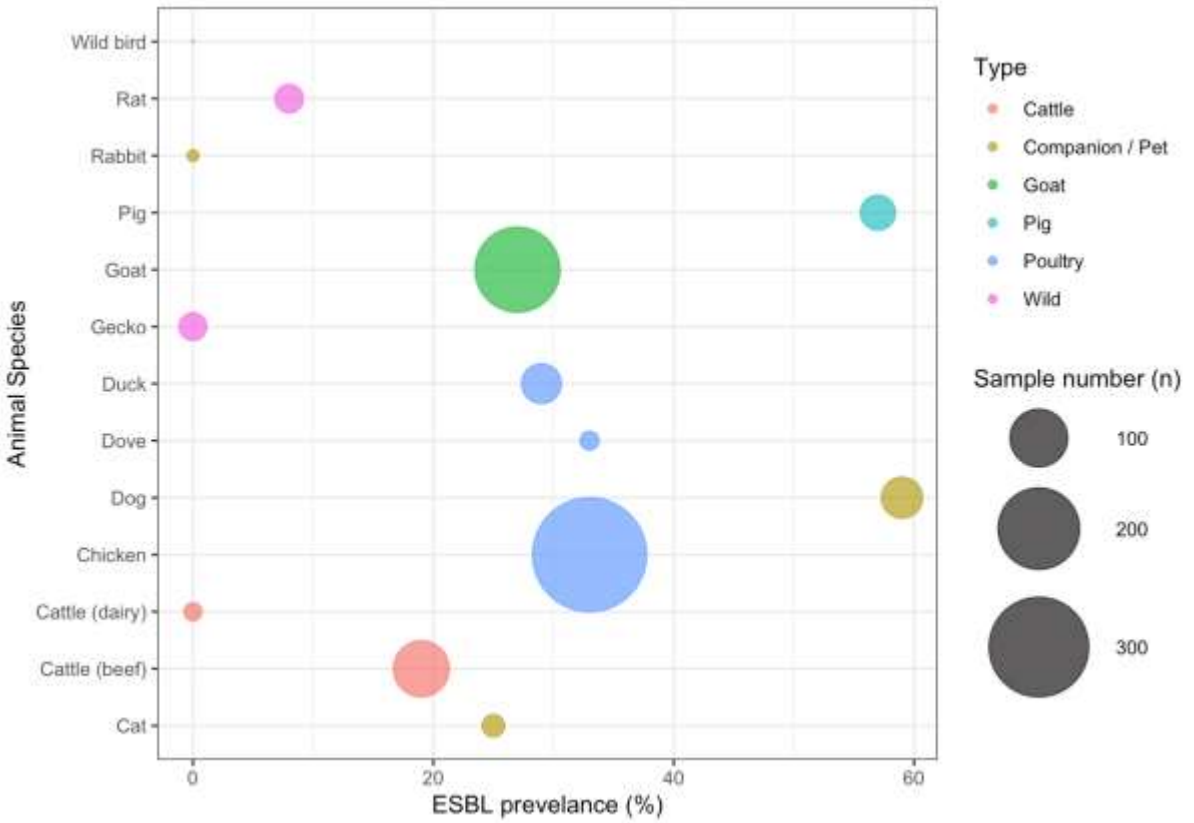


Figure 5.11. Bubble plot of ESBL (*E. coli* or *K. pneumoniae*) prevalence in stool samples obtained from a composite of urban, peri-urban and rural animals, stratified by species, and categorised by animal type. The volume of the circle represents the number of samples processed for each species.

There were regional differences seen in the prevelance of ESBL colonisation amongst poultry, goats and companion animals, with urban households having the highest ESBL rates for these species, at 64.7%, 100.0% and 65.8% respectively (**Table 5.7**). No regional differences existed between the prevelance of ESBL colonisation amongst pigs or wild animals.

Table 5.7. Regional comparison of ESBL (*E. coli* or *K. pneumoniae*) prevalence in stool samples obtained from animals, stratified by animal grouping.

		Region n (%)			
Animal group	**Total**	**Urban**	**Peri-urban**	**Rural**	***p***
Cattle*	n=18 (17.1%)	*NA*	*NA*	n=18 (17.1%)	*NA*
Companion	n=34 (46.6%)	n=25 (65.8%)	n=6 (22.2%)	n=3 (37.5%)	***.002***
Goat	n=59 (27.1%)	n=2 (100.0%)	n=10 (18.5%)	n=47 (29.0%)	***.023***
Pig*	n=21 (56.8%)	*NA*	n=5 (50.0%)	n=16 (59.3%)	*.716*
Poultry	n=148 (32.5%)	n=33 (64.7%)	n=48 (39.0%)	n=67 (23.8%)	***<.001***
Wild animal*	n=2 (3.8%)	n=2 (10.5%)	n=0 (0.0%)	n=0 (0.0%)	*.314*

*^p values generated by Fishers exact test. * Low species number in study (less than 5% of total animals).*

There were regional differences in ESBL-E colonisation rates amongst companion animals and poultry, where the urban setting has substantially higher rates of ESBL colonisation in poultry (62.7%) and pets (63.2%) than the other regions (**Table 5.8.**). For ESBL-K, limited returns in animals did not allow for a detailed analysis (**Table 5.9**)

Table 5.8. Regional comparison of ESBL ***E. coli*** prevalence in stool samples obtained from animals, stratified by animal grouping.

	Region n (%)			
Animal group	**Urban**	**Peri-urban**	**Rural**	***p***
Cattle*	*NA*	*NA*	n=15 (14.3%)	*NA*
Companion	n=24 (63.2%)	n=5 (18.5%)	n=2 (25.0%)	***<.001***
Goat	n=1 (50.0%)	n=10 (18.5%)	n=43 (26.5%)	*. 246*
Pig*	*NA*	n=5 (50.0%)	n=16 (59.3%)	*. 715*
Poultry	n=32 (62.7%)	n=44 (35.8%)	n=62 (22.0%)	***<.001***
Wild animal*	n=2 (10.5%)	n=0 (0.0%)	n=0 (0.0%)	*.314*

*^p values generated by Fishers exact test. * Low species number in study (less than 5% of total animals).*

Table 5.9. Regional comparison of ESBL ***K. pneumoniae*** prevalence in stool samples obtained from animals, stratified by animal grouping.

	Region n (%)			
Animal group	**Urban**	**Peri-urban**	**Rural**	***p***
Cattle*	*NA*	*NA*	n=7 (6.7%)	*NA*
Companion	n=6 (15.8%)	n=1 (3.7%)	n=1 (12.5%)	*.245*
Goat	n=1 (50.0%)	n=2 (3.7%)	n=7 (4.3%)	*.106*
Pig*	*NA*	n=0 (0.0%)	n=2 (7.4%)	*1.00*
Poultry	n=5 (9.8%)	n=7 (5.7%)	n=12 (4.3%)	*.249*
Wild animal*	n=2 (10.5%)	n=0 (0.0%)	n=0 (0.0%)	*.314*

*^p values generated by Fishers exact test. * Low species number in study (less than 5% of total animals).*

Overall, ESBL *E. coli* were more prevalent than ESBL *K. pneumoniae,* with 83.1% (n=261) of the total ESBL animal samples yielded *E. coli* compared to 16.9% (n=52) *K. pneumoniae*; 81.7% (n=237) of animal samples returned ESBL *E. coli* only, and 7.2% (n=21) returned ESBL *K. pneumoniae* only, with 11.1% (n=32) having both bacteria. There were some differences in animal species, with pigs rarely having ESBL-K, and wild animals invariably having the presence of both ESBL-E and ESBL-K (**Figure 5.12**).

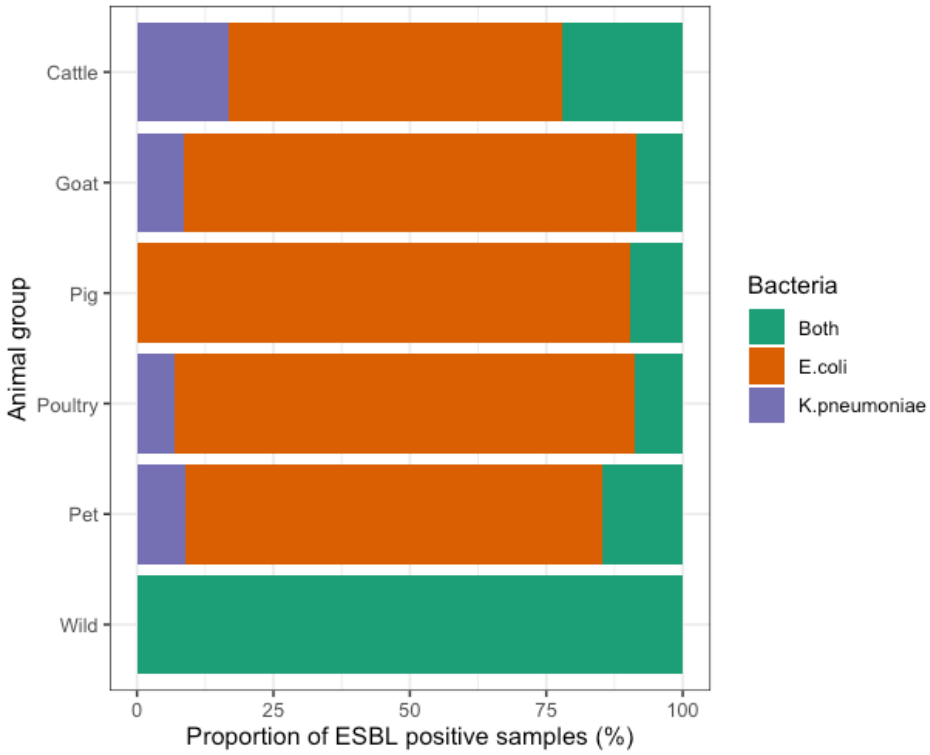


Figure 5.12. Proportion of the total household human stool, animal stool and environmental samples that were positive for ESBL *E. coli*, ESBL *K. pneumoniae* or both ESBL *E. coli* & ESBL *K. pneumoniae*, stratified by sample type and bacterial species.

There was no difference between the rates of ESBL colonisation in humans whether households reported owning animals or not (p=.842); this held true for poultry and companion animal ownership (p=.819 and p=.929).

5.7. Variations in ESBL contamination within the household environment.

ESBL-E or ESBL-K were detected on 6.8% (n=166) of the household environmental surfaces sampled, and 45.6% (n=89/195) of households had ESBL surface contamination at some point during the study. A broad range of internal household sampling points were selected, based on information from the WASH observations, and ESBL bacteria were found at 22/26 location types (**Figure 5.13**). The samples with the highest ESBL contamination were those relating to household waste management (i.e. drainage), items that had regular hand-contact (i.e. winnowers and implement handles) and surfaces or items associated with food-hygiene (cleaning area or rags/wasters). 9.1% (n=68) of the household floor samples were positive for ESBL-E or ESBL-K, and 29.2% (n=57/195) of households had ESBL contamination of the floors at some point during the study.

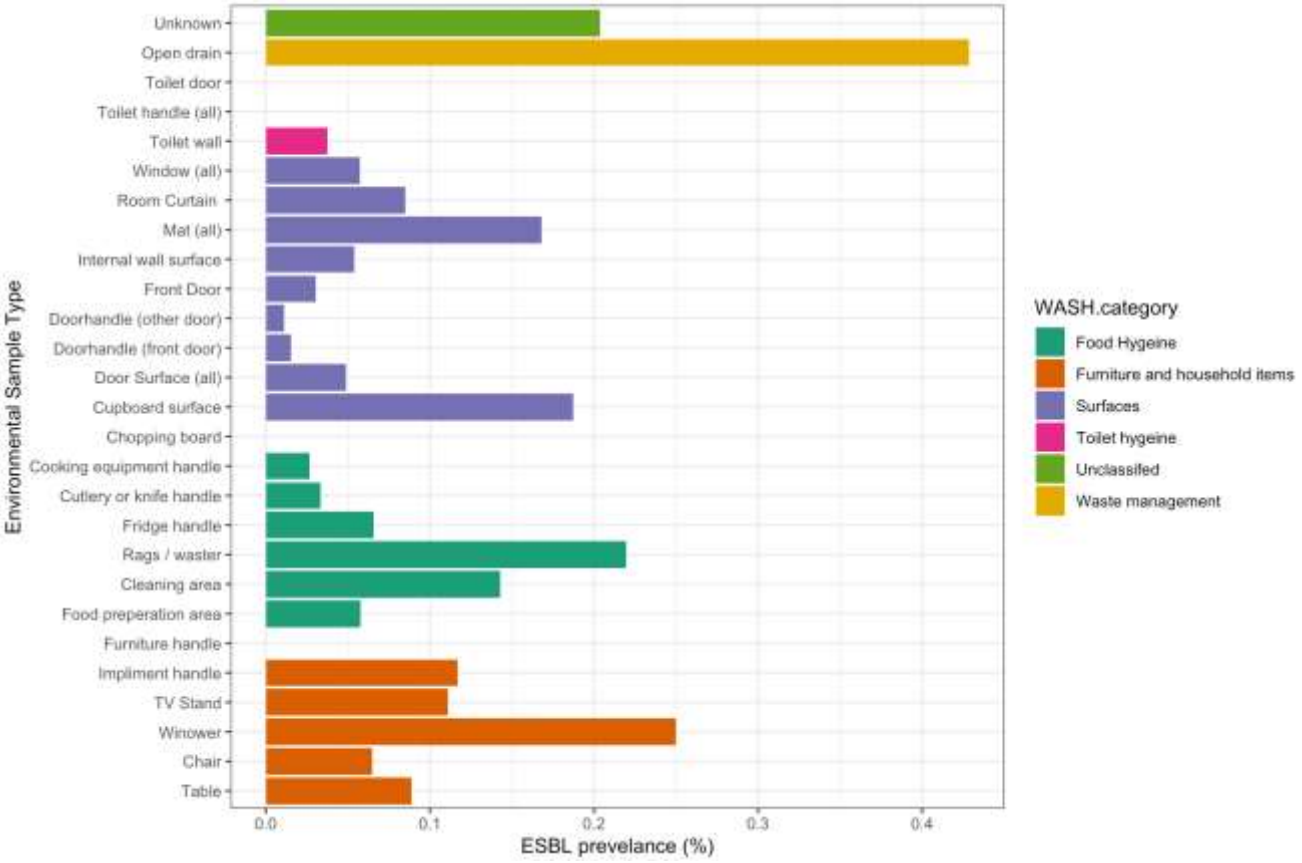


Figure 5.13. Plot of the ESBL (*E. coli* or *K. pneumoniae*) prevalence in household environmental samples, stratified by WASH category.

5.8. Variations in ESBL contamination within household food and drinking water.

There were variations in the ESBL contamination of fruit and vegetables, with 10.8% (n=59) of fruit and 15.7 % (n=96) of vegetables shown to be ESBL contaminated (*p=.016*). However, no difference in ESBL contamination was found between food that was cooked 14.3% (n=2) or uncooked 13.4% (n=153) (*p=1.00*), and there was little variance in the proportions of ESBL contamination of specific food produce (**Figure 5.14.**)

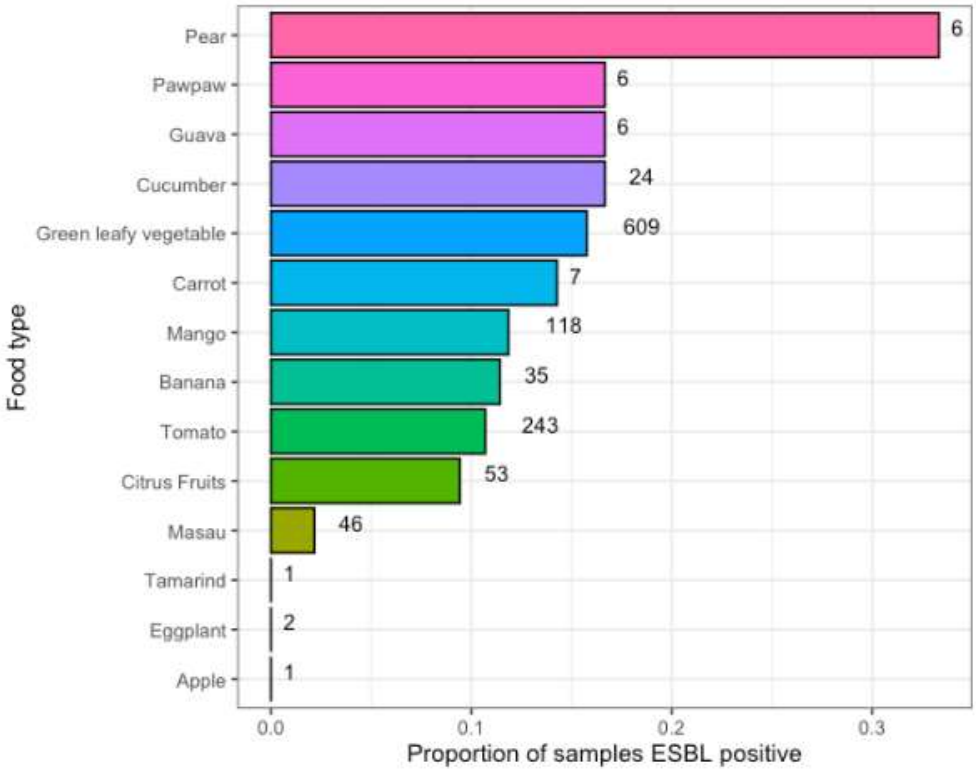


Figure 5.14. Plot of ESBL (*E. coli* or *K. pneumoniae*) identified in household food samples. The numbers of samples are expressed at end of columns.

21.3% of household drinking water was contaminated with ESBL-E or ESBL-K. There was a difference in the rates of ESBL identified in drinking water samples, depending on the primary water source used (X^2, p = .003), with the lowest contamination found in samples using tap or bottled water (**Figure 5.15a**). However, there was no statistical difference between the rates of ESBL contamination found in storage vessels that were uncovered compared to those that were covered (X^2, p = .578) (**Figure 5.15b**).

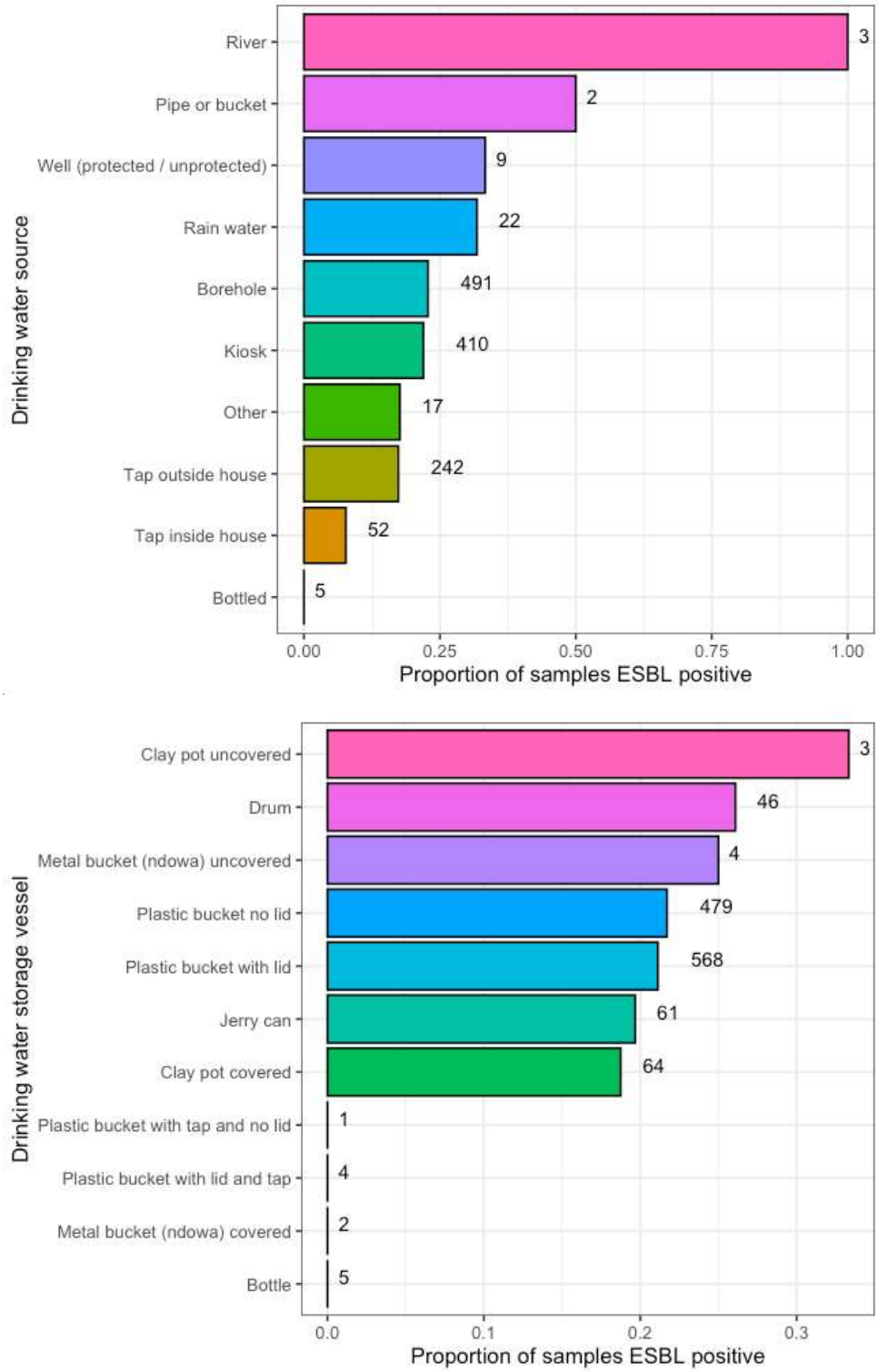


Figure 5.15. Plot of proportion of household drinking water samples positive for ESBL (*E. coli* or *K. pneumoniae*) classified by (a) primary source of drinking water used, and (b) storage vessel used. Numbers of samples expressed at end of columns.

5.9. ESBL contamination within the external environment.

88.7% (n=266) of wastewater samples collected were from open drains surrounding the house, with visible faecal effluent present at 11.3% (n=34) and plastic rubbish present at 41.7% (n=125) of the sampling sites. In total, 46.0% (n=138) of these drain samples yielded ESBL bacteria.

River water was collected from the points at which households interacted with the watercourse, or alternatively, for households that reported no river interactions, the nearest river site to households (**Chapter 2**). River water in the urban and peri-urban regions had the highest overall ESBL prevalence at 66.2% (n=338). There was a higher presence of ESBL-E (59.6%, n=305) compared to ESBL-K (30.9%, n=158) in river samples (**Figure 5.4**), however it should be noted that river water had the highest presence of either species in any of the sample types included in the study. There was a relationship between the physical properties of the rivers and the prevelance of ESBL bacteria, with increased turbidity and fast flow associated with higher levels of ESBL contamination (**Table 5.10**).

20.9% (n=52) of urban households and 37.0% (n=90) of peri-urban households reported using the river water that was sampled, and the most common reasons for river usage were cleaning clothes (59.9%, n=85) and interactions relating the commute to work (28.9%, n=41) or school (21.8%, n=31). No geospatial analysis was completed as part of this thesis.

Table. 5.10. Relationship between the physical properties of urban and peri-urban rivers and ESBL contamination

River water variable	Category				*p*
River water colour*	**Absent**	**Present (light)**	**Present (moderate)**	**Present (dark)**	
ESBL (any)	n=21 (53.8%)	n=81 (67.5%)	n=159 (64.1%)	n=65 (73.9%)	*.139*
ESBL-E	n=16 (41.0%)	n=73 (60.8%)	n=143 (57.7%)	n=60 (68.2%)	
ESBL-K	n=13 (33.3%)	n=38 (31.7%)	n=70 (28.2%)	n=30 (34.1%)	
River water turbidity*	**Clear**	**Cloudy (mild)**	**Cloudy (moderate)**	**Cloudy (severe)**	
ESBL (any)	n=29 (67.4%)	n=93 (56.0%)	n=145 (71.1%)	n=59 (72.0%)	***.012***
ESBL-E	n=23 (53.5%)	n=83 (50.0%)	n=131 (64.2%)	n=55 (67.1%)	
ESBL-K	n=16 (37.2%)	n=42 (25.3%)	n=68 (33.3%)	n=25 (30.5%)	
River water flow*	**Slow**	**Medium**	**Fast**		
ESBL (any)	n=174 (61.9%)	n=85 (66.4%)	n=67 (77.9%)		***.023***
ESBL-E	n=161 (57.3%)	n=73 (57.0%)	n=58 (67.4%)		
ESBL-K	n=76 (27.0%)	n=37 (28.7%)	n=37 (43.0%)		

**17/512 river samples missing metadata. p values obtained using X^2 test. Qualitative measurements. Detailed descriptions in the study SOPs (Chapter 2)*

5.10. Seasonal effects on ESBL prevalence

In Malawi the wet season falls sometime between Nov-Apr, with peaks rainfall in January and February and the dry season is between May-Oct. There was an apparent ESBL peak at the end of the wet season (**Figure 5.16**), and both ESBL-E and ESBL-K in the wet (26.1%, SD=43.9) compared to the dry (19.4%, SD=0.40) season across all samples collected (p=<.001). This was particularly true of ESBL carriage in both human and animal stool (**Table 5.11**). Furthermore, alongside this increased ESBL carriage, there is an associated higher presence of ESBL contamination in the household drinking water, floors and surfaces during the wet season (**Table 5.11**). No seasonal effects in ESBL prevelance are seen within household food items or the broader environments, inclusive of drains and rivers.

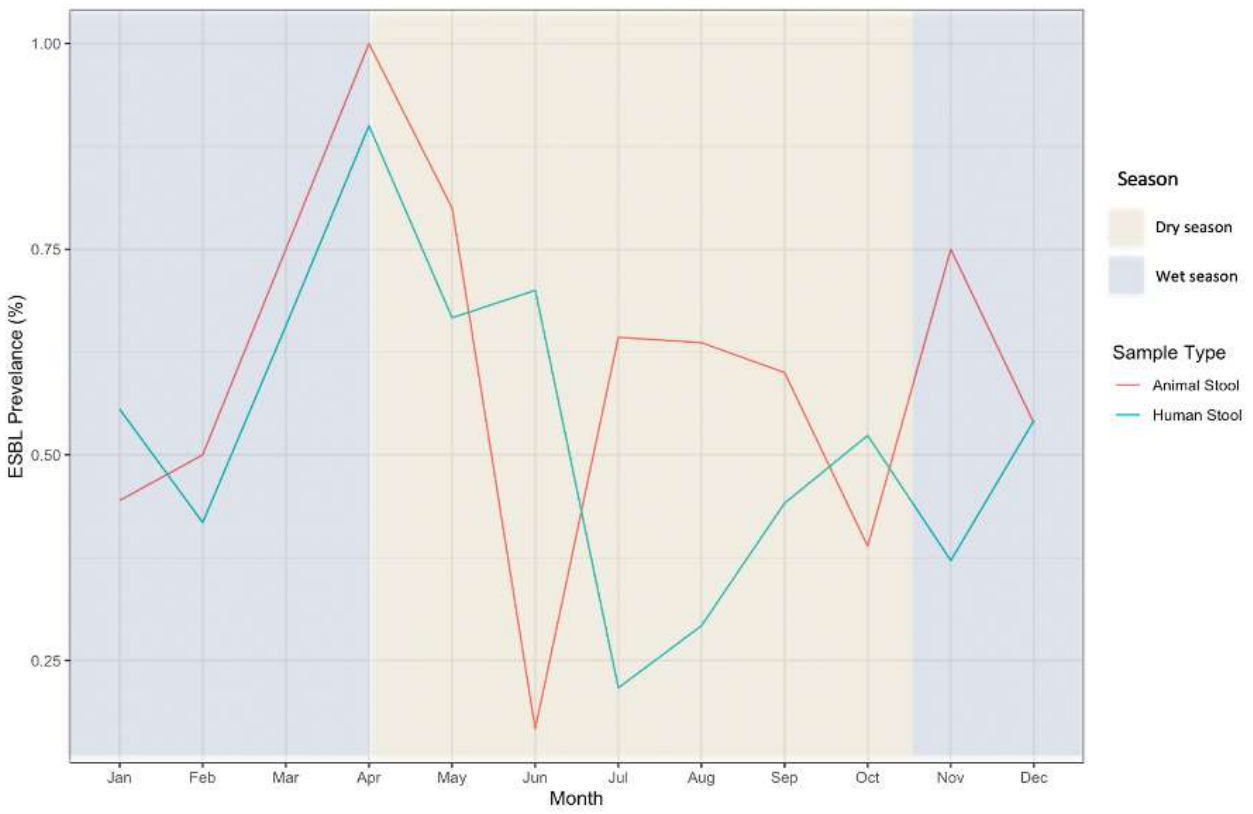


Figure 5.16. Monthly trend in ESBL (*E. coli* or *K. pneumoniae*) prevelance in human and animal stool, illustrating seasonal peaks and troughs.

Table 5.11. Seasonal variations in ESBL prevelance of household samples.

	ESBL prevelance by season (%, SD)		
Sample Type	**Wet**	**Dry**	***p***
Human stool	47.2% (SD=49.9)	36.6% (SD=48.2)	***<.001***
Animal stool	33.3% (SD=47.2)	25.5% (SD=43.6)	***.010***
Food	14.4% (SD=35.1)	12.3% (SD=32.9)	*.338*
Drinking water	26.2% (SD=44.0)	15.2% (SD=35.9)	***<.001***
Source water	6.5% (SD=24.7)	8.8% (SD=28.3)	*.413*
Household surfaces	8.8% (SD=28.3)	4.5% (SD=20.8)	***<.001***
Household floor	11.5% (SD=31.9)	6.6% (SD=24.9)	***.031***
Clothing	9.1% (SD=28.9)	5.6% (SD=23.0)	*.087*
Hand-contact samples	25.8% (SD=43.9)	17.9% (SD=38.4)	*.057*
Household drains	44.7% (SD=49.9)	48.2% (SD=50.2)	*.648*
River water	69.1% (SD=46.3)	62.9% (SD=48.4)	*.164*
Environmental survey	55.6% (SD=49.7)	52.9% (SD=50.0)	*.173*

^p values generated by X^2 test

5.11. Discussion

I have identified extremely high levels of ESBL *E. coli* and ESBL *K. pneumoniae* gut colonisation in humans and animals, alongside extensive ESBL contamination of the household and broader environments within urban, peri-urban and rural communities in southern Malawi. ESBL prevelance was found to be highest overall in the urban setting, particularly in the riverine network and in co-located animals, and there was a seasonal effect seen on the rates of human and animal ESBL carriage. These urban and seasonal effects are likely to result from interactions with a contaminated shared environment, which are in turn influenced by a deficiency of WASH infrastructure alongside key WASH behaviours pertaining to water usage, food-hygiene sanitation and waste management (**Chapter 4**).

Human ESBL (*E. coli* and *K. pneumoniae*) gut colonisation in southern Malawi is high. Previous reports of human ESBL colonisation from sub Saharan Africa (sSA) populations have ranged between 5-59%, with a pooled community ESBL *E. coli* colonisation estimate of 18% (99). Therefore, Malawian communities have a higher prevalence of ESBL colonisation than is typically seen across comparable sSA settings and is substantially higher than those rates seen in Europe (3-8%) or North America (3.4%), and on par with those reported is SE Asia (46%) (99).

There is a high degree of flux in the ESBL colonisation status of individuals over a 6-month period, which may, in turn, impact the accuracy of prevalence estimates. This phenomenon has previously been ascribed to ABU driving selection pressures in the gut (300). However, given the low ABU seen in the cohort (chapter 3), these findings may indicate that flux is a result of transmission between human-human or human to non-human sources. Equally, given the high levels of ESBL colonisation and flux seen within the cohort, we should remain open to the concept that ESBL is present in the microbiome of community participants most of the time, and this flux is stimulated by an absence of selection pressures combined with the insensitivity of phenotypic culture-based methods to identify ESBL bacteria.

A pre-enrichment step was employed to increase the chance of recovery of gram-negative bacteria, followed by CHROMagar™ media to select for ESBL bacteria. The manufactures documentation states CHROMagar™ has a 98% sensitivity, and independent evaluation against other commercially available ESBL medias has reported a 100% sensitivity for the detection of ESBL bacteria (362,363), however, there is less data on specificity of CHROMagar™ agar for speciating bacteria, and reports have ranged from 72-97% (363,364). In this regard, the microbiological results may under-report the presence of

ESBL bacteria, and CHROMagar™ has not been optimised for other classes of beta-lactamases i.e. AmpC.

Within the human and animal stool samples, I found a higher proportion of ESBL-E compared to ESBL-K (Human stool ESBL-E:ESBL-K ratio = 1.0:0.3). This ratio was not seen in environmental samples, where there was either a similar proportion of ESBL-E and ESBL-K (ESBL-E: ESBL-K = 1.0:0.9), or in the case of food and stored water an abundance of ESBL-K (Food ESBL-E:ESBL-K ratio = 1:0:1.5). This could be explained by differences in the ecological niches of ESBL-E and ESBL-K within our setting, and identification of clonal lineages via WGS will allow us to better characterise the niches for both ESBL *E. coli* and ESBL *K. pneumoniae*, the interconnections that exist between human, animal and environmental compartments and the relation to isolates from bloodstream infection.

ESBL prevalence in animals was between 0-56.8% depending on the species. In LMICs, *E. coli* ST131 containing *bla*CTX-M-15 have been identified in the guts of subsistence farmers and their food-production animals (122). I found a high rate of ESBL *E. coli* and *K. pneumoniae* in poultry samples, which are the most frequently owned animals in the study population, often sharing the household environment with participants (**Chapter 3 & 4**). Furthermore, poultry are often kept inside the household, whilst other food-production animals were kept in the household compound, nearby to the house, and high ESBL rates were seen in pigs (56.8%), goats (27.1%), and cattle (17.1%). Sharing of ESBL genes and bacteria between these animal species and humans has been documented (122,141,314,365).

Fewer data are available on companion animals in LMICs, however, pets have been shown to carry *bla*CTX-M genes on plasmids found in humans (i.e. IncF and IncI1), indicating that in HIC settings companion animals may be a source of plasmid-mediated ESBL resistance in humans (311,366). Here, I discovered ESBL colonisation rates of 46.6% in companion animals, and ESBL colonisation of this animal group has been shown in the literature to correlate with owner colonisation (39,367,368). Pets, in particular dogs, are not always kept inside the house. The role of companion and livestock animals that do not share the same living space, but shared nearby environments and have regular human contact may well be an important contributor to ESBL household transmission. Wild animals had the lowest prevelance of ESBL (3.8%), and this may be related to low sample numbers, the methodologies used for sampling or a true reflection of the ESBL prevelance in species with less human contact.

A limitation within the sampling method was that only poultry samples were taken directly from the animal, whilst others were obtained from the ground, and therefore spent varying lengths of time in contact with the external environment. This may have led to contamination of stool samples with environmental ESBL-E or ESBL-K. I chose to prioritise sampling from within the stool, to minimise contamination of the outer surface; however, there may have been some false positives, explaining some of the differences seen between poultry and other animal species.

Environmental reservoirs of ESBL-producing Enterobacteriaceae supplied by food-producing or companion animals regularly exchange clones and MGEs with the human reservoir by transposition or transduction, leading to clonal and epidemic plasmid spread within the community (122,369). Key factors in this process are the frequency and load to which these environments are contaminated with AMR bacteria of human and animal origin; a feature governed, in part, by local waste management practices. These results show that whilst the prevalence of ESBL-E or ESBL-K varies by environment, ESBL bacteria were found to some extent in all areas sampled, including 9.1% of household floors, 6.8% of household surfaces and 38.5% of external environmental samples. Given the absence of WASH infrastructure and variations in WASH behavioural practices seen within households, improper waste management may lead to environmental contamination, posing a risk for ongoing ESBL transmission within the household, which warrants further evaluation (**Chapter 6**). In this descriptive chapter, I cannot determine the direction of travel of ESBL bacteria, and the higher ESBL contamination rates seen in environmental samples where faecal contamination is highest (i.e. drains and rivers) may only represent a terminal sump, however, the paucity of WASH infrastructure and waste management practices raises the strong possibility that ESBL bacteria make their way back into humans from these reservoirs. Framing the environmental contexts into potential "sources" and "sinks" of AMR may be a useful approach in future analysis to contextualise the impacts of key environmental reservoirs on human transmission and infection/re-infection with ARB.

The relevance of EBSL bacteria in these sites and the role the riverine and drainage systems play in the ongoing transmission are complex and difficult to quantify due to dynamics in exposure routes and times, along with fluctuations in AMR bacterial load. An example of this complexity is the finding of similar ESBL bacterial prevelance rates in rivers from both the wet and dry season. Given rivers in Malawi undergo dramatic changes in water volumes between the wet and dry seasons (370,371) this seemingly similar prevelance in spot sampling may represent a huge difference in absolute bacterial load, leading to widespread dispersal across the environment. In this regard, dilution of AMR from rainwater highlights the scale of, rather than the solution to faecal pollution. Given that drainage

systems and rivers contain high levels of ESBL bacteria, and households frequently report human and animal interactions with these sites, I hypothesise that these sites serve as important reservoirs for the spread, maintenance and acquisition of ESBL bacteria; this will be evaluated through ongoing genomic analysis being undertaken by the DRUM consortium. Using phenotypic results alone, I cannot identify whether ESBL rates in these samples are related to human ESBL colonisation or are due to anthropogenic causes.

Another entry point into the household for ESBL bacteria is through contaminated food and water (181,349,350,372,373). ESBL contamination of drinking water sources has been previously seen in displaced populations and areas with reduced access to safe WASH infrastructure or adequate surveillance systems (181,230,243,374). Within southern Malawi, several sources of water are used, with some households using piped or bottled water, but ordinarily, participants utilise communal kiosks and boreholes located outside the household compound. According to the World Health Organization, a zero count of *E. coli* per 100 ml of water is considered safe for drinking (344). A count of 1–10 MPN/100 ml is regarded as low risk; 11–100 MPN/100 ml is medium risk. Safe drinking water should be free from *E. coli*, and while there are no recommended targets for AMR bacterial levels, clearly a zero count for ESBL (if not many other ARGs) in drinking water would be appropriate (344). In this study 5.5% of drinking water was contaminated with ESBL bacteria at source and 21.3% of stored drinking water had ESBL-E or ESBL-K present. I was unable to find a difference between the contamination rates of drinking water depending on the storage vessel used or whether it was covered but did see that the primary source of water used impacted on the probability of contamination. Given it has already been identified that drinking water is not treated prior to ingestion, this worrying finding highlights a daily entry point for ESBL acquisition, and potentially key reservoir within the household that is amenable to intervention.

Food can be contaminated with ESBL bacteria at any point from farming to ingestion, with the type of food impacting on the risk of contamination. Therefore, I focussed on green leafy vegetables and fruit, as these foods are handled by local market vendors and household participants and often eaten uncooked but recognise that this is a limitation. Here, I found 13.4% of the total food samples were contaminated with ESBL-E or ESBL-K, 15.7% of green leafy vegetables and 10.8% of fruits. There were only a few food items obtained that had been cooked, and there were no statistical differences in the presence of bacteria between the cooked and raw foods sampled. A market study is currently underway in Malawi to better evaluate the role of ESBL contamination in purchased goods.

Food and water are contaminated via ineffectual hygiene, especially hand hygiene (373,375,376). Recent modelling studies have suggested that hand hygiene improvements will have more impact on the reduction of household ESBL transmission than a reduction in ABU (106). Rinse water samples collected from the hands of household members in the study illustrate the contamination of people's hands and serve as a proxy for ineffectual hand hygiene. Here, 21.3% of samples from participants hands were found to be contaminated with ESBL *E. coli* or ESBL *K. pneumoniae*.

Previously I highlighted regional differences in WASH practices, with the poorest sanitation found in the urban setting. Informal urban sSA settlements have been proposed as hotspots of gram-negative AMR, and there is great interest in establishing the role of WASH and the broader environment (209). In this analysis, I identified a higher rate of ESBL colonisation in the urban setting compared to the other regions in both human and animal stool, food, household surfaces, floors and the external environment of the urban region compared to the peri-urban and rural sites. This was predicted based on the findings presented in Chapter 3 and 4.

In terms of urban WASH infrastructure, sanitation and food-hygiene practices, households often share their toilet with other households, and participants occasionally practice open defecation. Sharing of toilets with improper hygiene methods increases the risk of faecal-oral acquisition of ESBL bacteria between households, and between household members, as it does in other pathogens (377,378). Furthermore, the absence of space for home-grown crops leads to urban households relying on local markets for fresh fruit and vegetables and the regular purchasing of street food. Poor environmental health standards at urban street vendors and markets could be a regional driver of food contamination.

Animal ownership, and co-habitation is high amongst urban households, and urban households rarely dispose of animal waste appropriately, and this cycle of poor sanitation practices and high levels of contamination could maintain high levels of ESBL carriage in animals and humans. Modelling approaches will be undertaken on this dataset to determine the effects of animal co-habitation on ESBL carriage from the total dataset, adjusting for regional covariates and sample numbers. It should be noted that animals do not have to be owned by the household to share the same space. The nature of dense urbanisation permits environments where animals are free to roam between household compounds, and an illustration of this is evident from the households where animal stool has been found at the compound premises, but the household does not report owning any animals (*observational data not included in thesis*).

Within the framework of transmission and acquisition of AMR bacteria a shared environment is important (194,315–318). In the urban setting interactions with the broader environment, in particular rivers and drains are commonplace, and these are the areas which have the highest rates of ESBL seen in the study, at 74.0% and 70.9% respectively. The risks and role of the riverine network on human ESBL colonisation have not been fully described in sSA settings, but there is evidence from HIC settings of their importance, and therefore this could be an important local driver in the urban setting (53–56). The absence of a functioning sewerage network, poor investment in WASH infrastructure, the proximity of household toilets and drains all likely contribute.

A seasonal effect in ESBL colonisation of humans and animals was identified, with a higher likelihood of ESBL-E or ESBL-K colonisation in the wet season. Alongside increased ESBL carriage, there was an associated higher presence of ESBL contamination in the household drinking water, floors and surfaces during the wet season with no seasonal effects seen in ESBL contamination of food items or the broader environment. Drinking water safety in Malawi is known to alter with the season; *E. coli* contamination was shown to be higher in the wet season, reflecting a reduction in water quality from the point of collection to the point of consumption during the period (235).

The carriage of specific ESBL bacterial clades and ESBL genes between the shared human, animal, and environmental compartments in HICs has previously exhibited limited crossover (138). The DRUM consortium will therefore build on this dataset by using WGS techniques to determine the clonal strains of ESBL-E and ESBL-K in our setting and evaluate the interconnection between these compartments. Pairwise SNP distributions will then establish the level to which these bacteria are related. Further genomic analysis will be undertaken to assess the diversity of AMR genes in various sample types and between settings and identify whether human ESBL colonisation in healthy participants or non-human sources relate to those found in locally circulating blood stream infections. Lastly, given the inherent difficulty in inferring directionality, the DRUM consortium will input the genomic information obtained from these samples into agent-based models, along with key WASH data (**Chapters 4 & 6**)**,** to better understand the specific role of humans, animals and the environment in ESBL transmission and the key sites at which WASH interventions would have the greatest impact.

All of the data included within this chapter will be integrated into ABMs 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, as described in the methods section

(**Chapter 2**). Here, I have made a detailed One-Health microbiological summary of the urban, peri-urban and rural landscape for ESBL-E and ESBL-K at both a household and broader environmental level, evidencing the importance of urbanisation, seasonality and the contamination of key household and broader environments. In the next chapter, I will assess the role of individual-level and WASH factors on the rates of human ESBL colonisation.

Appendices

Appendix 5i. Overview of processing results for ESBL ChromAgar, indole and HRM-PCR, stratified by sample type.

Broad sample type	Broth	Chromogenic Agar			Additional tests	
	Visible growth in BPW^ n (%)	Pink colony n (%)	White colony n (%)	Blue colony n (%)	Indole positive n (% white)	PCR positive n (% blue)
Human stool	n=2581 (91.4%)	n=1046 (36.8%)	n=111 (3.9%)	n=517 (18.2%)	n=19 (17.1%)	n=341 (66.0%)
Animal stool	n=898 (92.3%)	n=266 (27.3%)	n=71 (7.3%)	n=105 (10.8%)	n=8 (11.3%)	n=53 (50.5%)
Environment	n=7548 (92.5%)	n=888 (10.9%)	n=2756 (33.8%)	n=1532 (18.8%)	n=66 (2.4%)	n=697 (45.5%)
Food	n=1093 (93.6%)	n=63 (5.4%)	n=666 (57.0%)	n=208 (17.8%)	n=17 (2.6%)	n=108 (51.9%)
Drinking water	n=1159 (92.4%)	n=145 (11.6%)	n=235 (17.6%)	n=350 (26.3%)	n=10 (2.4%)	n=160 (45.7%)
Source water	n=482 (91.3%)	n=21 (4.0%)	n=84 (15.9%)	n=54 (10.2%)	n=4 (4.8%)	n=19 (35.2%)
Household surfaces	n=2273 (92.5%)	n=90 (3.7%)	n=890 (36.2%)	n=219 (8.9%)	n=16 (1.8%)	n=93 (42.5%)
Household floor	n=696 (93.4%)	n=54 (7.2%)	n=239 (32.1%)	n=91 (12.2%)	n=3 (1.3%)	n=25 (27.5%)
Clothing	n=689 (94.1%)	n=28 (0.13%)	n=368 (49.6%)	n=69 (9.3%)	n=5 (1.4%)	n=27 (39.1%)
Hand-contact samples	n=409 (90.7%)	n=62 (13.7%)	n=104 (23.1%)	n=110 (24.4%)	n=3 (2.9%)	n=47 (42.7%)
Household drains	n=281 (94.0%)	n=120 (40.0%)	n=62 (20.7%)	n=129 (43.0%)	n=3 (4.8%)	n=58 (45.0%)
River water	n=466 (91.0%)	n=305 (59.6%)	n=108 (21.1%)	n=308 (60.2%)	n=5 (4.6%)	n=160 (51.9%)

^ Missing data for broth results from 20 human samples and 1 drain sample.

Chapter 6:

Results from PCA & mixed-effects modelling of individual, household and laboratory datasets delineating the key risks for ESBL colonisation.

6.0. Chapter Summary

In this chapter I use principal component analysis (PCA) to explore how individual-level, household-level and sample-level variables broadly interact to explain the microbiological findings described in Chapter 5 and have inputted these PCAs into multivariate models to assess for associations with a) ESBL colonisation (with either ESBL *E. coli* or ESBL *K. pneumoniae*) and b) separately for ESBL *E. coli* and ESBL *K. pneumoniae* colonisation.

ESBL colonisation has been shown to be dependent on a number of interlinked factors and have species specific (i.e. *E. coli* vs *K. pneumoniae*) associations. The outputs of the modelling illustrate a strong seasonal association with ESBL colonisation, and a trend towards an increased risk from household contamination, piped-water usage and in the case of *K. pneumoniae*, poor hand-hygiene, increased household density and drain-water exposure. There are independent effects from WASH and environmental factors, adding to the complexity of designing future interventions. Nevertheless, predictions made from these models suggest that future WASH interventions to curb ESBL transmission should consider integrating water management, hand-hygiene and environmental measures as part of their strategy for maximal effect.

My contributions to this chapter and those of others are included in Table 6.0.

Table 6.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted and analysed by the PhD candidate
Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborators, Tracy Morse and Chris Jewell.

	Statistical advice and coding help was sought from Chris Jewell, Barry Rowlingson and Joe Lewis.

6.1. Rationale and overview

There is limited evidence in the literature describing the effect of household factors and WASH practices on ESBL colonisation in LMIC communities (198). In Chapters 3, 4 and 5 I described individual-level, household-level and microbiological data of ESBL prevalence within urban, peri-urban and rural sites across southern Malawi. In this chapter, I will bring these data together to determine the effect of individual, household and independent (i.e. season) factors on human colonisation with ESBL bacteria.

Given the complexity and breadth of the dataset, I have initially used PCA to reduce the dimensionality of the data and then selected PCAs for inclusion into ESBL, ESBL-E and ESBL-K mixed-effects models through ANOVA testing and stepwise selection of model fit using Akaike information criteria (AIC). Within the mixed-effect models PCAs have been input as covariates alongside within-household and within-participant random effects to determine key risks for ESBL, ESBL-E and ESBL-K colonisation.

To evaluate the success of putative WASH interventions I have compared these models with and without sample-level data to determine whether environmental contamination is independent or dependant on WASH factors within the causal pathway for ESBL colonisation and made predictions of ESBL colonisation rates in response to alterations of pre-selected WASH parameters.

6.2. Variable selection process and rationalisation

There was a total of 41 individual questions and 167 (132/35) household/WASH questions within the database. These were refined *a priori* by members of the DRUM consortium including D Cocker, T Morse, K Chidwisano and N Feasey to determine key variables of interest, accounting for pre-existing knowledge on AMR risk factors and critical control points for faecal-oral transmission (Chapters 2 & 4). The household and WASH questions were ordered into WASH categories encompassing high-level sanitation, water usage, food-hygiene, hand-hygiene, animal and environmental factors alongside household specific factors (i.e. density and income). These were then classified as either reported or

observed depending on whether the variable outcome was witnessed by the study team or reported by a household member. Finally, microbiological samples (i.e. drinking water, animal stool, environmental surface) were assigned into the WASH or household categories depending on where they conceptually fit best. Household and WASH variables were predominantly obtained from the baseline questionnaire and linked to each of the 300 households. 8 observed WASH variables were obtained via the WASH checklists, and unlike the household questionaries completed at baseline, these were undertaken at up to 4 timepoints during the study period at the same house. Occasionally there were fluctuations in the response to these outcomes (i.e. households would not always have the presence of cleaning materials) and therefore aggregated values were used for these variables, assigned to each of the 300 households. ESBL microbiological data was collated from the laboratory records of all households, each of which had data for human stool, and 195 households had paired animal and environmental samples.

In total this generated a list of 19 individual variables (**Table 6.1.**), 29 household/WASH variables (**Table 6.2**.) and 10 sampling variables (**Table 6.2**.). Each variable response was converted into binary (or continuous) functions. Categorical answers were grouped into binary outcomes if necessary and the variable names were altered (i.e. ABU; Yes/No included a composite of any antibiotics in last 6 months). Details of these changes are included in the descriptions within **Table 6.1** and **Table 6.2**. Continuous variables were transformed log(x) to provide normalized distributions where possible. The tested outcome was human ESBL colonization status (positive / negative), and each episode was evaluated independently, to account for flux in colonization status **(Table 6.3.)**. For animal and environmental samples, a binary classification of positive (at any point) or negative (at all points) was implemented and linked to each household. Region and season (wet/dry) were determined for each stool sample result dependent on the household location and date of collection respectively **(Table 6.3.)**.

Table 6.1. Individual-level variables selected from the CRFs for analysis, including any groupings, outputs and transformations undertaken.

Variable name	Description
Age	Continuous, age at enrolment (years)
Male	Binary, {1 = male, 0 = female}
Religion (Christianity)	Binary, {1 = faith reported as Christianity, 0 = other religion practiced}
School (attendance)	Binary, {1 = attend school, 0 = do not attend school}
School (work)	Binary, {1 = work in/at school, 0 = do not work in/at school}
Healthcare (work)	Binary, {1 = work in/at hospital, 0 = do not work in/at hospital}
Hospital admission	Binary, {1 = admitted overnight to hospital in last 6 months, 0 = not admitted over last 6 months}
Hospital guardian	Binary, {1 = been a guardian at hospital in last 6 months, 0 = not been a guardian at hospital in last 6 months)
Employed*	Binary, {1 = have regular job at recruitment, 0 = no job at recruitment}
Residency	Binary, {1 = resident for year or more at household, 0 = not resident for year}
Travel (Outside region)	Binary, {1 = travel outside region (any purpose in last 6 months, 0 = no travel outside area in last 6 months}
HIV status*	Binary, {1 = HIV reactive, 0 = HIV non-reactive or unknown}
TB history	Binary, {1 = ever had diagnosis of TB, 0 = never had TB}
Comorbidities	Binary, {1 = 1 or more comorbidities, 0 = no comorbidities}
Medication	Binary, {1 = take any prescribed regular medication, 0 = do not take any regular medication}
Unwell 4 weeks	Binary, {1 = 1 or more unwell episodes in last 4 weeks, 0 = no illness episodes in last 4 weeks}
Unwell 3 months	Binary, {1 = 1 or more unwell episodes in last 3 months, 0 = no illness episodes in last 3 months}
ABU	Binary, {1 = 1 or more antibiotic courses taken in the last 6 months, 0 = no antibiotics taken in last 6 months}

**log-transformed*

Table 6.2. Household, WASH and sampling variables selected from the CRFs for analysis, including any outputs and transformations undertaken. Variables are grouped into reported, observed and laboratory categories and stratified by factor type.

		Variable	Description
Household Factors	**Reported**	Number of people living in house*	Continuous, number of people cohabiting at baseline.
		Household income*	Continuous, household income (MK) at baseline.
	Lab	Share household with ESBL colonised humans	Binary, {1 = yes [share with 1 or more ESBL colonised individuals within the same household], 0 = no [Do not share with ESBL colonised household members]}
Sanitation Factors	**Observed**	Presence of drop hole cover	Binary, {1 = drop hole cover present, 0 = drop hole cover absent}
		Cleansing materials at toilet	Binary, {1 = cleansing material [any type] present, 0 = cleansing material [any type] absent}
		Visible human defecation	Binary, {1 = visible human stool [adult or child], 0 = no visible human stool}
	Reported	Use of pit latrine	Binary, {1 = use pit latrine, 0 = use other toilet type, or do not have toilet}
		Toilet presence (any) at household	Binary, {1 = toilet present, 0 = toilet absent}
		Open human defecation	Binary, {1 = open defecation reported [by 1 or more household members], 0 = no open defecation reported [by all household member]}
		Sharing household toilet with non-household members	Binary, {1 = shared toilet used, 0 = do not share their toilet external to the household}
		Absence of disposal mechanism for animal waste	Binary, {1 = no disposal mechanism for animal faeces, 0 = dispose of animal faeces by either sweeping them away, putting into a refuse pit or re-using as manure}
	Lab	Household environmental ESBL contamination	Binary, {1 = yes [at any point in the household during study], 0 = no [at all points during study]}
Hygiene factors	**Reported**	Facilities for hand washing (all areas) at household	Binary, {1 = yes [present at one or more place within the household], 0 = no [present at no places within the household]}
	Observed	Presence of soap at (any) HWF	Binary, {1 = yes [present at one or more HWFs within the household], 0 = no [present at no HWFs within the household]}
	Lab	Household rinse water ESBL contamination	Binary, {1 = yes [at any point in the household during study], 0 = no [at all points during study]}
Food Factors	**Reported**	Eat street food	Binary, {1 = yes [supplement diet with street food at some points], 0 = no [never buy street food]}
		Eat from shared plates	Binary, {1 = yes [use shared plates], 0 = no [do not use shared plates]}
		Buy vegetables or fruit from the market	Binary, {1 = yes [use vegetables or fruit from the market on any occasion], 0 = no [do not use market food or fruit]}

	Lab	Household food ESBL contamination	Binary, {1 = yes [at any point in the household during study], 0 = no [at all points during study]}
Water Factors	Observed	Is water stored in the house covered?	Binary, {1 = yes [water stored at the house covered], 0 = no [water not stored at the house covered]}
	Reported	Is water stored in the house?	Binary, {1 = yes [water stored at the house], 0 = no [water not stored at the house]}
		Drinking water source piped into household	Binary, {1 = yes [water source from outside the household], 0 = no [water source from pipe inside or directly outside the household]}
		Drinking water source kiosk	Binary, {1 = yes [water source from outside the household], 0 = no [water source from pipe inside or directly outside the household]}
		Drinking water source tubewell	Binary, {1 = yes [water source from outside the household], 0 = no [water source from pipe inside or directly outside the household]}
		Alternative water used for cleaning utensils	Binary, {1 = yes [different water used for cleaning utensils than for drinking], 0 = no [same water used for cleaning utensils as drinking]}
	Lab	Household source water ESBL contamination	Binary, {1 = yes [at any point in the household during study], 0 = no [at all points during study]}
		Household stored water ESBL contamination	Binary, {1 = yes [at any point in the household during study], 0 = no [at all points during study]}
Animal Factors	Observed	Animal faeces seen around the area	Binary, {1 = yes [any animal faeces seen around the household at any point], 0 = no [no animal faeces ever seen around the household]}
		Evidence of animal contact with food	Binary, {1 = yes [animal seen in contact with food], 0 = no [no animal seen in contact with food]}
	Reported	Does the household own any animals?	Binary, {1 = yes [household owns 1 or more animals], 0 = no [no animals owned by household]}
		Own cattle or ruminant	Binary, {1 = yes [household owns 1 or more animals], 0 = no [no animals owned by household]}
		Own poultry	Binary, {1 = yes [household owns 1 or more animals], 0 = no [no animals owned by household]}
		Own pet / companion animal	Binary, {1 = yes [household owns 1 or more animals], 0 = no [no animals owned by household]}
		Own pigs	Binary, {1 = yes [household owns 1 or more animals], 0 = no [no animals owned by household]}
		Animals (any species) kept inside the house?	Binary, {1 = yes [if animals owned - they kept inside the house], 0 = no [if animals owned - they are not kept inside the house]}
	Lab	Household animal ESBL contamination	Binary, {1 = yes [at any point in the household animals during study], 0 = no [at all points during study]}
Broader Environment	Observed	Accumulation of water / wastewater (household environment)	Binary, {1 = yes [water seen external to the household], 0 = no [water not seen external to the household]}
	Reported	Household member interaction with river water	Binary, {1 = yes [any adult or child at the household reportedly interact with river water], 0 = no [no adult or child at the household even interact with river water]}

		Household member interaction with drains	Binary, {1 = yes [any adult or child at the household reportedly interact with drains], 0 = no [no adult or child at the household even interact with drains]}
	Lab	Drain ESBL contamination	Binary, {1 = yes [at any point during study], 0 = no [at all points during study]}
		River ESBL contamination	Binary, {1 = yes [at any point during study], 0 = no [at all points during study]}

**log-transformed*

Table 6.3. Outcome variables and covariates.

Dependant variable	Description
ESBL (positive)	Binary, {1 = ESBL positive at single episode [with either KPN or EC], 0 = ESBL negative at single episode [with either KPN or EC]}
ESBL-E (positive)	Binary, {1 = ESBL *E. coli* positive at single episode, 0 = ESBL *E. coli* negative at single episode}
ESBL-K (positive)	Binary, {1 = ESBL *K. pneumoniae* positive at single episode, 0 = ESBL *K. pneumoniae* negative at single episode}
Covariates	
Region	Categorical, {Urban / Peri-urban / rural}
Season	Binary, {1 = wet (October-April), 0 = dry (May-September)}

6.3. Handling missing data and homogeneity of responses

There was minimal missing data from the individual, household/WASH and laboratory datasets (**Figure 6.1**). It should be noted that antibiotic usage and illness responses were not captured for every individual due to participants either not having episodes of illness or of antibiotic prescriptions. Further, HIV status was not known by 51% (n=492) of participants, although this is not missing data *per se* (**Figure 6.1A**). A total of 9 household or WASH variables had no responses for specific animal metrics, because these questions are only asked when the households reported animals present. In relation to the laboratory data, some households did not have a linked river water or animal sample, due to recruitment occurring during the dry season or the absence of animal faeces available at household visits (**Figure 6.1B-C**). Lastly, rinse water and source water were not always obtained from each household, and these 4 sample types have been interpreted as missing data at the household level.

The distribution of individual and household variables was evaluated through density plots (**appendix 6i/ii**) highlighting that most variables exhibited skewed data. Near zero variance predictors were used to assess variables with a high degree of homogeneity in response outcomes, and these were found in the individual (**appendix 6iii**) and household (**appendix 6iv**) dataset. No near zero variance

predictors were identified in the laboratory dataset. The variables identified either had limited unique values or high frequency ratios between the first and second response outcome and would not be useful to model as they will tend towards zero, so they were removed from the dataset prior to further analysis.

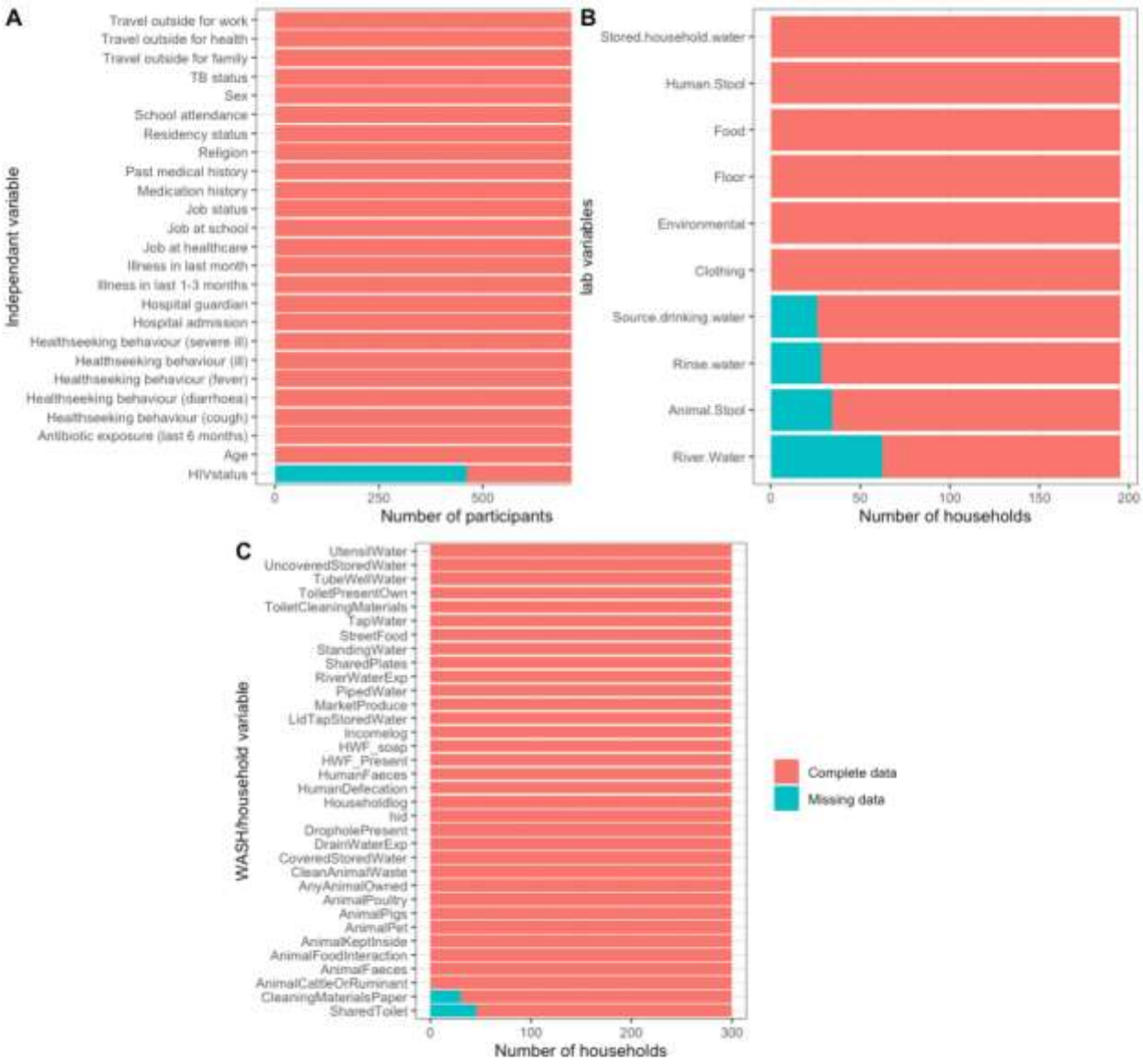


Figure 6.1. Missing data from individual, laboratory and household variables

6.4. Correlation between variables (either individual or household)

Associations between variables were calculated using Pearson correlation coefficients and expressed as a coloured matrix (heatmap) of correlations amongst individual (**Figure 6.2**), household (**Figure 6.3**) or microbiological (**Figure 6.4**) variables.

A high degree of correlation was found between unemployment and school attendance, and antibiotic usage and periods of illness in the individual dataset. These finding are consistent with associations that we would expect to see. In the household dataset associations were found between household toilet presence and pit latrine toilets, reflecting the predominance of pit latrines used in these settings, and hand washing facilities and either clean toilets, or drophole cover and cleansing material presence. This is likely to suggest that households that have a hand washing facility also own or use other forms of household sanitation equipment.

Animal ownership was correlated with manure usage or the presence of animal faeces seen in the household compound, and this is again consistent with what we would expect to find as linked outcomes from owning animals. Shared plates, owning cattle and accessing a tube well are all associated because the practice of sharing plates is common in the rural district, which is the only area in our study where cattle were owned and also where the predominant water source is from boreholes. There was little correlation between the microbiological sampling types, other than a weak effect seen between environmental surface and floor contamination.

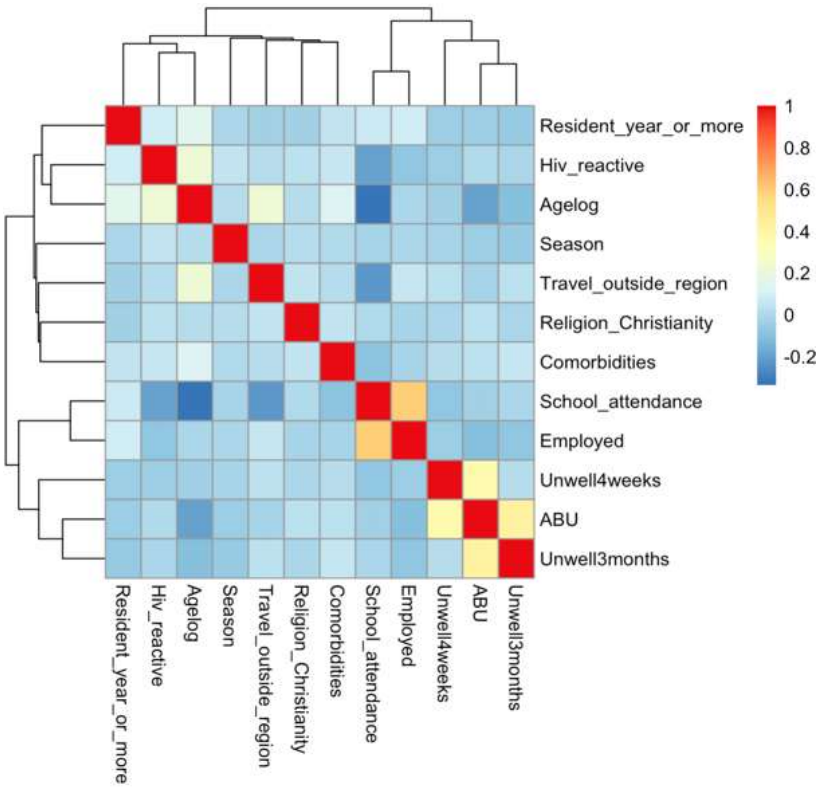


Figure 6.2. Heatmap of variables relating to individuals using Pearson's coefficient to identify variables that are associated

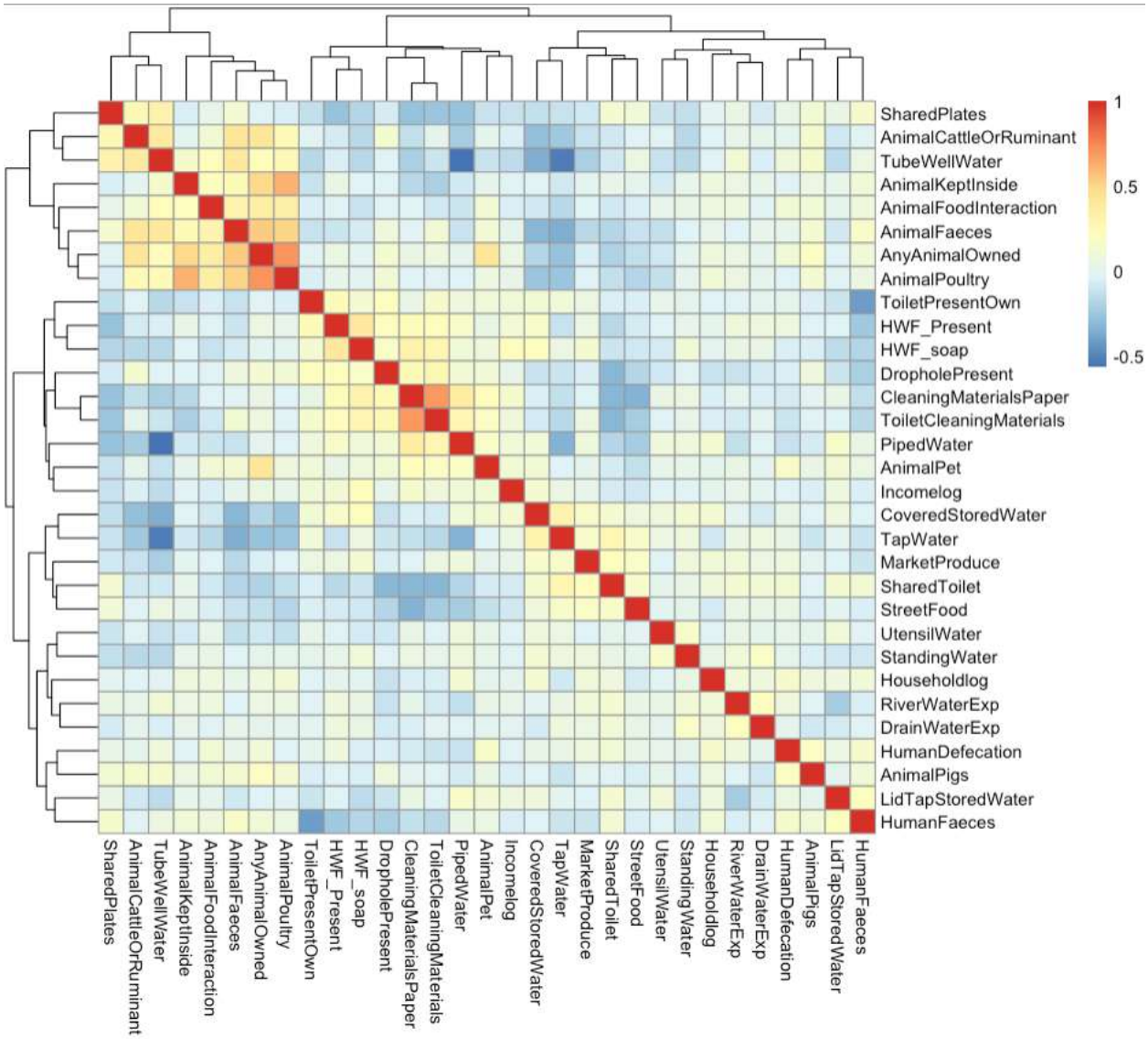


Figure 6.3. Heatmap of variables relating to the household and household WASH using Pearson's coefficient to identify variables that are associated

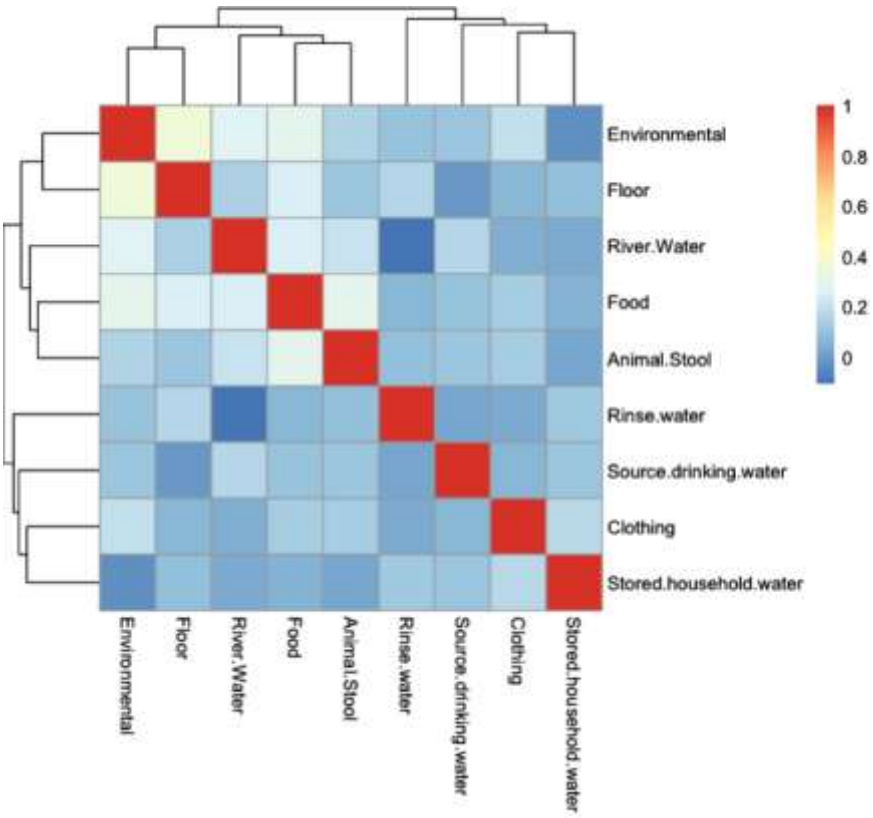


Figure 6.4. Heatmap of variables relating to microbiological sampling results using Pearson's coefficient to identify variables that are associated

6.5. Principal component analysis

Given the large number of variables, 3 PCAs were undertaken to reduce the dimensionality of the data, including one at the individual-level, another on the household-level, and a third one at the microbiology sampling-level. PCAs were performed using the FactoMine package in R (v4.1.2) for both analysis and visualization, and inbuilt scaling within the package meant that no preceding scaling of variables was required.

The proportion of variances (eigenvalues) for the individual-level PCA show that 94.0% of the data can be described by 10 principal components and the first 2 principal components describe 30.5% of the data (**Figure 6.5**). Quality of representation (Cos2) of the 10 dimensions is shown on the correlation plot in **Figure 6.5,** indicating the dimensions in which variables of interest lie, and a full list of variable contributions for PCAs 1-10 are included in **appendix 6v**. The factor map of the first 2 PCAs demonstrates that PCA1 defines an axis of age (school attendance, employment, and age) and PCA2 defines an axis of antibiotic exposure (illness episodes and ABU). In the household-level PCA, the eigenvalues show that 72.0% of the data can be described by 10 principal components and the first 2

principal components describes 27.2% of the data (**Figure 6.6**). Again, contributions of the variables are shown within the correlation plot in **Figure 6.6**, and a full list of the variable contributions for each PCA are included in **appendix 6vi**. A factor map of the first 2 principal components of the household data illustrates PCA1 defining an axis of animal ownership and household water usage, and PCA2 describing an axis of sanitation and food-hygiene (**Figure 6.6**). Within the sample-level PCA analysis, 2 principal components described 38.7% of the data, and here is evident that river water, hand-hygiene samples (i.e. rinse water) and household environment are important contributors within the dataset. (**Figure 6.7 & appendix 6vii**).

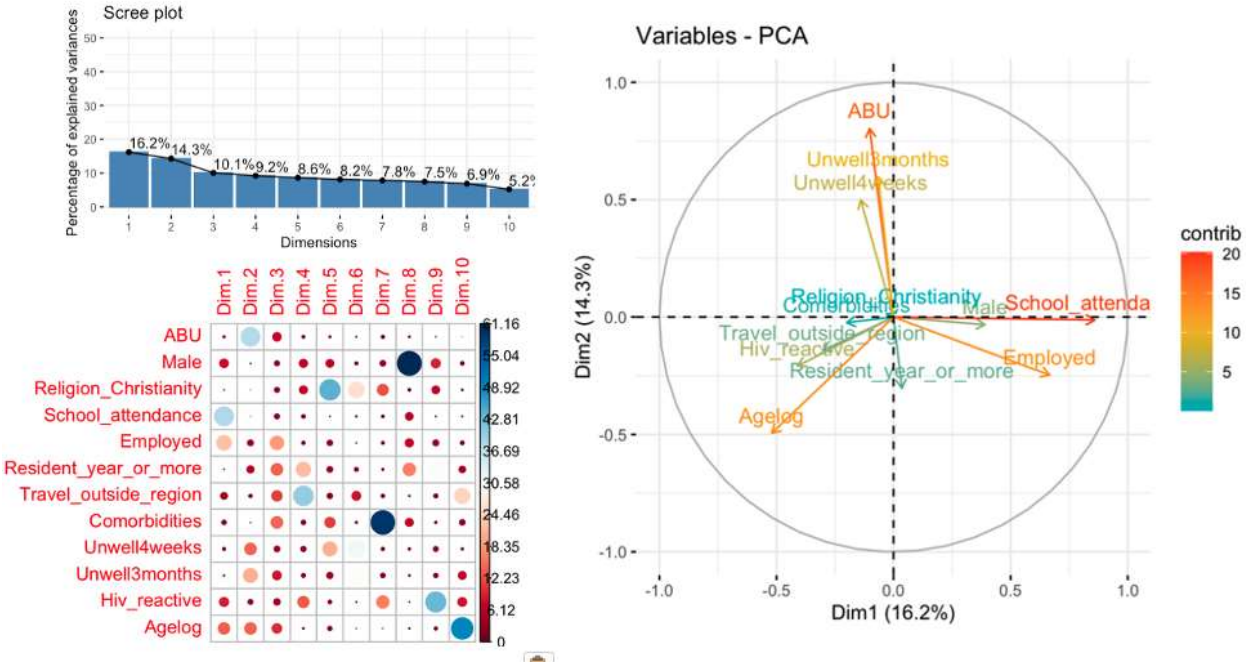


Figure 6.5. PCA analysis of individual variables, including a scree plot of the eigenvalues (top left), weighting of the variables by PCA (bottom left), and factor map (right).

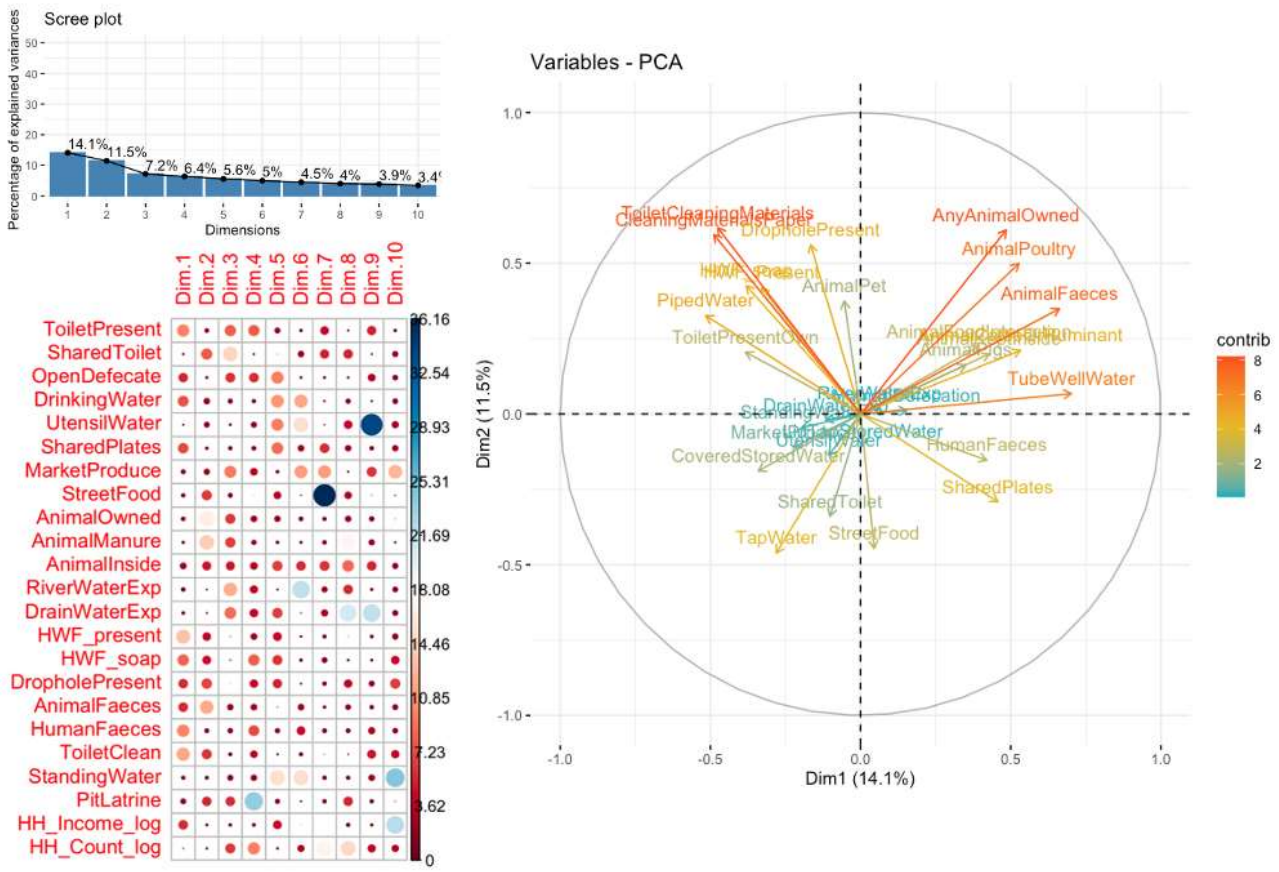


Figure 6.6. PCA analysis of household variables, including a scree plot of the eigenvalues (top left), weighting of the variables by PCA (bottom left), and factor map (right).

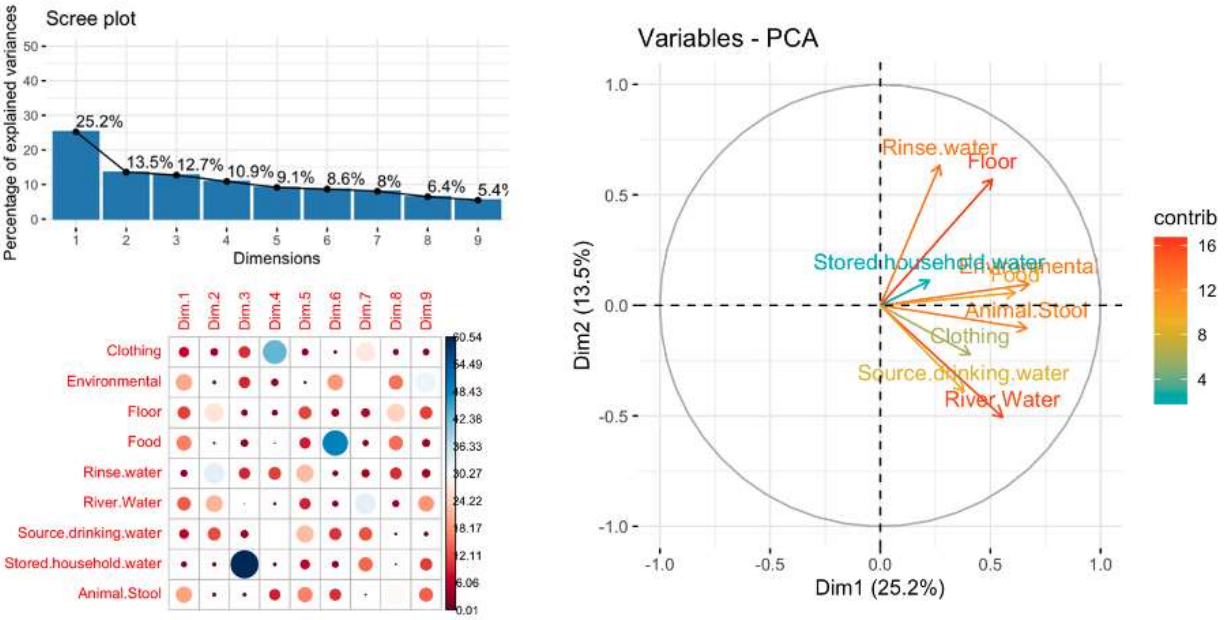


Figure 6.7. PCA analysis of laboratory variables, including a scree plot of the eigenvalues (top left), weighting of the variables by PCA (bottom left), and factor map (right).

6.6. Determining PCA selection for input into multivariate models

To select individual, household and sample PCAs to input into logistic regression models evaluating ESBL colonisation, 2543 stools from 745 participants at 195 households were attached to PCA vectors, and ANOVA testing against a null reference was used to determine which PCAs should be included. 179 stool returns from 178 participants at 105 households were excluded given incomplete metadata (**Section 6.4**). This method highlighted that PCAs 1, 5 and 7 for the individual-level variables, PCAs 2, 3, 4, 5, 6, 7, 8 and 9 from the household-level variables and PCAs 1, 2, 6, 7 and 9 from the sample-level variables best fit to models of ESBL colonisation, as determined by the lowest AIC value, and these results were validated by comparing them to results of other methods of determining model fit, including manual plotting of the AIC and automated (backwards) stepwise selection process using the stepAIC function in the Mass package in R (v4.1.2).

6.7. Bayesian multivariate models of ESBL colonisation

A mixed-effects model was constructed using the brm package in R (v4.1.2) and included within-household and within-participant random effects and season and site (urban / peri-urban / rural) fixed effects alongside the PCAs selected in section 6.6.

Caterpillar plots were generated for the random effects using posterior estimates, and here, I found a limited contribution of within-participant level effects on ESBL colonisation, and more within-household effects (**Figure 6.8c**). Parameter estimations of the fixed effects were expressed as odds ratios (ORs) with a point estimate (posterior median) and 95% credible intervals (CrI). Trace plots were run to test for convergence (**appendix 6viii**).

From this analysis it was identified that household environmental, stored water and hand contamination alongside piped-water usage and the season (wet) were strongly associated with ESBL colonisation (**Figure 6.9**). Participant (younger) age had a protective effect and there were no other WASH or individual-level factors that had a relationship to ESBL colonisation.

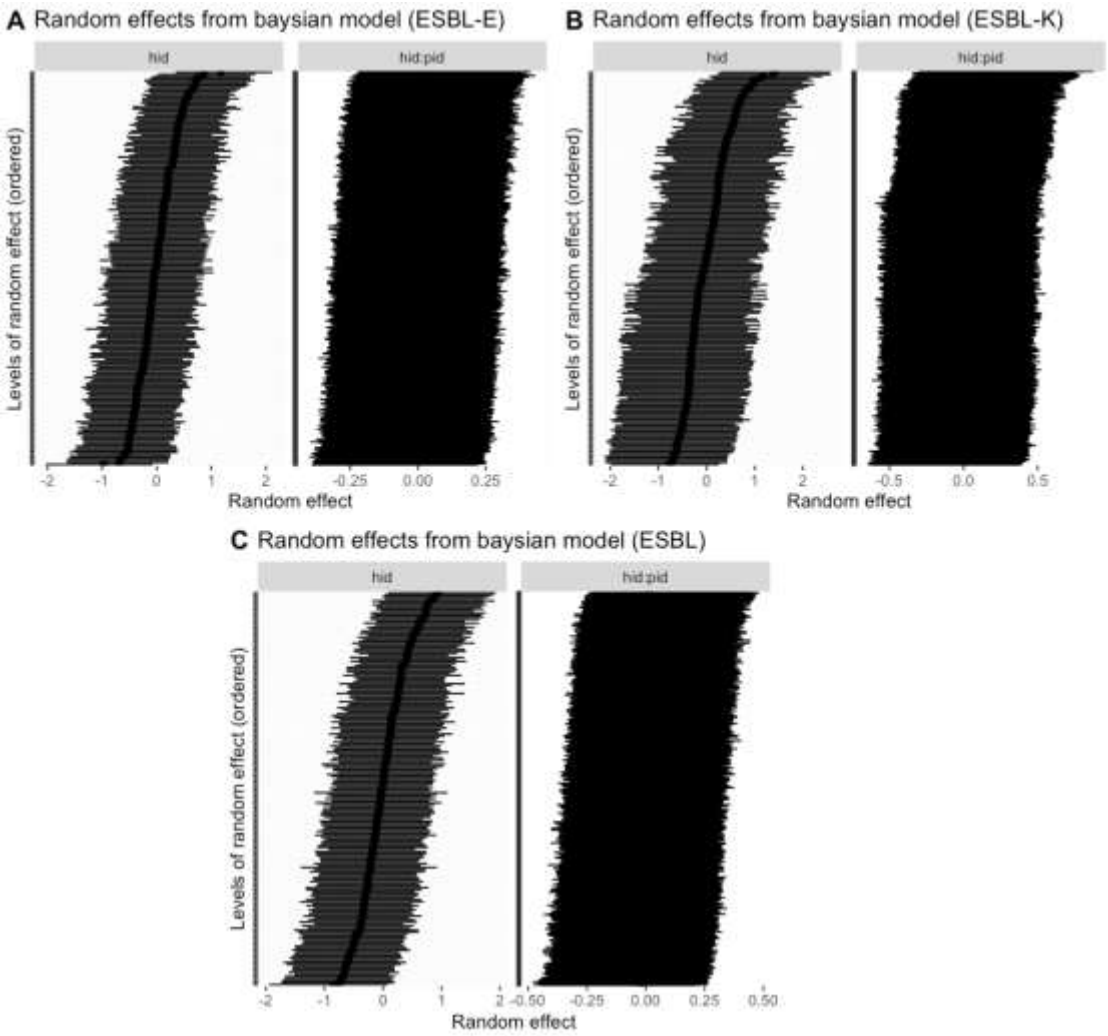


Figure 6.8. Caterpillar plot of random effects in Bayesian models of (a) ESBL-E colonisation, (b) ESBL-K colonisation, and (c) ESBL colonisation.

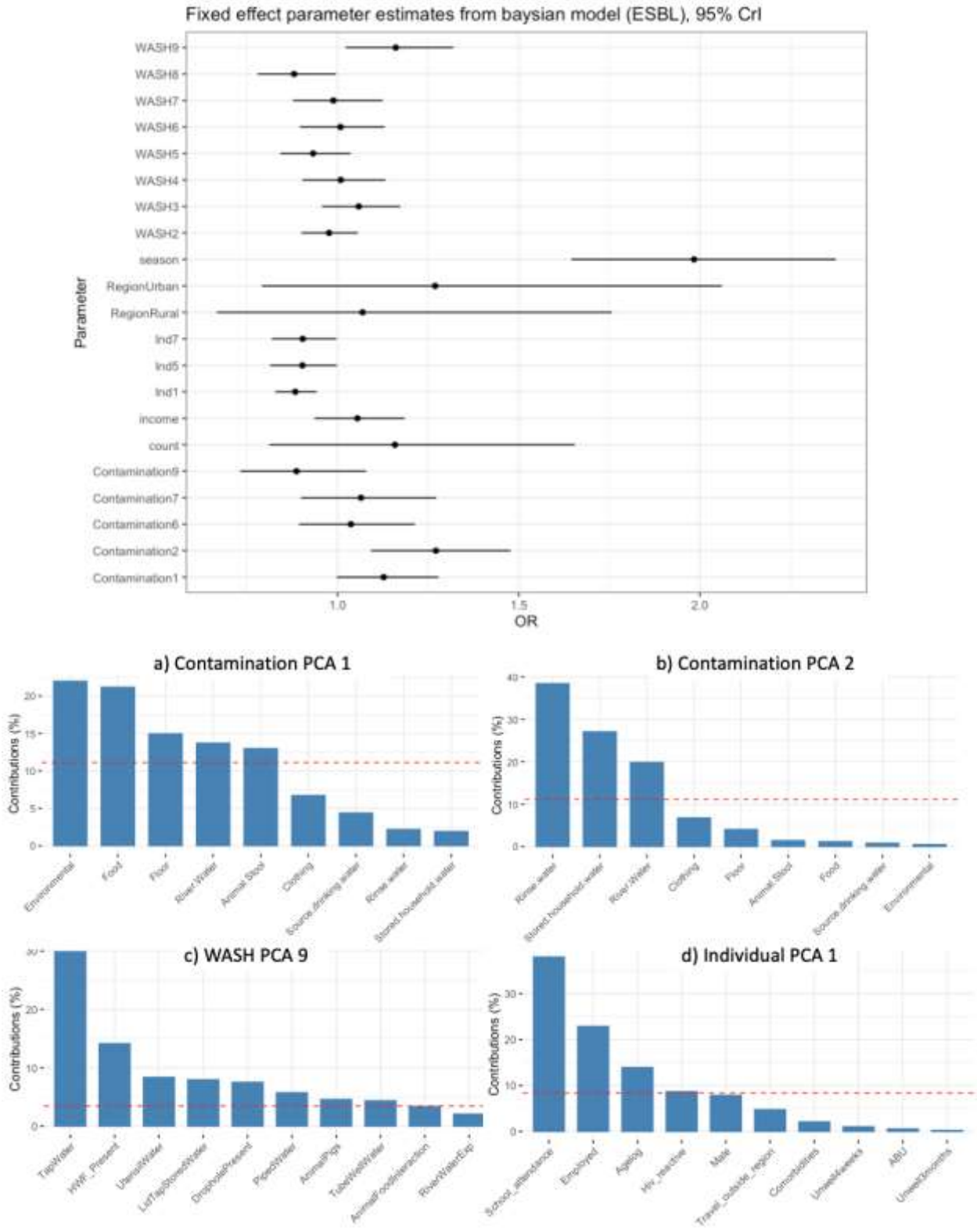


Figure 6.9. Parameter estimates for the fixed-effects used in a multivariate model of ESBL colonisation, including individual, household and laboratory datasets, expressed as odds ratios with 95% CrI (top). Examples of key PCA contributions with increased (a/b/c) and decreased (d) odds for ESBL colonisation (bottom).

6.8. Bayesian multivariate models for PCA associations with either ESBL *E. coli* or ESBL *K. pneumoniae* colonisation

The processes in Section 6.7 were repeated with the outcome of interest set as either colonisation with ESBL *E. coli* or colonisation with ESBL *K. pneumoniae* to look for species specific factors. The selection process illustrated that PCAs 1, 5 and 7 from the individual-level, PCAs 2, 3, 5 and 8 from the household-level and PCAs 1, 2, and 7 from the sample-level best fit to models of ESBL *E. coli* colonisation, and PCAs 2, 3 and 6 from the individual-level, PCAs 6 and 8 from the household-level and PCAs 2, 3 and 9 from the sample-level best fit to models of ESBL *K. pneumoniae* colonisation. Models were constructed using the same approach as previously set out for ESBL colonisation and accounted for within-household and within-participant random effects and season and site (urban / peri-urban / rural) fixed effects. Trace plots again exhibited convergence (**appendix 6ix-x**). Akin to ESBL colonisation I found a limited contribution of within-participant level effects and higher within-household-level effects on ESBL *E. coli* (**Figure 6.8a**) and ESBL *K. pneumoniae* (**Figure 6.8b**) colonisation.

Parameter estimations for ESBL *E. coli* mirrored those for all ESBL, and showed that contaminated hand and household environments, piped-water usage and the wet season were associated with ESBL *E. coli* colonisation (**Figure 6.10**). Young age had a protective effect on ESBL colonisation, as did WASH PCA8. WASH PCA8 could not easily be classified into a single conceptual category as it was composed of assumed negative and positive factors for ESBL acquisition, such as drain water exposure/ animal food interaction vs coverage of stored water/ shared plates (independent protective factor Chapter 7). Nevertheless, lower odds of ESBL colonisation were associated with this principal component.

For ESBL *K. pneumoniae* a slightly different spectrum of risk profiling was identified compared to ESBL *E. coli* or ESBL. Again, the wet season and poor hand-hygiene were strongly associated with gut colonisation, however, there was an association with increased household density and PCA8 (comprising of drain water exposure and coverage of stored water) and there was a reduced chance of ESBL *K. pneumoniae* colonisation if the household environment was contaminated with the ESBL bacteria. (**Figure 6.11**).

In the models of ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* colonisation it is evident that there are regional effects. These have not been explored in detail here but will be subject to testing in Chapter 7, where I look for similarities and differences in risk factors between the regions.

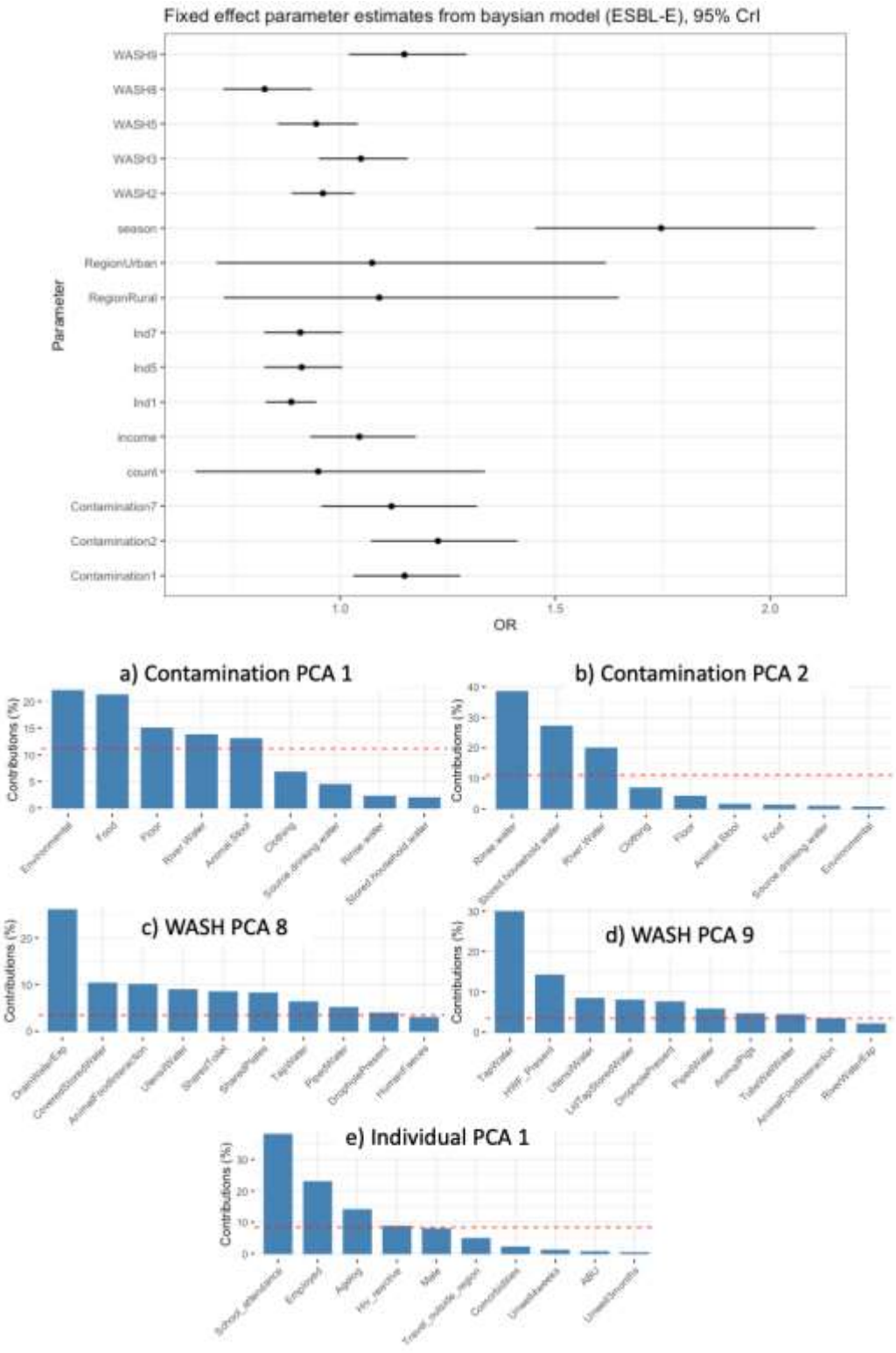


Figure 6.10. Parameter estimates for the fixed-effects used in a multivariate model of ESBL *E. coli* colonisation, including individual, household and laboratory datasets, expressed as odds ratios with 95% CrI (top). Examples of key PCAs with increased (a/b/d) and decreased (c/e) odds for ESBL *E. coli* colonisation (bottom).

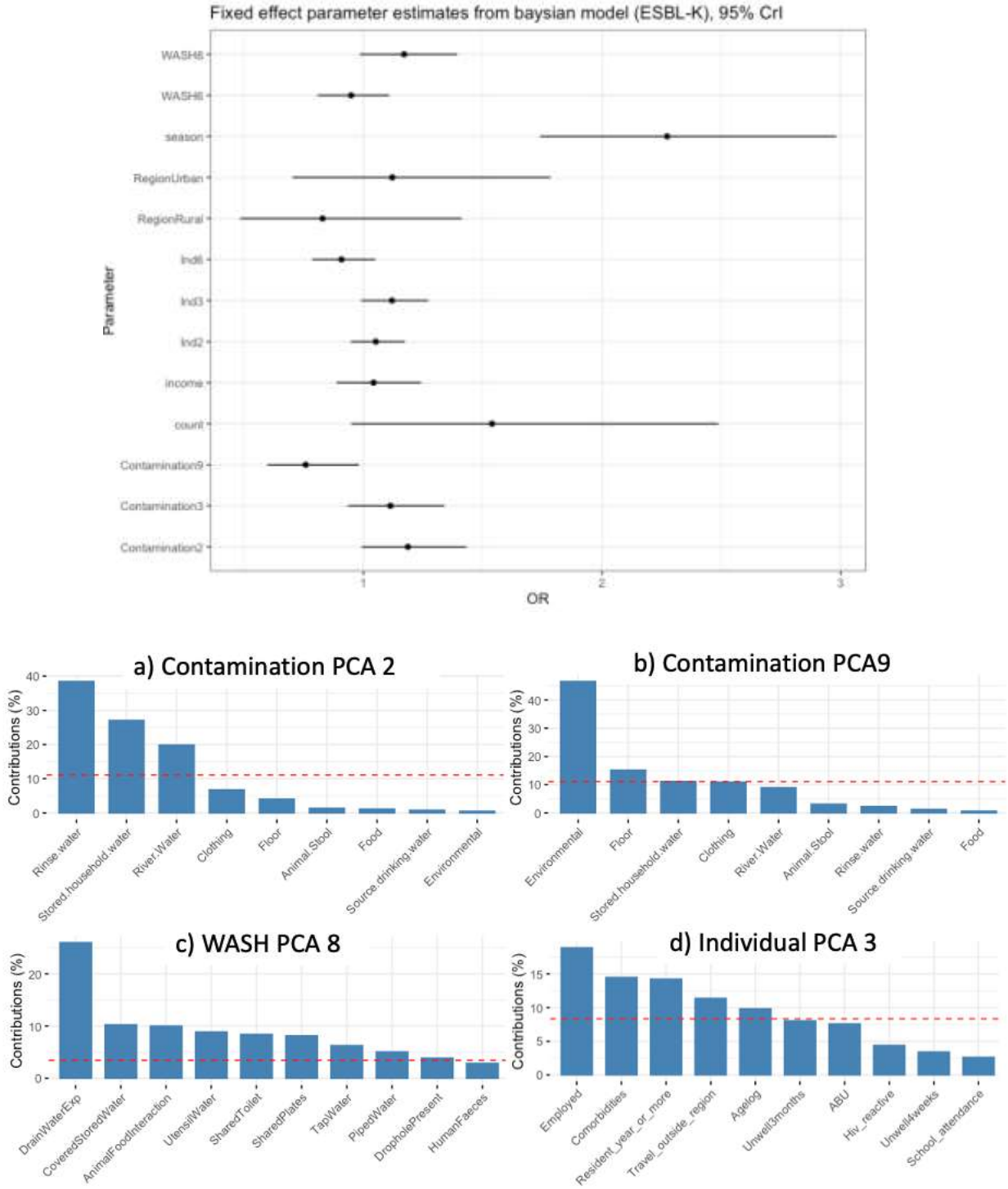


Figure 6.11. Parameter estimates for the fixed-effects used in a multivariate model of ESBL *K. pneumoniae* colonisation, including individual, household and laboratory datasets, expressed as odds ratios with 95% CrI (top). Examples of key PCAs with increased (a/b/c/d) odds for ESBL *K. pneumoniae* colonisation (bottom).

6.9. Independent effects of WASH and environmental contamination.

From the models of ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* colonisation specific WASH and environmental factors were associated with EBSL colonisation. To test whether these were independent (**Figure 6.12a**) or dependant (**Figure 6.12b**) on each other in the causal chain, models were tested with and without integration of environmental contamination data (**Figure 6.13**).

Within these models I found that removal of the environmental data had limited effect on the odds of ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* colonisation. This finding is consistent with WASH and environmental contamination having independent effects on the causal pathway (**Model A, Figure 6.12**) and illustrates the value that microbiological surveillance data adds.

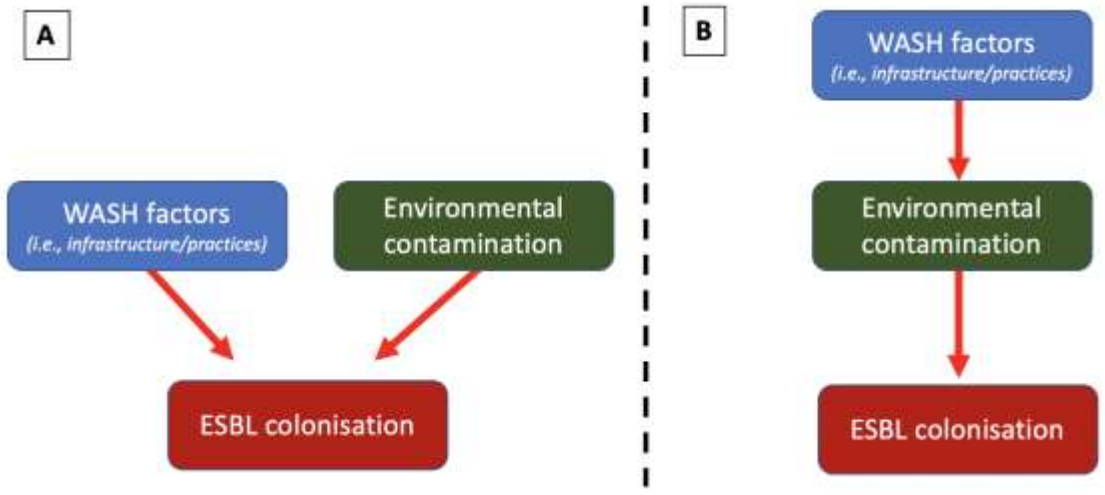


Figure 6.12. Theoretical causal frameworks for ESBL colonisation considering WASH factors and environmental contamination as independent (a) and dependant (b) effects.

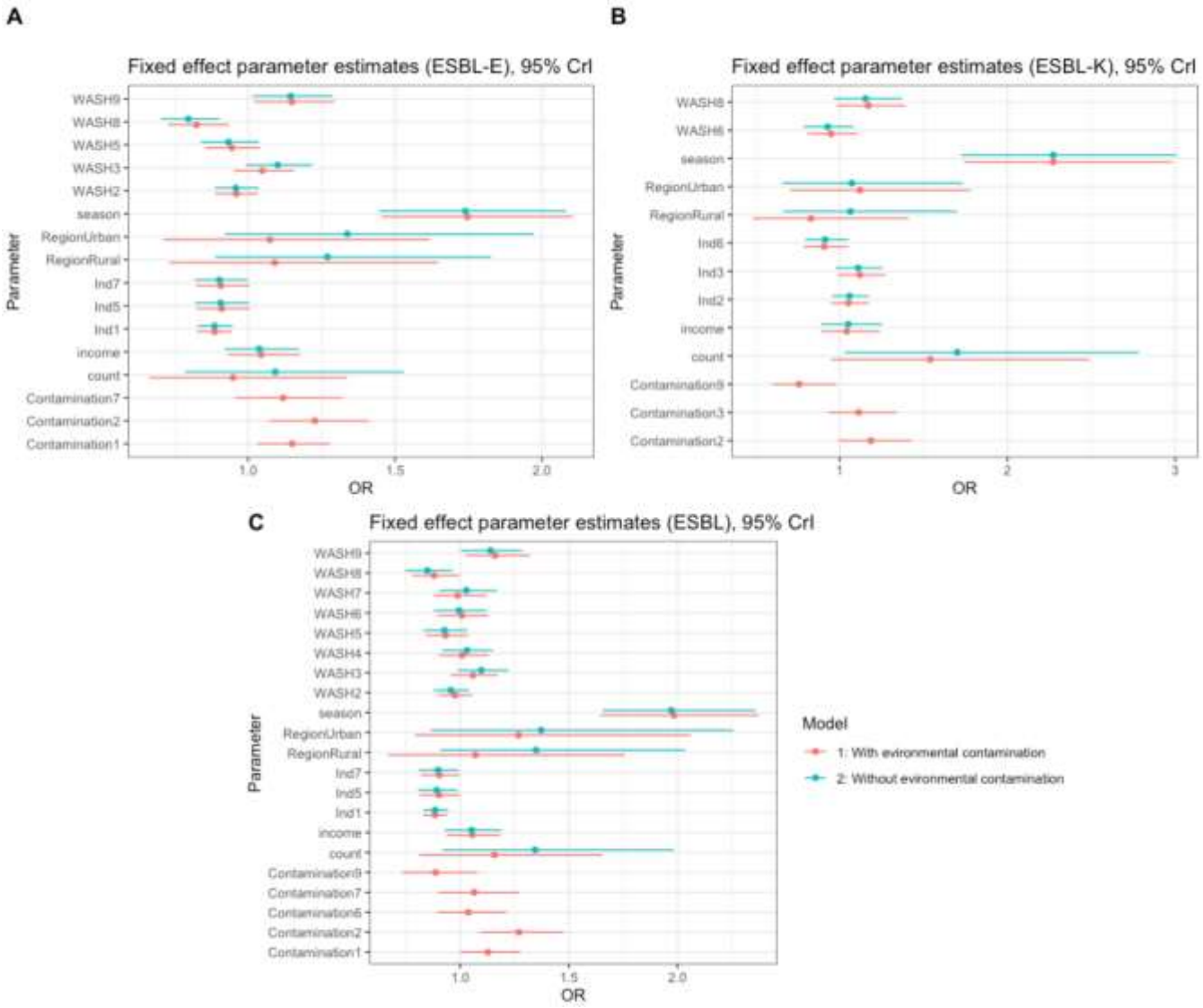


Figure 6.13. Parameter estimates for the fixed-effects in multivariate models of (a) ESBL *E. coli,* (b) ESBL *K. pneumoniae* and (c) ESBL colonisation, expressed as odds ratios with 95% CrI, for models with and without integration of environmental level contamination data. Red = Model 1 (original model, inclusive of environmental factors) and turquoise = Model 2 (original model ran without environmental factors).

6.10. Predicting rates of ESBL in response to changes in WASH infrastructure and behaviours

To determine whether variations in WASH practices would alter ESBL prevelance within our cohort, predictions were made from the posterior distributions of the ESBL model. To enable this, WASH variables within the dataset were selected *a priori* by consensus opinion of a panel of WASH specialists

which were considered to pose protective effects (i.e. use of drophole covers) or potential risks (i.e. use of shared toilet) for faecal-oral acquisition of ESBL bacteria; streamlined to include only those which could pragmatically be interrupted by future WASH interventions or policy interventions. WASH variables were grouped by category, as in Table 6.2 and binary outcomes were assigned for the desired optimal and suboptimal outcome responses (**Table 6.4 & appendix 6xi**). A typical individual was then generated using response variable means from within the dataset and average response outcomes were plotted against the individual and household principal components (**appendix 6xii**). Hypothetical "dummy" data of optimal and sub-optimal WASH practices were fed into the ESBL model for each WASH category, and the predicted probabilities of ESBL colonisation were generated (**Figure 6.14**).

The output of this approach illustrates that there is maximal difference seen in the predicted probability of ESBL colonisation with optimal changes made to water usage, household environmental and to a lesser extent hand-hygiene parameters. Little difference in the predicted probability of ESBL colonisation is seen in alterations in food-hygiene, animal management or sanitation factors.

Table 6.4. Optimal and suboptimal WASH variable settings, stratified by category

WASH category	Classification	Variables of interest
Water management	Optimal	Source water samples free from ESBL bacteria
		Drinking water samples free from ESBL bacteria
		Covered stored water
	Sub-optimal	Source water samples contamination with ESBL bacteria
		Drinking water samples contamination with ESBL bacteria
		Uncovered stored water
Sanitation	Optimal	Human defecation not practiced
		Toilet not shared
		Drophole cover present
		Toilet cleansing materials present
		Human or animal faeces seen
	Sub-optimal	Human defecation practiced
		Toilet shared
		Drophole cover absent
		Toilet cleansing materials absent
		Human or animal faeces not seen
Hand hygiene	Optimal	HWF available
		Soap available
		Hand samples not contaminated with ESBL bacteria
	Sub-optimal	HWF unavailable
		Soap unavailable
		Hand samples contaminated with ESBL bacteria
Food hygiene	Optimal	Street food not consumed
		Shared plates not used
		Food samples not contaminated with ESBL bacteria
	Sub-optimal	Street food consumed
		Shared plates used
		Food samples contaminated with ESBL bacteria
Environmental hygiene	Optimal	No interaction with river water
		No interaction with drains
		No standing water present
		No ESBL contamination of household environment
		No ESBL contamination of household floor
	Sub-optimal	Interaction with river water
		Interaction with drains
		Standing water present
		ESBL contamination of household environment
		ESBL contamination of household floor
Animal management	Optimal	No animals owned by household
		No animals interacting with food
		No animals kept inside the house
		No ESBL animal stool identified at household
	Sub-optimal	Animals owned by household
		Animals interacting with food
		Animals kept inside household
		ESBL identified in household animal stool

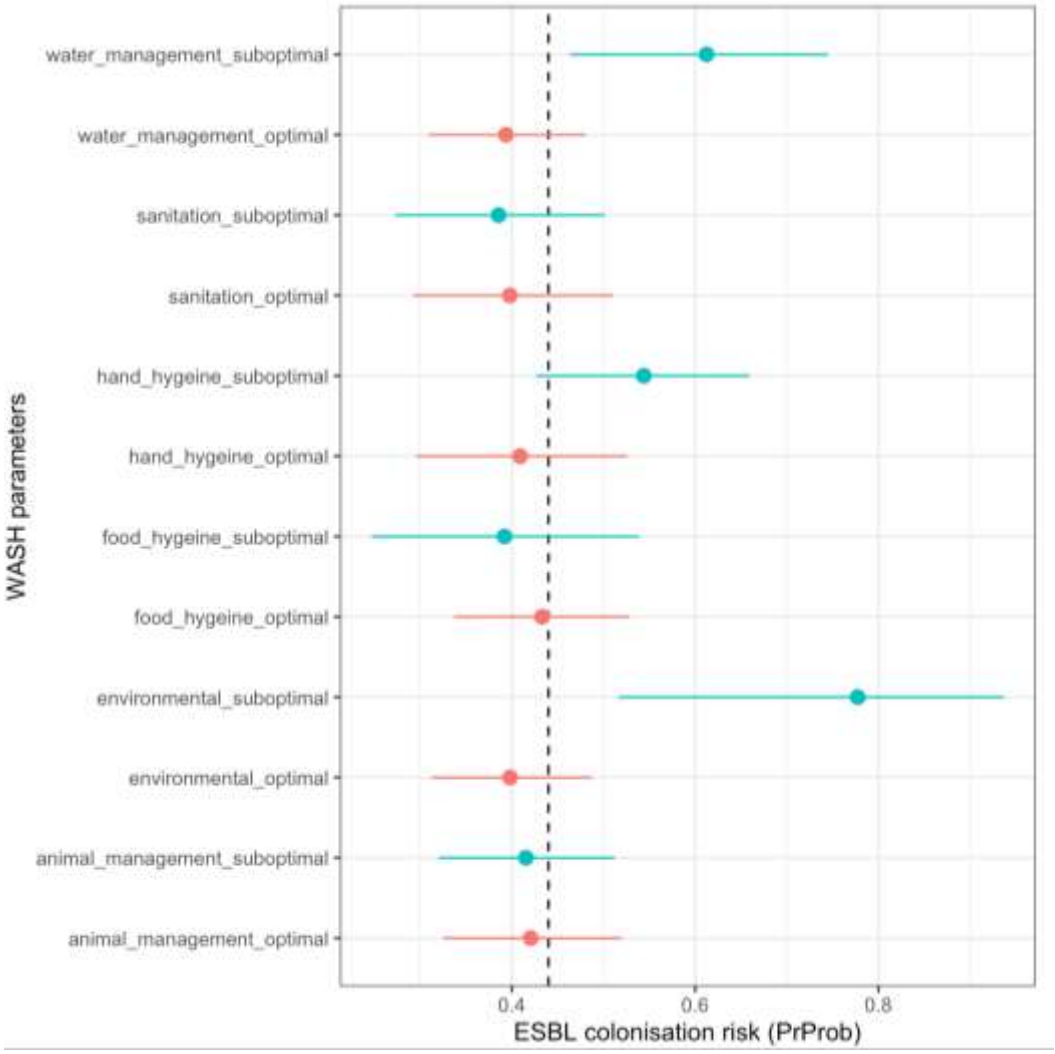


Figure 6.14. Plot of the predicted probability of ESBL colonisation attributed to alterations in key optimal (red) and suboptimal (blue) WASH parameters, adapted from posterior estimates of the ESBL Bayesian multivariate model.

6.11. Discussion

The results of the PCA analysis illustrated the variables which broadly describe the individual-level, household-level and sample level data. Age and antibiotic usage describe the individual level, animal co-habitation (its affiliated effects) and WASH practices on household water usage, sanitation and food hygiene describe the household-level, and environmental contamination describes the sample-level data. There was some degree of collinearity in responses for animal parameters in households that own animals and for sanitation materials in households that had access to hand washing facilities. Otherwise, the relationships between the variables, as determined by Pearson's coefficients were

small. Pragmatic selection of PCAs for inclusion into regression models was completed using sensitivity analysis against model fit (AIC) (379,380).

PCAs identified by this method were input into Bayesian mixed-effect models, which allow us to consider the probability of a parameter, permit *a priori* knowledge to be incorporated and enable us to fit models that include both constant (fixed) and varying (random) effects (381). This is particularly useful within this dataset, as it allows us to generalize the results and enables nested longitudinal measurements of the repeated sampling of participants and households (381).

Using this method, I found a very strong association with ESBL colonisation and the wet season (aOR = 1.96, 95% CI 1.60 - 2.34), and that season was the highest independent risk. When broken down into species-specific data, this seasonal risk is found with ESBL *K. pneumoniae* (aOR =2.32, 95% CI 1.75-2.98), and to a lesser extent with ESBL *E. coli* (aOR =1.74, 95% CI 1.48-2.10), highlighting that the seasonal effect is a key risk factor which is more pronounced with ESBL-K than ESBL-E. Within Malawi, the role of the wet season has been documented previously, with increased ESBL-E colonisation noted in unselected patients admitted to the QECH hospital in the wet season (aOR 2.21, 95% CI 1.07-8.75) (300). Increased ESBL gut colonisation in the wet season has also been reported in One-Health studies elsewhere in the world (382), including higher prevalence of ESBL-E colonisation in the wet season in community members from Bangladesh (383) and Madagascar (384,385). Furthermore, higher environmental contamination of ESBL-E have been reported in the wet season than the dry season, most recently from the WHO tricycle (pilot) study in Indonesia (386).

Seasonal variations are seen in particular with diarrhoeal pathogens that are faecal-orally acquired, including cryptosporidium, enterotoxigenic *E. coli* (ETEC) and *Salmonella* spp. (211,213,382,387–389). ESBL *E. coli* and *K. pneumoniae* are also faecal-orally acquired, and therefore it is likely that wet season corresponds with alterations in WASH risks factors that promote the faecal-oral acquisition of gut bacteria. Temperature and rainfall are the two components that are likely to be of greatest impact (390). Malawi has a sub-tropical climate, with high average temperatures (28^{o}C) and a warm wet season between November and April (391,392). Higher temperatures in the environment promote the growth of enteric bacteria and increases the rate of cell-cell plasmid conjugation, thereby increasing the risk of HGT, and subsequently, ARB (393,394). High temperatures are also associated with increases in the prevelance of diarrheal disease, and globally it is reported that for every 1°C increase in temperature there is an 8% increase in the incidence of diarrheagenic *E. coli* (390), influencing both antibiotic usage and household transmission risks. In relation to precipitation, the

annual rainfall in Malawi fluctuates year-to-year, but is consistently at its highest levels between November and March (392). The average precipitation during this period is 225mm per month, falling to just 12mm per month between the period June and September (392). In Malawi, this heavy period of rainfall puts pressure on fragile WASH infrastructure, particularly in urban settings, and flooding events during this time promote the spread and transmission of faecal-oral bacteria (395). Poor drainage systems, and the flooding of open drains in in the wet season has been shown elsewhere to lead to increased risks of bacterial colonisation and diarrhoeal disease (210,395–397), and transient drain flooding events in the urban environment have been linked to increased numbers of paediatric infections (210). The effects of climate change are likely be most keenly felt in countries like Malawi (392,394,398), and given the seasonal effects seen in this data, we might expect this will only be exacerbated with time. We may therefore consider classifying AMR colonisation alongside malaria and enteric fever (398) as a climate sensitive condition.

Waste management and sanitation in LMICs presents a major challenge to public health, given the absence of piped waste services and reliance on pit latrines. For example, whilst owning a toilet leads to less diarrheal disease overall (320), accumulated faecal sludge still requires management (399,400). Effective sewerage and sludge management has been shown to reduce the incidence of diarrhoeal disease in flood-prone areas (401). Improvements in waste management should be a key focus of policymakers in LMIC settings, when considering the immediate and downstream risks to health. Discharge of sewerage into aquatic environments, such as rivers has been shown to lead to high amounts of recoverable ARGs and ARB (402), and this is further compounded by the expelling of resistance driving chemicals such as antibiotics, pesticides and heavy metals (194,403,404). Human interactions with these contaminated waterways increases the acquisition of resistant bacteria, and has been shown to increase the rates of gut colonisation with ESBL *E. coli* (405). Urban environments in Malawi provide the perfect storm for acquisition of ESBL bacteria during the wet season, given the paucity of WASH infrastructure and inadequate waste management practices (Chapter 4), the high circulating levels of ESBL gut colonisation in humans and animals (Chapter 5), and the high frequency of human interactions with urban rivers (Chapter 3).

Seasonal effects vary from place to place; for example, in some settings, including Blantyre there is an increase in the incidence of diarrhoeal disease in the dry season, especially following a short episode of heavy rainfall (406,407). This suggests that accumulation of faecal contamination occurs during dry periods, which is then followed by a “flushing” effect, and this in turn provides a strong environmental risk factor of bacterial acquisition. This effect is most prominently shown in urban areas with dense

populations and impervious surfaces; illustrating how the rural-urban geography can alter climate-associated risks (407). For example, in Ecuador, heavy rainfall in the wet season has been shown to increases the risk of diarrhoea in households that use *unimproved water sources* and in the dry season low rainfall provides an increased risk of diarrhoeal disease in households that have *unimproved sanitation* (408). This opposing seasonal risk illustrates the importance of accounting for unique household and regional-specific factors and supports the use of complex multifaceted interventions that are tailored to each setting, when designing interventions that combat the effects of climate insecurity.

A greater risk of ESBL carriage was seen with increased household density (denominated by "count" in figures 6.9-6.11 & 6.13), predominantly as a result of ESBL *K. pneumoniae* carriage. Within household transmission is a well-recognised driver of AMR spread within the community (105,409). In fact, human-human transmission directly as a result of household density is thought to be the predominate route attributed to community carriage of ESBL *E. coli* in LMIC and HIC settings (83,87,104–106). The greater effect of household density on ESBL *K. pneumoniae* carriage is a novel observation and requires further investigation (410). The other household effect evaluated was income. Here, no change was seen in ESBL colonisation risk associated with differences in household income, although it is worth noting that the average household income across all sites was well below the internally accepted poverty line, and so small differences in low income may not be wide enough to determine an effect size.

Non-modifiable individual risk factors including age and sex were assessed in PCAs 1, 2 and 3, alongside the modifiable risk factors ABU and healthcare exposure. Factors associated with young age (Ind1 PCA) were somewhat protective against ESBL colonisation in our cohort (aOR=0.86, 95% CI 0.80-0.92), whilst sex was not a factor in ESBL, ESBL-E or ESBL-K colonisation via this analysis. Hospital and healthcare admission has been shown to increase colonisation in community households (411), however there was a low frequency of healthcare exposure in our cohort and determining the effect of healthcare exposure would require an alternative study design. ABU was more frequent, with 15.2% (n=147) of people receiving an antibiotic within 6 months of baseline (Chapter 3), here no link to ESBL colonisation is seen, other than a small increase in risk with ESBL-K colonisation. This is supported by recent modelling data where reduction in ABU makes little difference to ESBL colonisation in the community and better success obtained from modifying household WASH factors such as hand-hygiene measures (106).

Overall, there were minimal effects seen in association with most household-level WASH factors, apart from tap water usage (WASH PCA9), and variations in the risks of drain water exposure (WASH PCA8) between ESBL-E and ESBL-K. This may seem counter-intuitive, however when water is intermittently piped to taps as is often the case in Blantyre, there can be environmental ingress of faecal-contaminated groundwater into cracked pipes (398,412,413). Equally these results might reflect post-collection contamination within households that use and store tap water. A detailed analysis of household source water and drinking water storage in the future might unpick some of the factors leading to this finding. Lastly, the variations in risk from drain water most likely reflect the regional differences in drain water exposures, and regional variations are explored in the next chapter.

I have highlighted the significance of environmental contamination, particularly the association of ESBL colonisation with contaminated household surfaces and floors (Cont PCA1). The modelling approaches undertaken in this chapter do not assess the directionality, and additional modelling or experimental designs would be needed to assess for directionality. However, it was possible to run models with and without environmental contamination. These results illustrated minimal change in risk difference and are suggestive that environmental data provides additional information external to WASH. Therefore, management of WASH in the absence of improved environmental hygiene may not alter community ESBL colonisation rates, and this finding promotes the incorporation of environmental AMR surveillance within local and national AMR surveillance campaigns.

In the models used, WASH factors may not have been associated with risks in ESBL colonisation, but this could be related to the combination and weighted contributions of WASH factors in each PCA, or a reduction in effect size due to associations between WASH and seasonal effects. This illustrates a clear limitation of the use of PCAs for interpretating risks in multivariate models. An example of this is WASH PCA8, which comprises of drain water exposure and coverage of stored water. By condensing the dataset through PCAs, these unlinked, non-complimentary variables may cancel out positive and negative effects on ESBL colonisation, and falsely illustrate the absence of their individual impact. A more nuanced approach would be preferable that expresses them independently, rather than non-real-world scenarios generated by PCA groupings, and this has been undertaken in Chapter 7.

Here, I used Bayesian models to unpack some of the complexity of WASH by predicting changes in ESBL colonisation from dummy data, using hypothetical individuals. Whilst dummy data are artificially generated for maximal worst case and best-case scenarios, and predictions are subject to intrinsic biases from model fitting, these simulations predict the greatest change in ESBL colonisation status

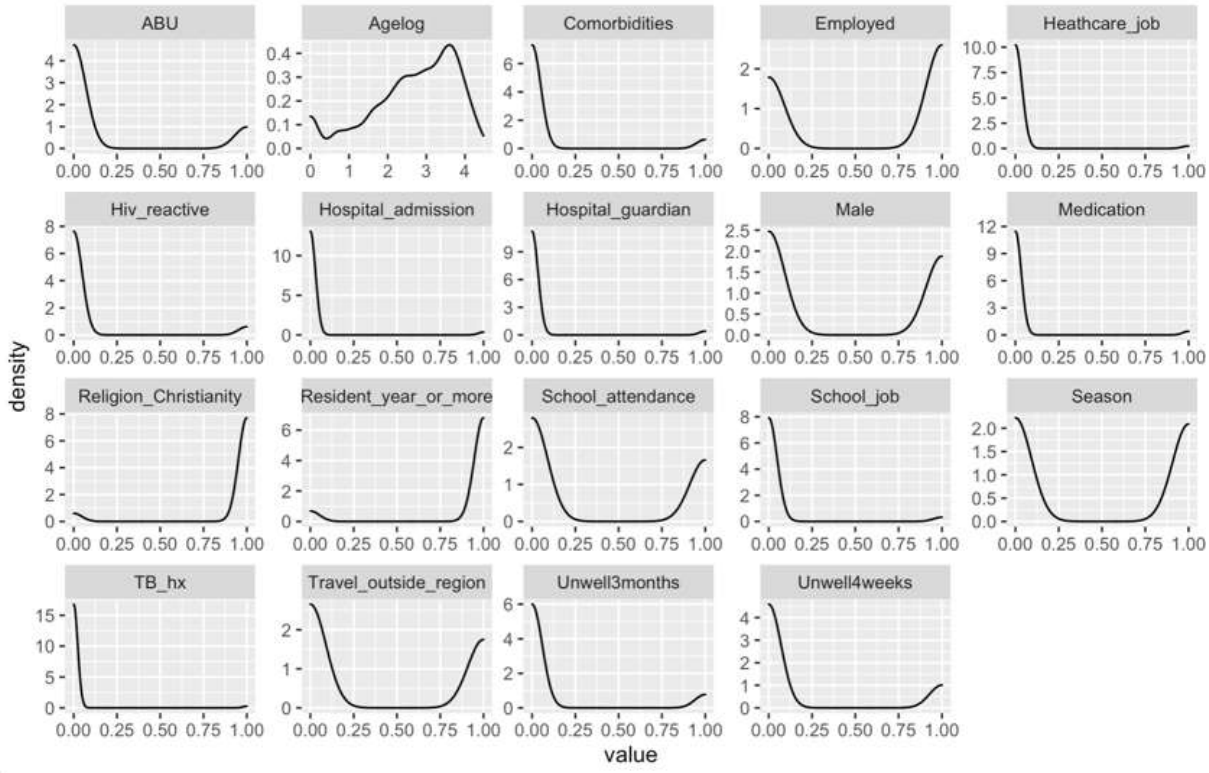
following optimisation of environmental, water management and hand-hygiene factors. This corroborates the findings of independent effects from environmental interactions, and stresses the importance of addressing contaminated water and hand hygiene measures, as previously reported by models exploring community ESBL colonisation risks in LMICs (106).

This exploratory analysis is subject to a number of limitations. Firstly, the use of PCAs to reduce the dimensionality of the data groups variables together in combinations that are sometimes difficult to attach real-world meaning to. In the next chapter I will perform a univariate and multivariate analysis of the variables, to unpack some of these complexities. The definition of ESBL colonisation status I used in these models also warrants consideration when interpretating the results. ESBL colonisation status was determined for each stool sample independently. This pragmatic choice was made because multiple samples were obtained from each individual and often included both positive and negative results. Given the high degree of intra-individual flux in ESBL status described, it could be argued that colonisation should be assessed from the household level, or alternatively from a weighted composite at the individual level. While these are possible, this pragmatic choice allowed me to account for independent events and adjust for within-participant and within-household factors by including them as random effects. Next, the models I used have some degree of intrinsic biases and therefore I cannot determine a causal chain for ESBL colonisation. So, while I have identified individual, household and sample level associated risks, I am unable to determine the causality or directionality of these relationships. Future analysis of this dataset may be performed using models designed to delineate the causal chain, or alternatively, prospective studies could be undertaken which are designed to explore the route of ESBL acquisition within this setting. Dovetailed with this, phenotypic data alone is not precise enough to directly link transmission events between human, animal and environmental compartments, nor can it assess for inter-household transmission. Bacterial genomic analysis will better explore this relationship and modelling of ARGs, or ARG-ARB combinations may provide unique insights into the risks of ESBL transmission.

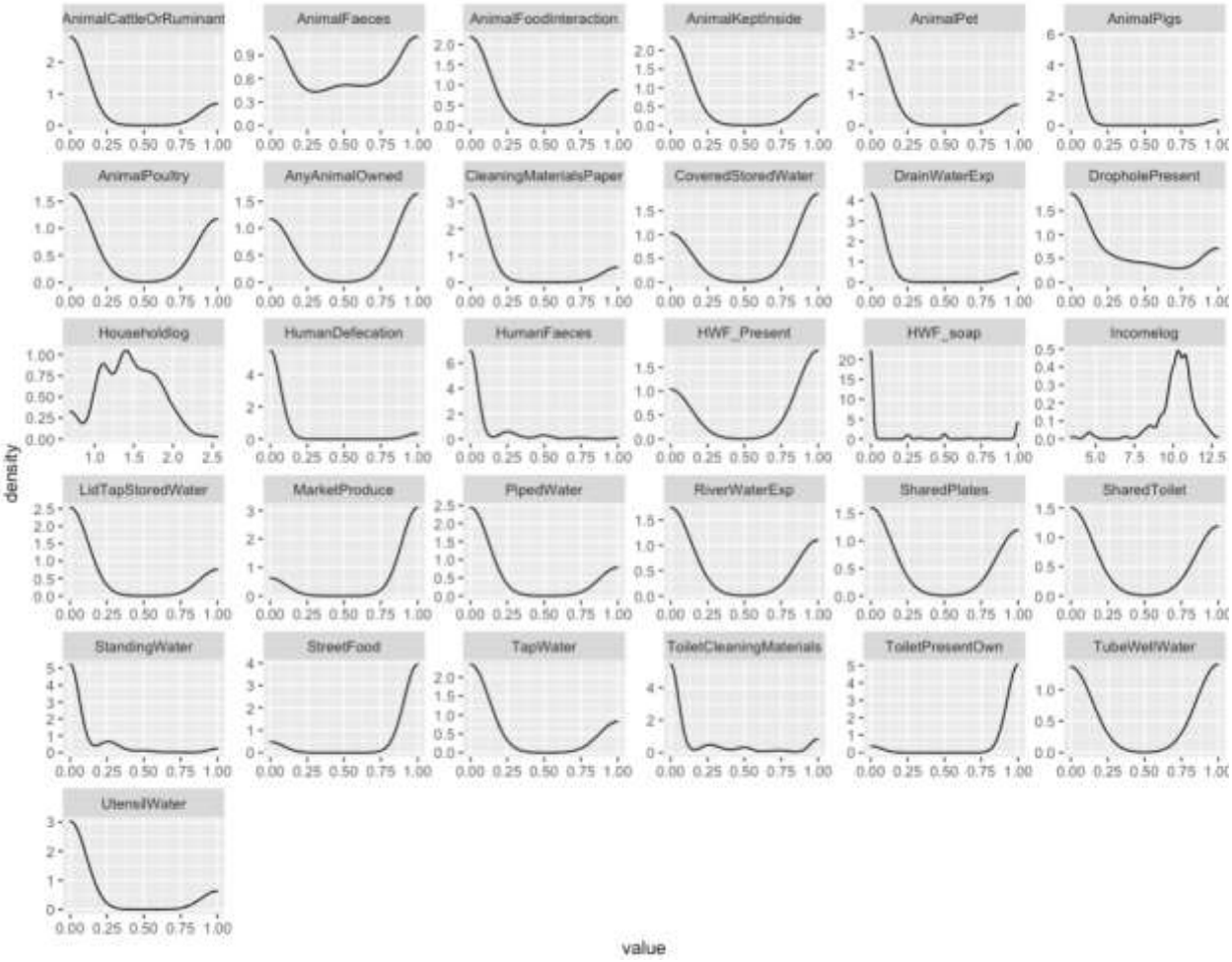
Within this chapter I have identified the key role of seasonal, environmental and specific WASH factors on ESBL, ESBL-E and ESBL-K colonisation using Bayesian mixed-effects models. In the next chapter I will explore further whether regional differences in the environment, infrastructure, animal co-habitation or behavioural practices provide alterations in the risks of ESBL colonisation.

Appendices

Appendix 6i. Density plots for individual variables, including those that are transformed.



Appendix 6ii. Density plots for household variables, including those that are transformed.



Appendix 6iii. Non-zero-sum table of individual variables illustrating homogeneity in the dataset

Non Zero Values

Indervidual variables

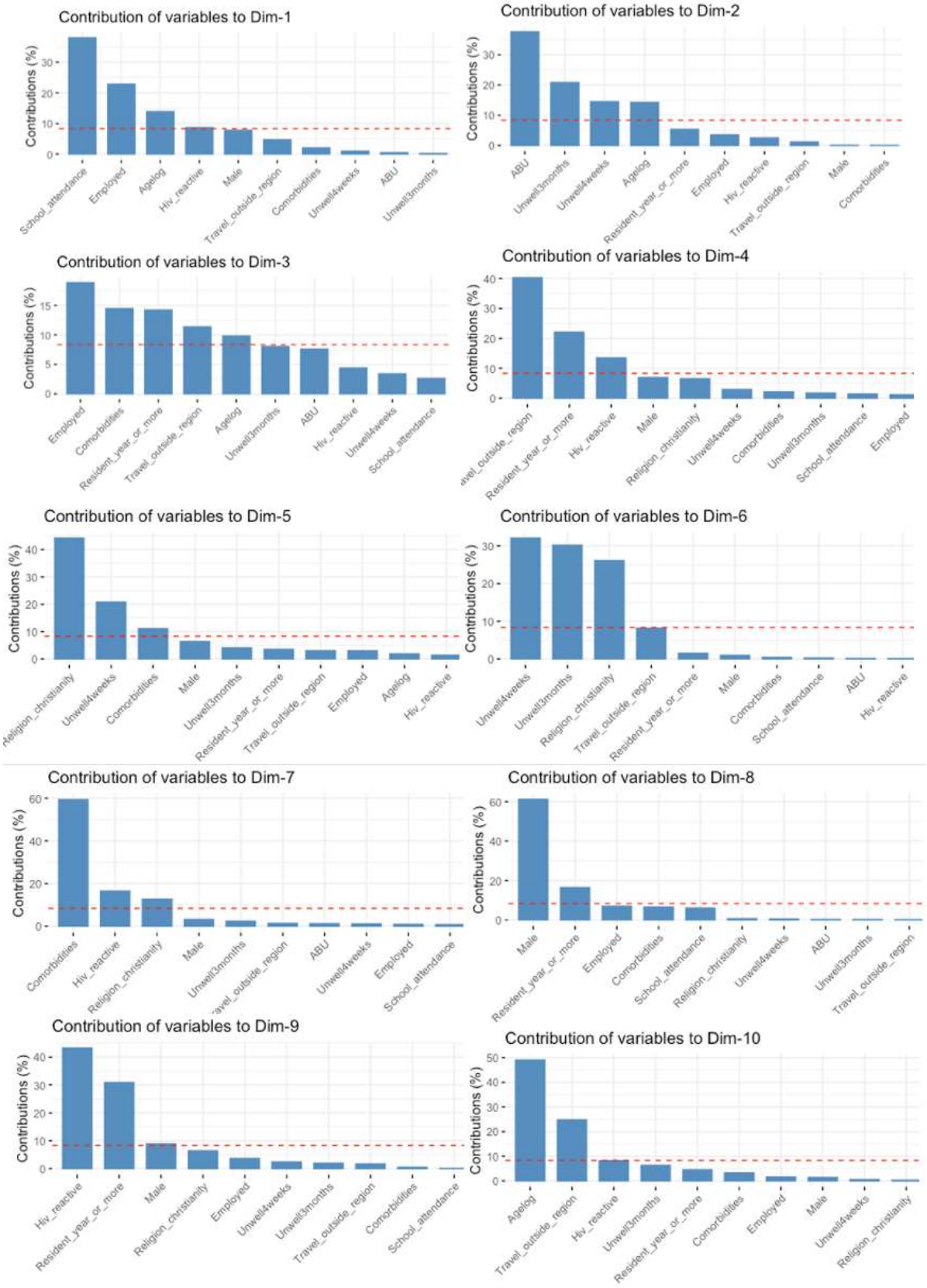
Variable	freqRatio	percentUnique	zeroVar	nzv
sample_id	1.000	100.000	FALSE	FALSE
pid	1.600	33.946	FALSE	FALSE
hhid	1.026	9.735	FALSE	FALSE
Region	1.035	0.110	FALSE	FALSE
age	1.260	2.976	FALSE	FALSE
ABU	4.804	0.073	FALSE	FALSE
esbl	1.394	0.073	FALSE	FALSE
season	1.064	0.073	FALSE	FALSE
Male	1.315	0.073	FALSE	FALSE
Religion_christianity	12.817	0.073	FALSE	FALSE
School_attendance	1.687	0.073	FALSE	FALSE
School_job	117.348	0.073	FALSE	TRUE
Heathcare_job	193.429	0.073	FALSE	TRUE
Hospital_admission	36.806	0.073	FALSE	TRUE
Hospital_guardian	27.062	0.073	FALSE	TRUE
Employed	1.454	0.073	FALSE	FALSE
Resident_year_or_more	9.976	0.073	FALSE	FALSE
Travel_outside_region	1.516	0.073	FALSE	FALSE
TB_hx	60.864	0.073	FALSE	TRUE
Medication	28.269	0.073	FALSE	TRUE
Comorbidities	11.544	0.073	FALSE	FALSE
Unwell4weeks	4.544	0.073	FALSE	FALSE
Unwell3months	7.752	0.073	FALSE	FALSE
Hiv_reactive	12.542	0.073	FALSE	FALSE
Agelog	1.492	2.939	FALSE	FALSE

Appendix 6iv. Non-zero-sum table for household-level variables illustrating homogeneity in the dataset

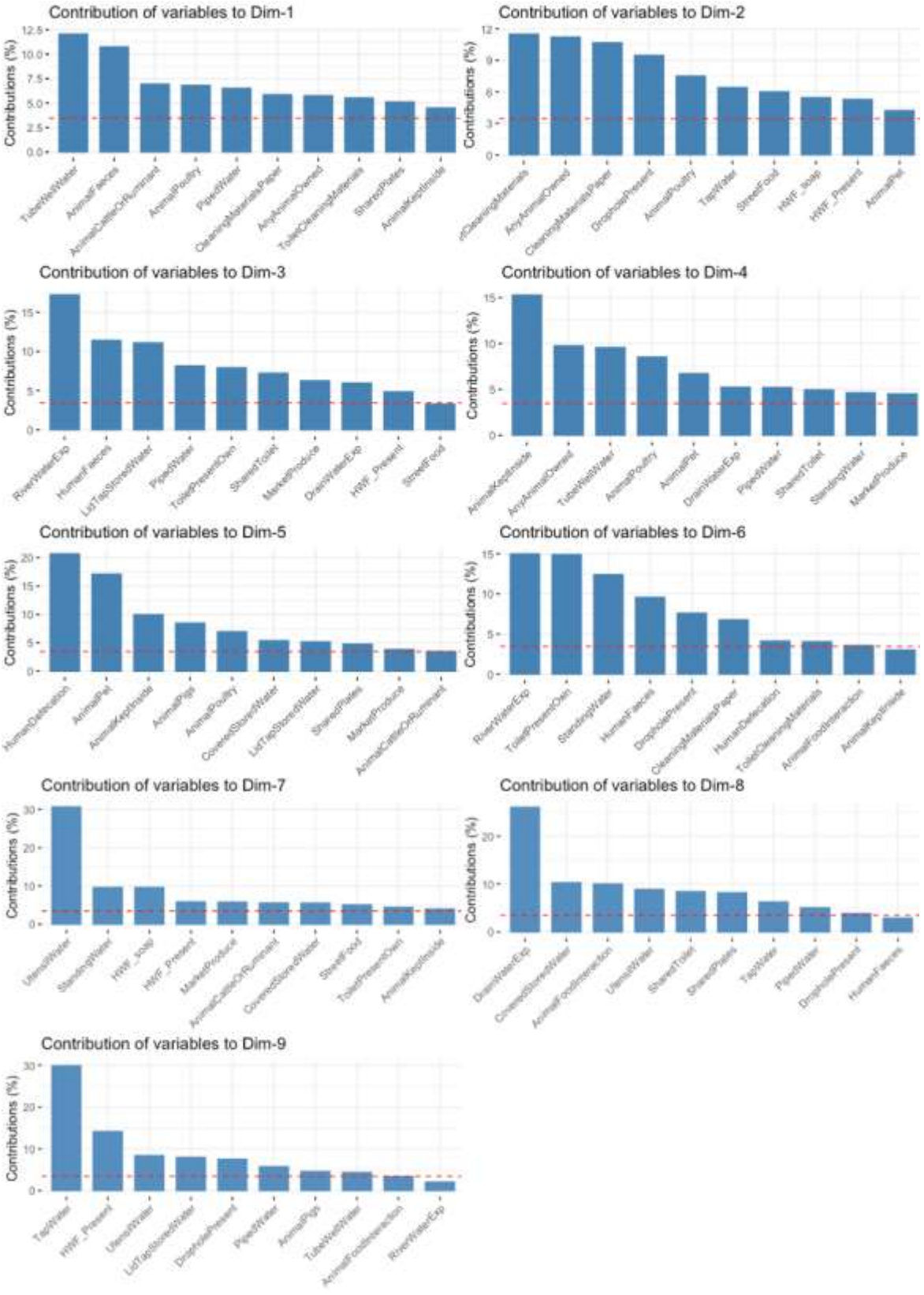
Non Zero Values
WASH variables

Variable	freqRatio	percentUnique	zeroVar	nzv
hid	1.000	100.000	FALSE	FALSE
SharedToilet	1.268	0.667	FALSE	FALSE
CleaningMaterialsPaper	5.923	0.667	FALSE	FALSE
HWF_Present	1.778	0.667	FALSE	FALSE
CoveredStoredWater	1.804	0.667	FALSE	FALSE
UncoveredStoredWater	32.333	0.667	FALSE	TRUE
LidTapStoredWater	3.348	0.667	FALSE	FALSE
AnimalFoodInteraction	2.488	0.667	FALSE	FALSE
ToiletPresentOwn	13.286	0.667	FALSE	FALSE
HumanDefecation	15.667	0.667	FALSE	FALSE
CleanAnimalWaste	22.077	0.667	FALSE	TRUE
StreetFood	8.091	0.667	FALSE	FALSE
SharedPlates	1.344	0.667	FALSE	FALSE
MarketProduce	5.000	0.667	FALSE	FALSE
RiverWaterExp	1.586	0.667	FALSE	FALSE
DrainWaterExp	9.714	0.667	FALSE	FALSE
AnimalKeptInside	2.896	0.667	FALSE	FALSE
UtensilWater	4.769	0.667	FALSE	FALSE
AnyAnimalOwned	1.400	0.667	FALSE	FALSE
AnimalCattleOrRuminant	4.085	0.667	FALSE	FALSE
AnimalPoultry	1.400	0.667	FALSE	FALSE
AnimalPet	4.263	0.667	FALSE	FALSE
AnimalPigs	17.750	0.667	FALSE	FALSE
PipedWater	3.110	0.667	FALSE	FALSE
TapWater	2.846	0.667	FALSE	FALSE
TubeWellWater	1.027	0.667	FALSE	FALSE
Incomelog	1.184	16.000	FALSE	FALSE
Householdlog	1.099	4.000	FALSE	FALSE
HWF_soap	5.357	2.667	FALSE	FALSE
DropholePresent	2.617	3.000	FALSE	FALSE
AnimalFaeces	1.020	3.000	FALSE	FALSE
HumanFaeces	13.474	2.667	FALSE	FALSE
ToiletCleaningMaterials	6.441	3.000	FALSE	FALSE
StandingWater	9.423	2.667	FALSE	FALSE

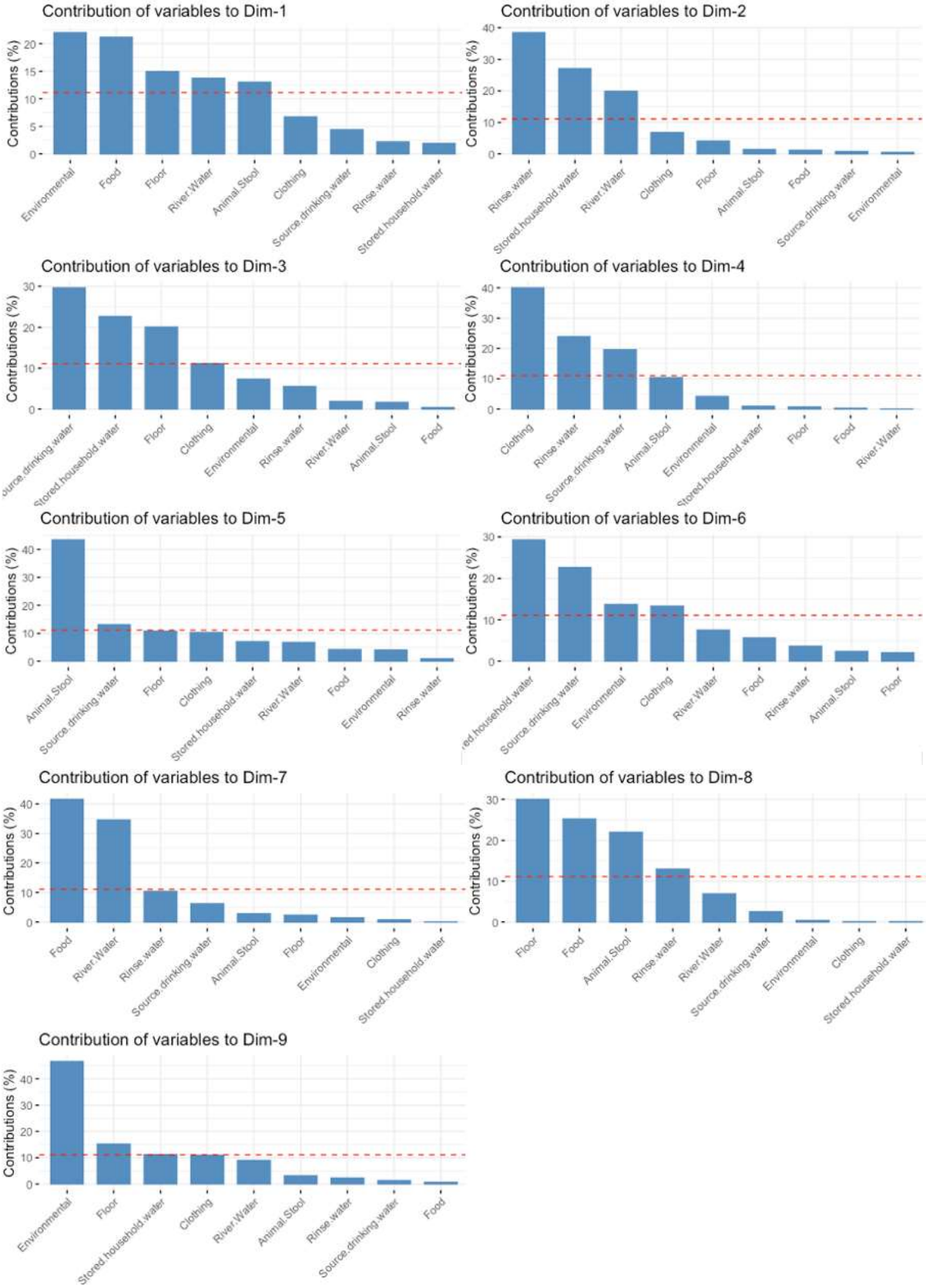
Appendix 6v. Individual PCA contributions.



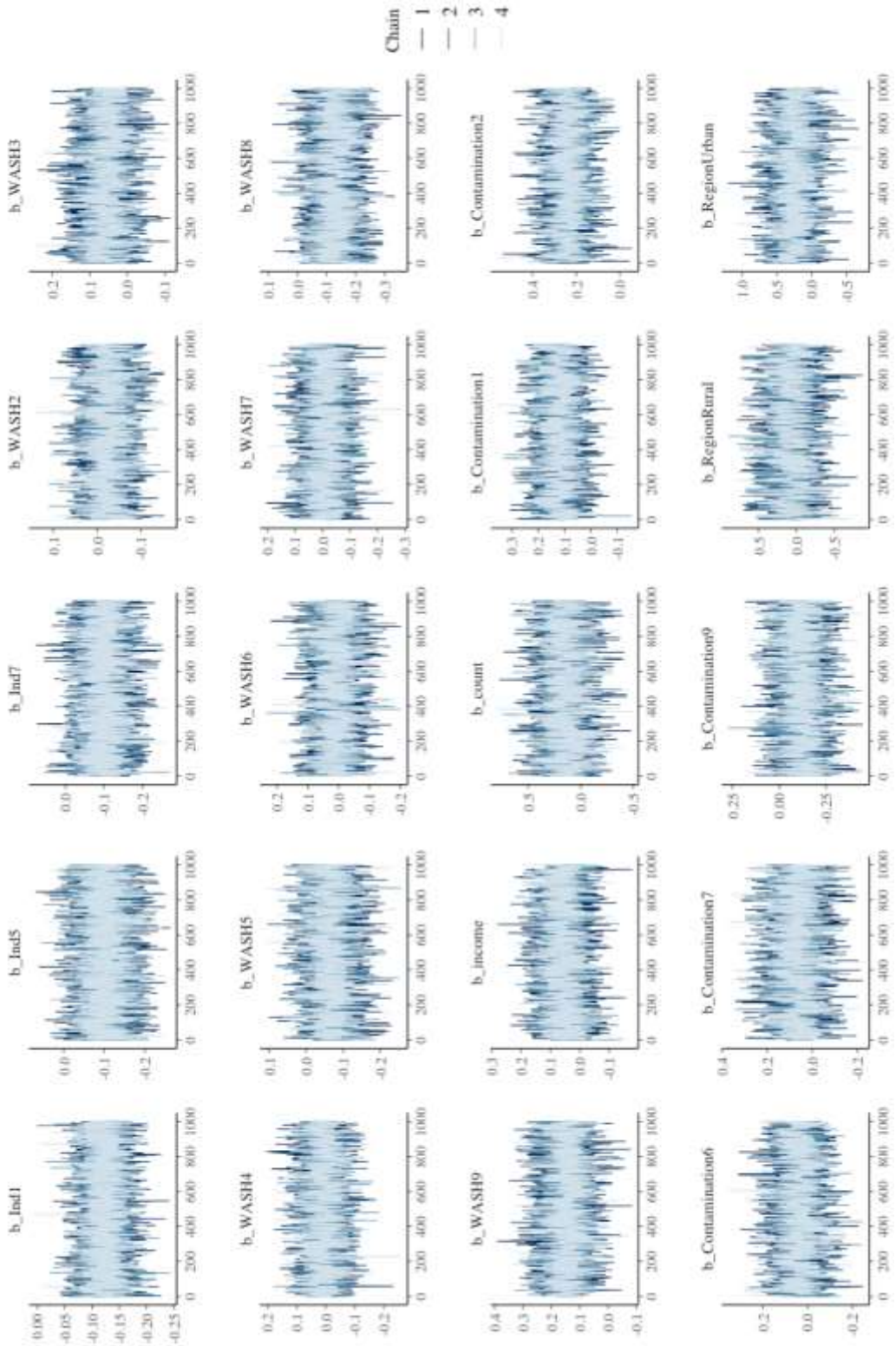
Appendix 6vi. Household PCA contributions.



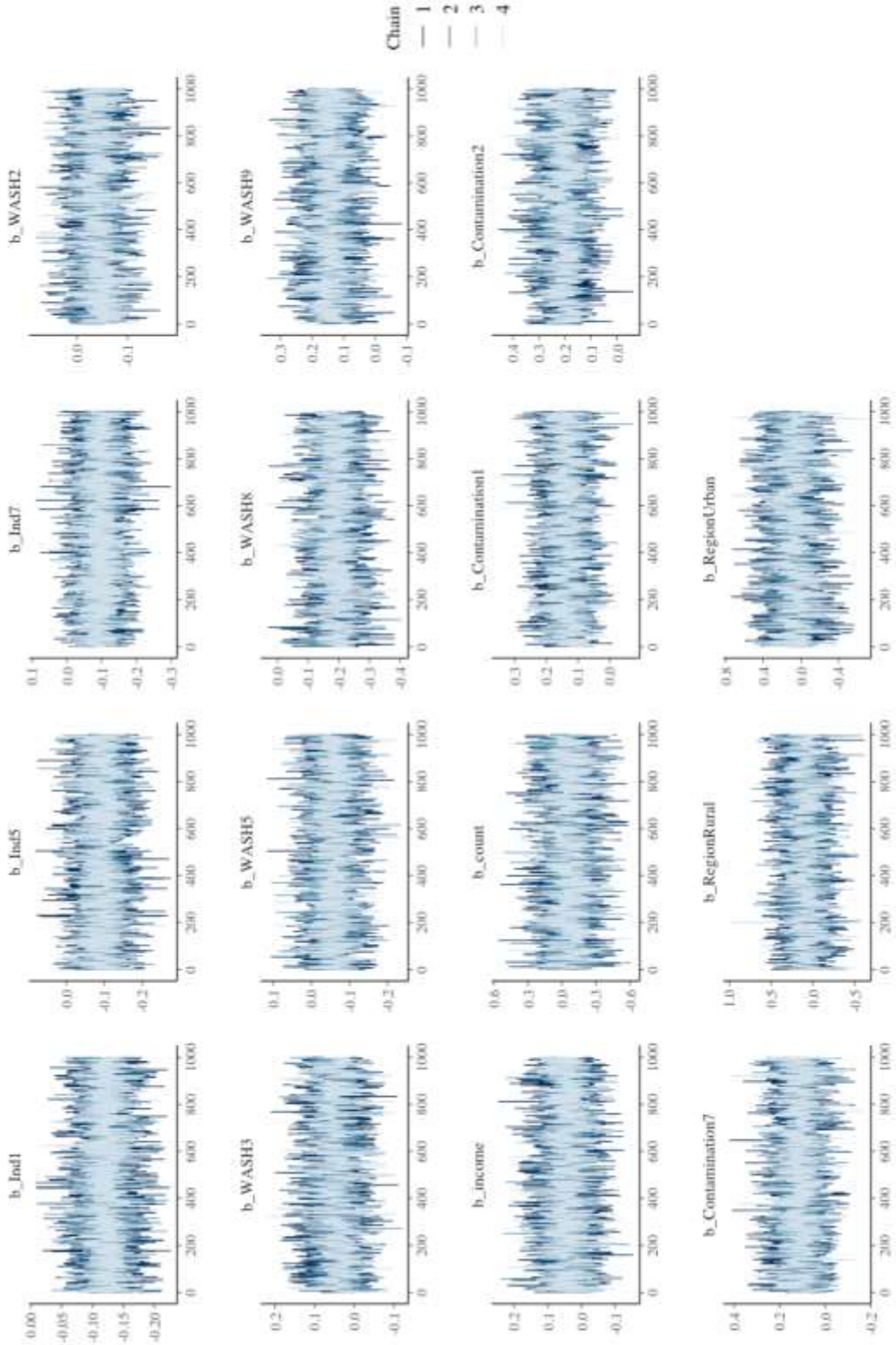
Appendix 6vii. Laboratory PCA contributions.



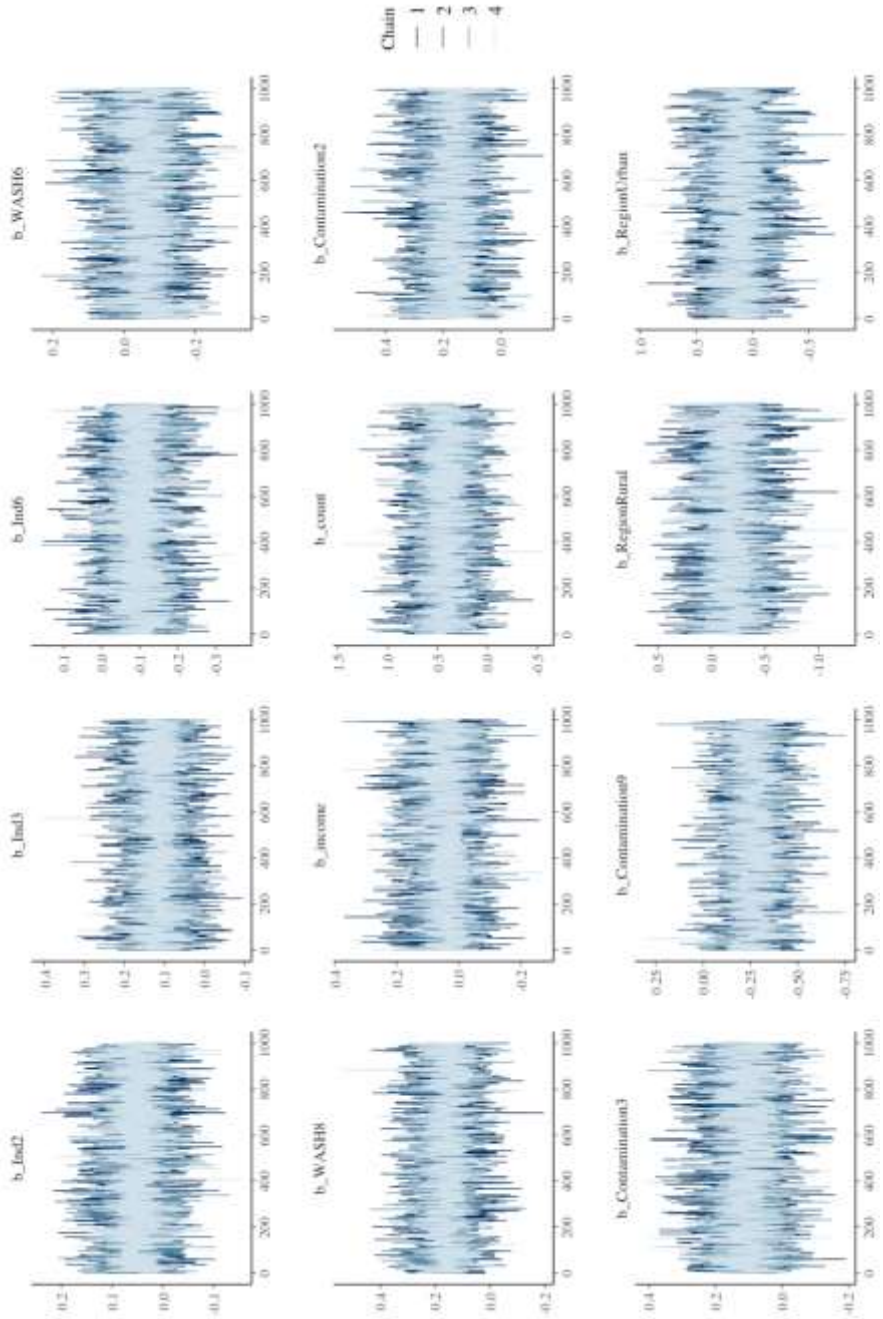
Appendix 6viii. Trace plots for ESBL model. Model was fit with Stan v2.21.0 via the R *brms* v2.13.5 package with 4 chains per dataset each with 2000 iterations in total, with 1000 warm up iterations.



Appendix 6ix. Trace plots for ESBL *E. coli* model. Model was fit with Stan v2.21.0 via the R *brms* v2.13.5 package with 4 chains per dataset each with 2000 iterations in total, with 1000 warm up iterations.



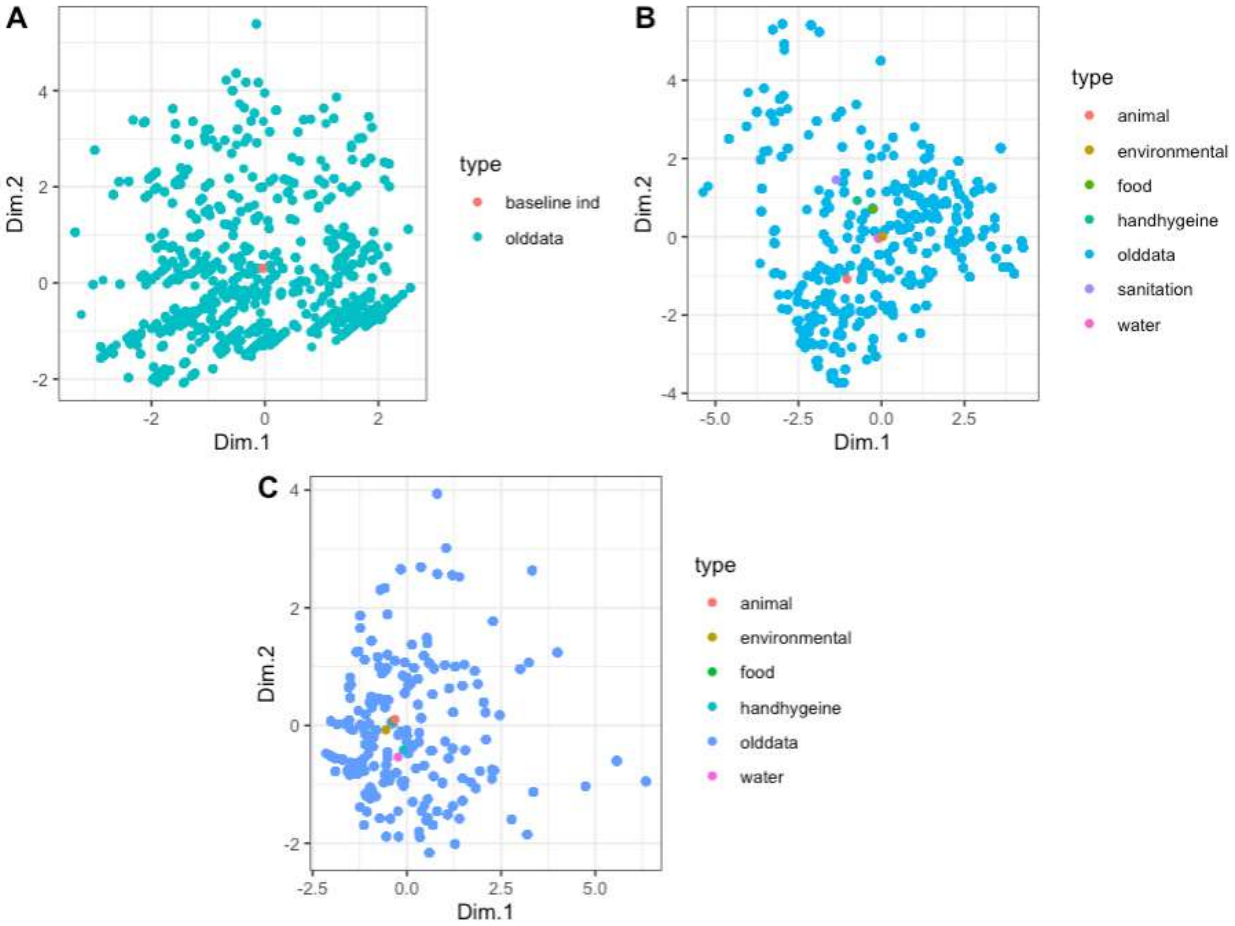
Appendix 6x. Trace plots for ESBL *K. pneumoniae* model. Model was fit with Stan v2.21.0 via the R *brms* v2.13.5 package with 4 chains per dataset each with 2000 iterations in total, with 1000 warm up iterations.



Appendix 6xi. Optimal and suboptimal WASH variable settings, stratified by category, including outcome response settings used in prediction modelling.

WASH category	Classification	Variables of interest	Set outcome response
Water management	Optimal	Source water samples	0 = Source water free from ESBL bacteria
		Drinking water samples	0 = Drinking water free from ESBL bacteria
		Covered stored water	1 = Drinking water covered when stored
	Sub-optimal	Source water samples	1 = Source water has contamination with ESBL bacteria
		Drinking water samples	1 = Drinking water has contamination with ESBL bacteria
		Covered stored water	0 = Drinking water not covered when stored
Sanitation	Optimal	Human defecation practice	0 = Human defecation not practiced
		Toilet sharing	0 = Toilet not shared
		Drophole cover	1 = Drophole cover present
		Toilet cleansing materials	1 = Toilet cleansing materials present
		Human or animal faeces seen	0 = Human or animal faeces not seen in the household environment
	Sub-optimal	Human defecation practice	1 = Human defecation practiced
		Toilet sharing	1 = Toilet shared
		Drophole cover	0 = Drophole cover not present
		Toilet cleansing materials	0 = Toilet cleansing materials not present
		Human or animal faeces seen	1 = Human or animal faeces seen in the household environment
Hand hygiene	Optimal	HWF availability	1 = HWF available
		Soap availability	1 = Soap available
		Hand hygiene samples	0 = Hand samples not contaminated with ESBL bacteria
	Sub-optimal	HWF availability	0 = HWF unavailable
		Soap availability	0 = Soap unavailable
		Hand hygiene samples	1 = Hand samples contaminated with ESBL bacteria
Food hygiene	Optimal	Street food	0 = Street food not consumed
		Shared plates	0 = Shared plates not used
		Food samples	0 = Food not contaminated with ESBL bacteria
	Sub-optimal	Street food	1 = Street food consumed
		Shared plates	1 = Shared plates used
		Food samples	1 = Food contaminated with ESBL bacteria
Environmental hygiene	Optimal	River water interactions	0 = No interaction with river water
		Drain water interactions	0 = No interaction with drains
		Standing water	0 = No standing water present
		Household environment	0 = No ESBL contamination of household environment
		Household floor	0 = No ESBL contamination of household floor
	Sub-optimal	River water interactions	1 = Interaction with river water
		Drain water interactions	1 = Interaction with drains
		Standing water	1 = Standing water present
		Household environment	1 = ESBL contamination of household environment
		Household floor	1 = ESBL contamination of household floor
Animal management	Optimal	Animal ownership	0 = No animals owned by household
		Animal interactions with food	0 = No animals interacting with food
		Animals kept inside the house	0 = No animals kept inside the house
		Animal stool samples	0 = No ESBL animal stool identified at household
	Sub-optimal	Animal ownership	1 = Animals owned by household
		Animal interactions with food	1 = Animals interacting with food
		Animals kept inside the house	1 = Animals kept inside household
		Animal stool samples	1 = ESBL identified in household animal stool

Appendix 6xii. Scatter plot of (a) individual (b) household and (c) laboratory parameters for the first 2 principal component dimensions with new "dummy data" for baseline individual WASH and laboratory-level parameters included (coloured by WASH category type).



Chapter 7:

Regional contrasts between individual-level and household-level parameters on risks of ESBL colonisation.

7.0. Chapter Summary

A key question I have sought to address, is what are the similarities and differences in risk for ESBL colonisation between regions? Here I evaluate regional-specific variations in ESBL, ESBL-E and ESBL-K colonisation risks related to season, animal-cohabitation, environmental exposures and WASH infrastructure, access and behaviours.

Seasonal effects varied between setting, with the peri-urban site being the most climate sensitive and having the highest odds of ESBL colonisation in the wet season. Animal-associated risks were dependant on the combination of the site, species and bacteria. Individual-level differences were minimal between the regions, however household infrastructure, WASH practices and environmental exposures provided distinct regional-risks for ESBL colonisation. Site-dependant water management, sanitation and hand-hygiene practices influenced ESBL colonisation status and across all regions there were risks associated with sharing toilets, river water exposures and with regards to ESBL-K, increased household density.

These results indicate that the geographic location and associated variations in regional WASH infrastructure, practices and environmental exposures impact upon ESBL, ESBL-E and ESBL-K colonisation risk. Future interventions and policy designed to interrupt AMR transmission should be cognisant of these differences, and adaptions made wherever possible which are tailored to the local population for maximal effect.

My contributions to this chapter and those of others are included in Table 7.0.

Table 7.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted and analysed by the PhD candidate

Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborators, Tracy Morse and Chris Jewell. Statistical advice and coding help was sought from Chris Jewell, Barry Rowlingson and Joe Lewis.

7.1 Rationale and methodological overview

In Chapter 6 I identified key individual-level, WASH, environmental and seasonal factors associated with ESBL *E. coli* and ESBL *K. pneumoniae* colonisation across the study cohort. Given the clear differences in the physical landscapes and variations in WASH infrastructure and practices seen between urban, peri-urban and rural Malawi (**Chapters 3 & 4**), it is important to consider what the regional similarities and differences in risk of ESBL carriage are.

To do this, I have re-analysed outputs of the PCA and multivariate models in Chapter 6 to visualise regional differences and have performed univariate analysis of the variables stratified by region. Variables that were significantly associated with ESBL colonisation by univariate analysis ($p<0.05$) were placed in a regional context via likelihood ratio tests of model fit and included in a multivariate analysis as either independent or regionally adjusted covariates. A value of $p <0.05$ was used in preference to 0.1 or alternatives as these less stringent values failed to reduce the number of covariates included in the model. Models were fit with Stan v2.21.0 via the R *brms* v2.13.5 package with 4 chains per dataset each with 2000 iterations in total, with 1000 warm up iterations. Outputs have been expressed as odds ratios (OR) with 95% CrI.

7.2 Regional differences in the individual-level, household-level and sample-level data.

PCA on the study dataset was completed as described in Chapter 6, and outputs were stratified by setting (urban, peri-urban and rural). Confidence ellipses were drawn on the first 2 principal-component dimensions to evaluate broad regional differences in the individual-level, household-level, and sampling-level data (**Figure 7.1**). This approach highlighted that there was little variation in the individual-level data between regions (**Figure 7.1a**), but regional differences were seen in the household-level (**Figure 7.1b**) and sample-level (**Figure 7.1c**) data. These results imply that regional variations amongst participants are small, but that regional differences in WASH factors and ESBL

contamination are large, corroborating what was identified in the descriptions from Chapters 4 & 5 and emphasising the importance of site stratification when evaluating WASH and household ESBL contamination.

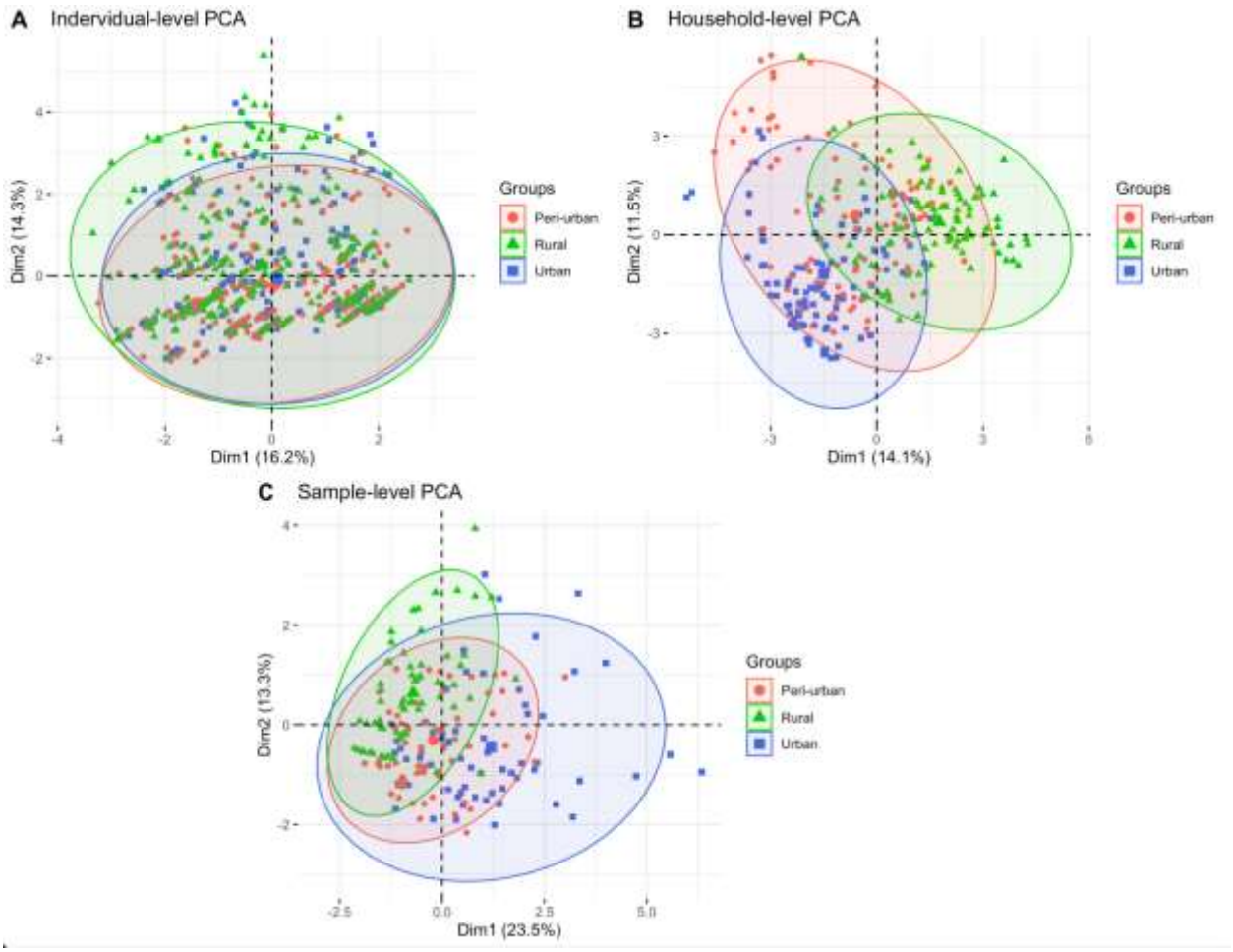


Figure 7.1. Confidence ellipses of regional effects exhibited by the (a) individual-level dataset, (b) household level dataset and (c) sample level dataset, from the first 2 PCAs.

7.3 Regional effects on ESBL colonisation from outputs of mixed effects models

To determine the regional contributions to ESBL colonisation from individual, household and sample-level data I repeated the multivariate models for ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* colonisation in Chapter 6, and plotted kernel density estimates and intervals for the posterior distributions of each PCA or independent variable, stratified by region (**Figures 7.2, 7.3 & 7.4**).

This approach identified that regional differences in the risk of ESBL colonisation were seen by season (**Figure 7.2, season**) and within household environmental or food contamination (**Figure 7.2,**

Contamination PCA1), with the greatest regional difference noted in the effect of ESBL colonisation in the wet season (**Figure 7.2)**. Wet season has already been identified as a risk for ESBL colonisation (**Chapter 6**), however, a higher odds of colonisation was seen in the peri-urban region compared to the other two regions during the wet season. Smaller regional effects were identified in risks of ESBL colonisation associated with household environmental and food contamination, with the highest risk being in the rural area. There were limited differences seen in the effect of region on the rest of the components tested, inclusive of WASH, participant (i.e. Ind1, Ind2, Ind3) or independent (i.e. income or household density) factors.

Regional effects on ESBL *E. coli* colonisation were similar to those represented in the ESBL model, with site being important in relation to wet season or environmental and food contamination risk (**Figure 7.3)**. Again, the peri-urban site showed a higher chance of ESBL colonisation in the wet season compared to the other sites and the rural site had an increased risk associated with contamination of the household environment or food. There were similarities seen in the effect on WASH, participant, or independent factors across the regions. Regional effects of ESBL *K. pneumoniae* colonisation were less notable, and similarities were seen across all covariates tested, inclusive of season (**Figure 7.4)**.

These results highlight that region is likely to be important in relation to ESBL *E. coli* colonisation, but less so with ESBL *K. pneumoniae* colonisation, and that the greatest effects of region are felt seasonally. I was unable to determine regional effects on WASH associated risk factors from this approach and it will be necessary to consider the WASH variables independently rather than as composites within principal components (section 7.4).

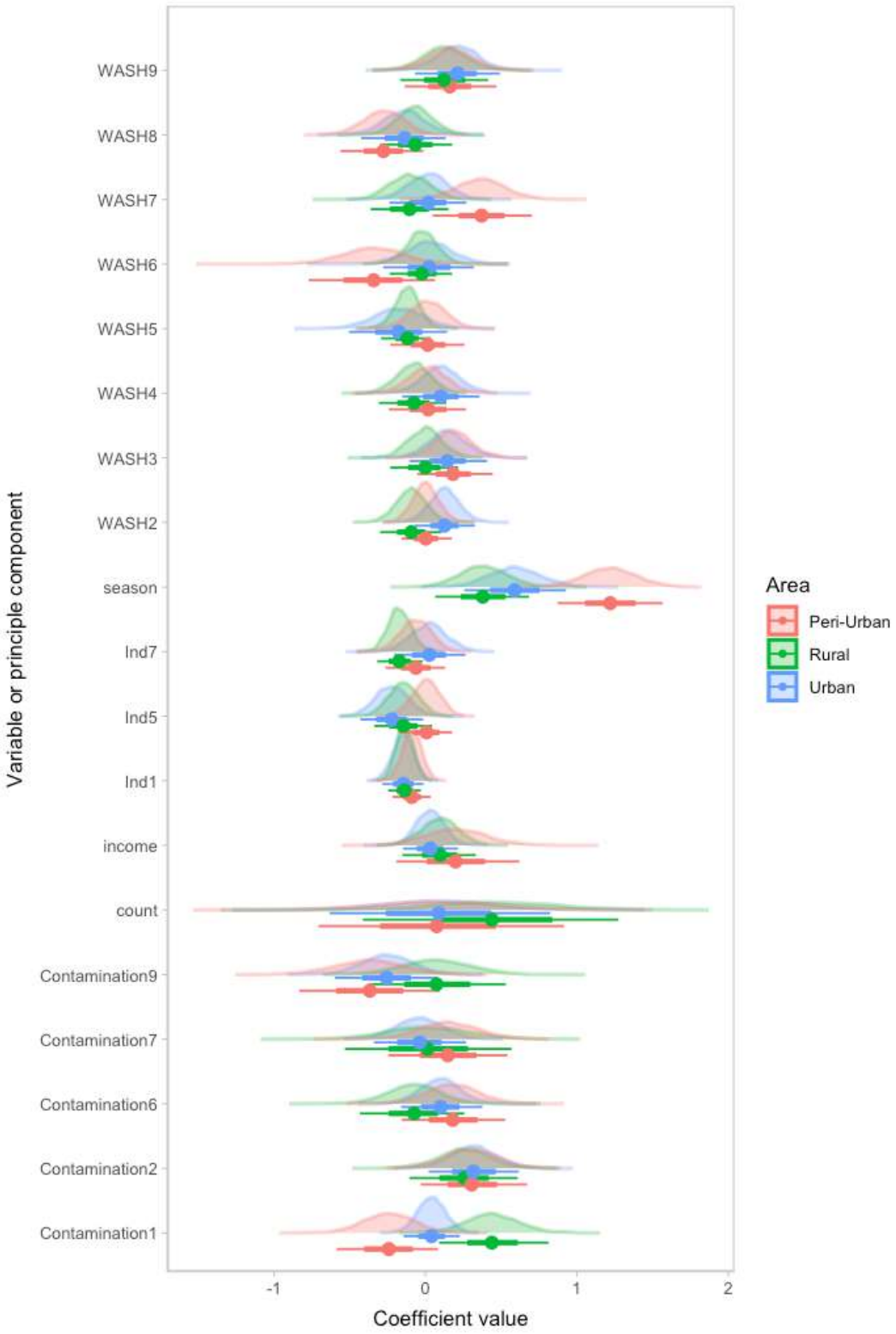


Figure 7.2. Half-eye plot of the Bayesian posterior distributions from principal components of the ESBL model, stratified by region. The shaded regions illustrate the kernel density estimations for urban (blue) peri-urban (orange) and rural (green) regions. Below this, the dot represents the median, with the thick and thin lines representing the 95% and 99% values of each posterior distribution.

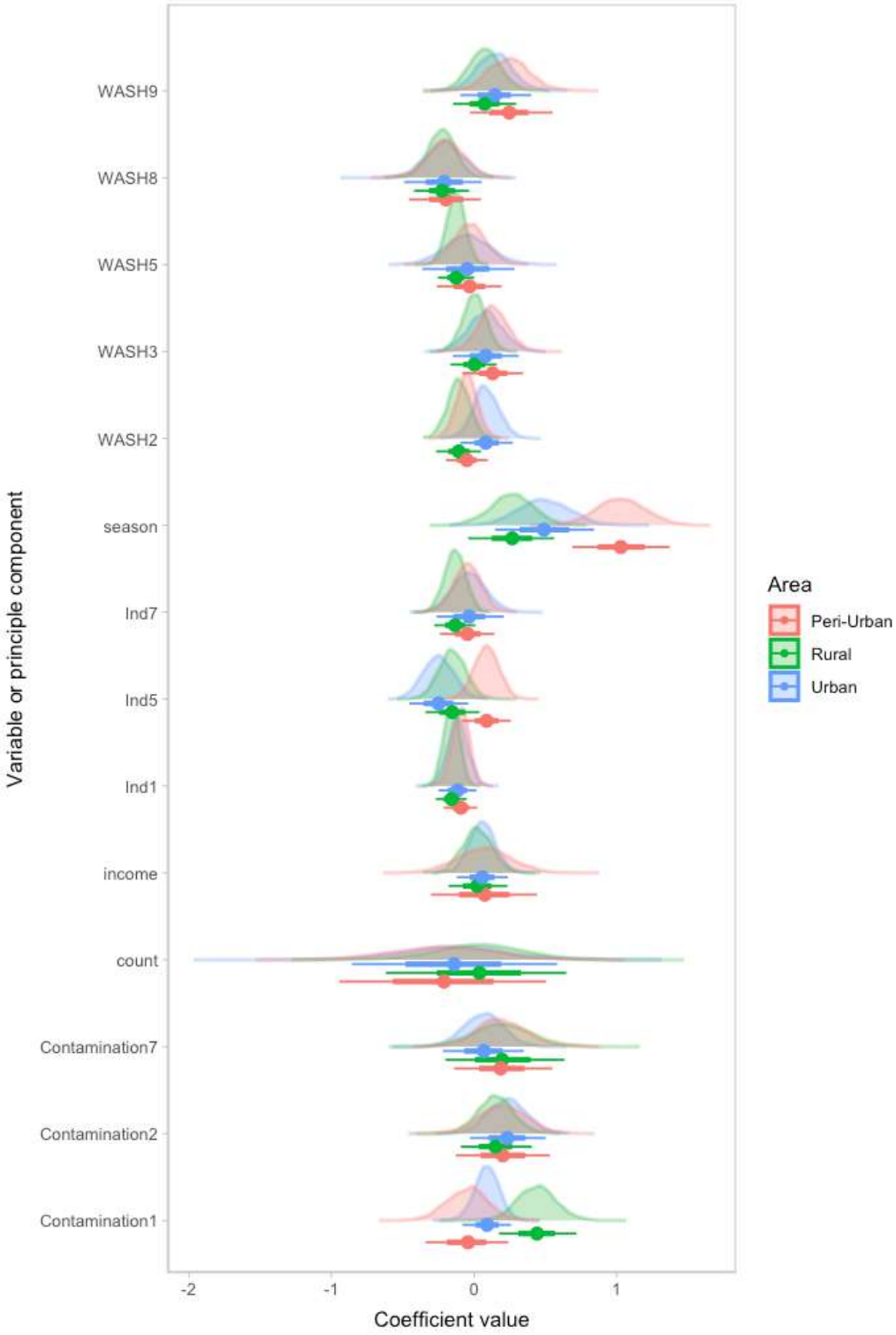


Figure 7.3. Half-eye plot of the Bayesian posterior distributions from principal components of the ESBL-E model, stratified by region. The shaded regions illustrate the kernel density estimations for urban (blue) peri-urban (orange) and rural (green) regions. Below this, the dot represents the median, with the thick and thin lines representing the 95% and 99% values of each posterior distribution.

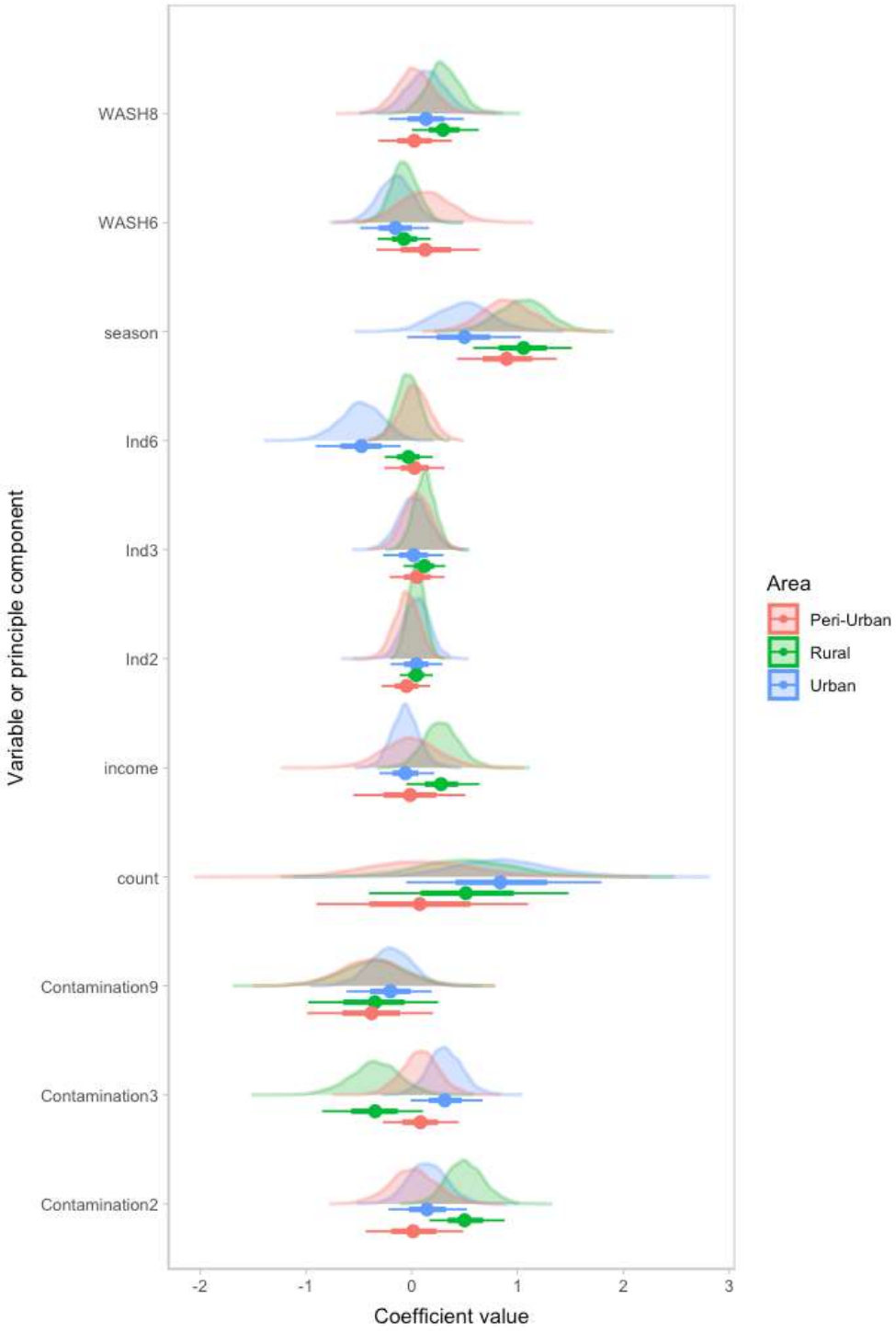


Figure 7.4. Half-eye plot of the Bayesian posterior distributions from principal components of the ESBL-K model, stratified by region. The shaded regions illustrate the kernel density estimations for urban (blue) peri-urban (orange) and rural (green) regions. Below this, the dot represents the median, with the thick and thin lines representing the 95% and 99% values of each posterior distribution.

7.4. Univariate analysis of regional risks in ESBL colonisation

To screen for regional risks of ESBL colonisation, a univariate analysis was performed on the variables identified in Chapter 6 for ESBL (**Table 7.1**), ESBL *E. coli* (**Table 7.2**), and ESBL *K. pneumoniae* (**Table 7.3**).

From this analysis, regional variations in the risks of ESBL colonisation were seen. In the urban site there was an increased risk of ESBL colonisation associated with any animal ownership (OR =1.55, 95%CrI =1.20,2.09, p = 0.001), especially poultry (OR = 1.85, 95%CrI:1.30,2.62, p = <0.001), and from households that choose to keep animals inside the house rather than outside (OR = 1.59, 95%CrI: 1.20,2.09 p = 0.011). The relationship to animal co-habitation within the other settings was not as strong, with animal ownership not posing a risk in the peri-urban site and potentially providing a protective effect in the rural setting. Only co-habitation with ruminants in the peri-urban region was associated with a species-specific risk, and there were examples of animal ownership being seemingly protective, such as owning pigs in the peri-urban region. One of the potential reasons why animals may pose a risk is through contamination of the household food or environment. Here, it was identified that in households where animals were visualised interacting with food items there was a higher chance of ESBL colonisation, especially in the urban setting (OR =1.48, 95%CrI: 1.06-2.07 *p =0.023*).

Water management was also important in the urban site, and individuals that used tube well (borehole) water as their primary source of drinking water had higher odds of ESBL colonisation (OR =2.05, 95%CrI: 1.17-3.68 p =0.013). Tube well water also provided a small increase in risk within the peri-urban region (OR =1.33, 95%CrI: 1.01-1.75 p =0.041), and in this setting, communal piped water (i.e. kiosk) usage was associated with a protective effect (OR =0.66, 95%CrI: 0.49-0.90 p =0.008). The other less common source of water used at households is from a private tap inside or outside the house, and this was identified in the PCA contributions of Chapter 6 as a possible risk factor for ESBL colonisation. In this analysis, no clear association with ESBL colonisation was seen, but a trend towards an increased risk within the rural setting was identified, which may be related to species-specific risks (see section 7.5). Using a different (secondary) water source for cleaning utensils was shown to provide no additional risk, and in relation to coverage of stored drinking water, fluctuations in regional-associated differences were identified, with coverage of stored water in the rural setting providing a strong benefit (OR =0.69, 95%CrI: 0.53-0.90 p =0.006), but coverage of stored water in the urban site associate with an increased risk (OR =1.79, 95%CrI: 1.17-2.78 p =0.008).

Peri-urban risks were more typically associated with seasonal, sanitation and hand-hygiene factors. While seasonality was noted in Chapters 5 and 6 as an important factor across the study, I found higher odds of ESBL colonisation in the wet season within the peri-urban site (OR =2.14, 95%CrI: 1.64-2.79 p = <0.001) compared to the other two regions, highlighting that seasonal effects may be more keenly felt in households residing in this area. Sanitation and hand-hygiene factors had varying degrees of effect in the urban and rural areas, but in the peri-urban site households that shared a toilet (OR =1.38, 95%CrI: 1.05-1.80 p =0.021) or had visible human faecal contamination (OR =1.44, 95%CrI: 1.06-1.96 p =0.019) had a higher risk of colonisation, and households that had a hand washing facility (OR =0.67, 95%CrI: 0.48-0.96 p =0.026), access to soap (OR =0.72, 95%CrI: 0.55-0.95 p =0.018) or toilet cleansing materials (OR =0.70, 95%CrI: 0.54-0.90 p =0.006) and owned a drophole cover (OR =0.63, 95%CrI: 0.47-0.83 p =0.001) had a lower risk of colonisation.

Other than season, the rural risks were predominately associated with food-hygiene and environmental factors. I found an increased risk in ESBL colonisation associated with households that ate street food on a regular basis (OR =1.53, 95%CrI: 1.13-2.09 p =0.007) and a protective effect amongst individuals that used shared plates (OR =0.68, 95%CrI: 0.52-0.90 p =0.006). The protective effect of shared plates is limited to the rural site, and risks associated with the consumption of street food differ by setting. Interestingly higher risks in street food from the rural or peri-urban regions were seen, and the opposite in the urban site. There were no differences associated with the use of market produce in any setting.

The key interactions with river and sewerage environments were explored. Participants who regularly interacted with local rivers (i.e for washing clothes) had a higher risk of ESBL colonisation, particularly in the peri-urban (OR =1.38, 95%CrI: 1.04-1.83 p =0.024) and rural (OR =1.41, 95%CrI: 1.04-1.90 p =0.027) sites. I did not find any association in increased ESBL presence from household individuals who reported contact with drains.

Finally, the individual-level factors of ABU (in the last 6 months) or HIV status did not have a significant effect on the risk of ESBL colonisation in any region.

Table 7.1. Regional univariate analysis of key WASH and individual variables against ESBL colonisation

Characteristic	Region	n	OR	95% CI	p value	Model inclusion
Season (wet)	Urban	813	1.25	0.95,1.66	0.11	**Yes**
	Peri	971	**2.14**	**1.64,2.79**	**<0.001**	
	Rural	938	**1.35**	1.04,1.75	**0.025**	
Male sex	Urban	813	**0.74**	**0.56,0.99**	**0.042**	**Yes**
	Peri	971	0.89	0.69,1.15	0.4	
	Rural	938	0.82	0.63,1.07	0.14	
Age (log)	Urban	813	**1.14**	**1.01,1.28**	**0.035**	Yes
	Peri	971	1.06	0.94,1.20	0.3	
	Rural	938	1.05	0.93,1.17	0.4	
ABU *(Last 6 months)*	Urban	813	1.07	0.75,1.53	0.7	No
	Peri	971	0.96	0.63,1.45	0.8	
	Rural	938	1.11	0.81,1.51	0.5	
HIV reactive	Urban	813	0.89	0.50,1.54	0.7	No
	Peri	971	1.02	0.60,1.70	>0.9	
	Rural	938	0.98	0.61,1.54	>0.9	
Household density (log)	Urban	813	**1.43**	**1.00,2.03**	**0.049**	**Yes**
	Peri	971	1.12	0.80,1.55	0.5	
	Rural	938	0.97	0.67,1.39	0.8	
Income *(>40,000MK/month)*	Urban	813	0.95	0.72,1.25	0.7	No
	Peri	971	1.16	0.89,1.50	0.3	
	Rural	938	0.85	0.65,1.10	0.2	
Shared Toilet	Urban	813	1.12	0.85,1.48	0.4	**Yes**
	Peri	971	**1.38**	**1.05,1.80**	**0.021**	
	Rural	938	1.07	0.79,1.44	0.7	
Drophole Present	Urban	813	0.83	0.58,1.48	0.3	**Yes**
	Peri	971	**0.63**	**0.47,0.83**	**0.001**	
	Rural	938	1.09	0.83,1.43	0.6	
Cleaning Materials available	Urban	813	1.07	0.77,1.47	0.7	**Yes**
	Peri	971	**0.70**	**0.54,0.90**	**0.006**	
	Rural	938	0.80	0.52,1.20	0.3	
Human Faeces visible	Urban	813	1.11	0.84,1.46	0.5	**Yes**
	Peri	971	**1.44**	**1.06,1.96**	**0.019**	
	Rural	938	0.86	0.66,1.12	0.3	
Human defecation practiced	Urban	813	1.37	0.67,2.87	0.4	No
	Peri	971	1.02	0.68,1.50	>0.9	
	Rural	938	**0.57**	**0.36,0.86**	**0.009**	
HWF present	Urban	813	1.19	0.90,1.57	0.2	**Yes**
	Peri	971	**0.67**	**0.48,0.96**	**0.026**	
	Rural	938	1.14	0.87,1.48	0.3	
Soap present	Urban	813	1.62	1.00,2.65	0.051	**Yes**

	Peri	971	**0.72**	**0.55,0.95**	**0.018**	
	Rural	938	**0.36**	**0.16,0.71**	**0.006**	
Stored water covered	Urban	813	**1.79**	**1.17,2.78**	**0.008**	**Yes**
	Peri	971	1.25	0.94,1.66	0.13	
	Rural	938	**0.69**	**0.53,0.90**	**0.006**	
Stored water covered and tap	Urban	813	0.82	0.62,1.09	0.2	No
	Peri	971	1.25	0.46,1.16	0.13	
	Rural	938	0.86	0.66,1.13	0.3	
Utensil water	Urban	813	0.96	0.72,1.29	0.8	No
	Peri	971	1.33	0.87,2.03	0.2	
	Rural	938	1.09	0.76,1.55	0.6	
Piped water (i.e. kiosk)	Urban	813	1.09	0.83,1.45	0.5	**Yes**
	Peri	971	**0.66**	**0.49,0.90**	**0.008**	
	Rural	938	0.85	0.60,1.19	0.3	
Tap water (i.e. household tap)	Urban	813	0.81	0.61,1.07	0.13	No
	Peri	971	1.14	0.76,1.72	0.5	
	Rural	938	1.82	0.90,3.70	0.094	
Tube well water	Urban	813	**2.05**	**1.17,3.68**	**0.013**	**Yes**
	Peri	971	**1.33**	**1.01,1.75**	**0.041**	
	Rural	938	1.11	0.80,1.54	0.5	
Animal owned by household	Urban	813	**1.58**	**1.20,2.09**	**0.001**	**Yes**
	Peri	971	0.99	0.76,1.29	>0.9	
	Rural	938	**0.51**	**0.35,0.74**	**<0.001**	
Cattle or ruminant owned	Urban	813	NA	NA	NA	**Yes**
	Peri	971	**2.17**	**1.53,3.09**	**<0001**	
	Rural	938	0.91	0.70,1.18	0.5	
Poultry owned	Urban	813	**1.85**	**1.32,2.60**	**<0.001**	**Yes**
	Peri	971	0.83	0.63,1.08	0.2	
	Rural	938	1.04	0.77,1.39	0.8	
Pet owned	Urban	813	1.13	0.83,1.55	0.4	No
	Peri	971	0.93	0.68,1.27	0.7	
	Rural	938	0.91	0.67,1.22	0.5	
Pig owned	Urban	813	NA	NA	NA	**Yes**
	Peri	971	**0.25**	**0.07,0.64**	**0.010**	
	Rural	938	1.00	0.71,1.39	>0.9	
Animal kept inside house	Urban	813	**1.59**	**1.12,2.28**	**0.011**	**Yes**
	Peri	971	1.07	0.80,1.42	0.6	
	Rural	938	1.26	0.97,1.63	0.089	
Animal interacting with food	Urban	813	**1.48**	**1.06,2.07**	**0.023**	**Yes**
	Peri	971	1.29	0.99,1.68	0.063	
	Rural	938	1.24	0.95,1.61	0.11	
Animal faeces seen	Urban	813	0.95	0.72,1.25	0.7	No
	Peri	971	1.13	0.81,1.59	0.5	

	Rural	938	NA	NA	NA	
River water exposure	Urban	813	1.06	0.78,1.44	0.7	**Yes**
	Peri	971	**1.38**	**1.04,1.83**	**0.024**	
	Rural	938	**1.41**	**1.04,1.90**	**0.027**	
Drain water exposure	Urban	813	0.77	0.45,1.29	0.3	No
	Peri	971	1.24	0.83,1.85	0.3	
	Rural	938	1.08	0.67,1.74	0.7	
Street food use	Urban	813	**0.48**	**0.31,0.74**	**<0.001**	**Yes**
	Peri	971	**1.57**	**1.02,2.45**	**0.043**	
	Rural	938	**1.53**	**1.13,2.09**	**0.007**	
Shared plates	Urban	813	0.80	0.59,1.09	0.2	**Yes**
	Peri	971	1.07	0.82,1.39	0.6	
	Rural	938	**0.68**	**0.52,0.90**	**0.006**	
Market produce used	Urban	813	0.72	0.44,1.16	0.2	No
	Peri	971	0.81	0.54,1.23	0.3	
	Rural	938	1.04	0.79,1.37	0.8	

7.5. Univariate analysis of regional risks in ESBL *E. coli* and ESBL *K. pneumoniae* colonisation

Univariate analysis was undertaken to evaluate the individual and WASH factors associated with ESBL colonisation with either ESBL *E. coli* (**Table 7.2**) or ESBL *K. pneumoniae* (**Table 7.3**), to determine whether there are species-specific risks across the regions. I broadly found that ESBL *E. coli* risks paralleled those in the ESBL analysis from section 7.4, with notable differences in the importance of drinking water sources, animal interactions, sex and HWF presence. ESBL *K. pneumoniae* had a slightly different pattern of risk, with a focus more on individual level factors (i.e. ABU or HIV status) and the management of human waste.

For ESBL *E. coli* colonisation, sanitation factors were crucial, particularly in the peri-urban region with use of drophole covers (OR =0.59, 95%CrI: 0.44-0.79 p =<0.001), access to cleansing materials (OR =0.65, 95%CrI: 0.49-0.84 p =0.001) and soap (OR =0.74, 95%CrI: 0.56-0.98 p =0.034) important in reducing ESBL *E. coli* colonisation. In contrast to the overall analysis of ESBL, there were no differences in risk associated with sex and a protective benefit from having a hand washing facility. Furthermore, there was a high risk associated with animal-food interactions in all regions and a change in the spectrum of animal co-habitation risks dependant on species. With regards to drinking water sources, a benefit was identified from using piped (kiosk) water in all regions and a higher risk from using tube-well water. The use of tap water was associated with a very high odds of ESBL *E. coli* colonisation in the rural region only (OR =2.38, 95%CrI: 1.19-4.86 p =0.015).

For ESBL *K. pneumoniae* colonisation, the presence of human faecal contamination of the urban household environment (OR =1.58, 95%CrI: 1.03-2.44 p =0.039) and interaction with drains in the rural site (OR =2.82, 95%CrI: 1.59-4.83 p = <0.001) were associated with a higher risk of ESBL *K. pneumoniae* colonisation. Also, unlike the ESBL *E. coli* analysis there was increased risk of colonisation associated with antibiotic exposure in the rural region (OR =1.54, 95%CrI: 0.99-2.35 p =0.048) and a positive HIV status in the peri-urban participants (OR =2.29, 95%CrI: 1.32-3.99 p =0.003).

Table 7.2. Regional univariate analysis of key WASH and individual variables against ESBL *E. coli* colonisation

Characteristic	Region	n	OR	95% CI	p value	Model inclusion
Season (wet)	Urban	813	1.11	0.84,1.47	0.5	**Yes**
	Peri	971	**1.92**	**1.47,2.53**	**<0.001**	
	Rural	938	1.29	0.98,1.69	0.067	
Male sex	Urban	813	0.82	0.61,1.09	0.2	No
	Peri	971	0.93	0.71,1.22	0.6	
	Rural	938	0.97	0.66,1.14	0.3	
Age (log)	Urban	813	**1.14**	**1.01,1.29**	**0.030**	**Yes**
	Peri	971	1.07	0.94,1.21	0.3	
	Rural	938	1.07	0.95,1.21	0.3	
ABU *(Last 6 months)*	Urban	813	1.01	0.71,1.45	>0.9	No
	Peri	971	0.89	0.57,1.37	0.6	
	Rural	938	1.15	0.83,1.57	0.4	
HIV reactive	Urban	813	0.85	0.48,1.49	0.6	No
	Peri	971	0.86	0.49,1.47	0.6	
	Rural	938	1.23	0.77,1.94	0.4	
Household density (log)	Urban	813	1.17	0.82,1.67	0.4	**Yes**
	Peri	971	0.99	0.71,1.39	>0.9	
	Rural	938	**0.66**	**0.45,0.97**	**0.034**	
Income *(>40,000MK/month)*	Urban	813	0.91	0.69,1.21	0.5	No
	Peri	971	1.04	0.8,1.37	0.8	
	Rural	938	0.84	0.64,1.11	0.2	
Shared Toilet	Urban	813	1.02	0.77,1.35	0.9	**Yes**
	Peri	971	**1.37**	**1.04, 1.81**	**0.026**	
	Rural	938	0.86	0.63,1.18	0.4	
Drophole Present	Urban	813	0.80	0.55, 1.15	0.2	**Yes**
	Peri	971	**0.59**	**0.44,0.79**	**<0.001**	
	Rural	938	1.08	0.81,1.44	0.6	
Cleaning Materials available	Urban	813	1.17	0.84,1.62	0.3	**Yes**
	Peri	971	**0.65**	**0.49,0.84**	**0.001**	
	Rural	938	0.80	0.51,1.23	0.3	
Human Faeces visible	Urban	813	0.95	0.72,1.25	0.7	**Yes**
	Peri	971	**1.47**	**1.08.2.01**	**0.015**	
	Rural	938	0.82	0.63,1.08	0.2	
Human defecation practiced	Urban	813	1.47	0.71,3.04	0.3	**Yes**
	Peri	971	1.02	0.68,1.52	>0.9	

	Rural	938	**0.59**	**0.37,0.91**	**0.021**	
HWF present	Urban	813	1.15	0.87,1.52	0.3	No
	Peri	971	0.74	0.53,1.06	0.1	
	Rural	938	1.07	0.82,1.40	0.6	
Soap present	Urban	813	**1.89**	**1.17,3.08**	**0.010**	**Yes**
	Peri	971	**0.74**	**0.56,0.98**	**0.034**	
	Rural	938	**0.47**	**0.21,0.94**	**0.044**	
Stored water covered	Urban	813	**2.16**	**1.38,3.47**	**<0.001**	**Yes**
	Peri	971	1.30	0.97,1.75	0.082	
	Rural	938	**0.62**	**0.46,0.81**	**<0.001**	
Stored water covered and tap	Urban	813	0.83	0.62,1.10	0.2	No
	Peri	971	0.76	0.46,1.21	0.3	
	Rural	938	0.76	0.57,1.01	0.056	
Utensil water	Urban	813	0.95	0.71,1.27	0.7	No
	Peri	971	1.43	0.93,2.18	0.10	
	Rural	938	1.22	0.84,1.74	0.3	
Piped water (i.e. kiosk)	Urban	813	0.97	0.73,1.29	0.9	**Yes**
	Peri	971	**0.64**	**0.47,0.88**	**0.006**	
	Rural	938	**0.68**	**0.47,0.97**	**0.036**	
Tap water (i.e. household tap)	Urban	813	0.87	0.66,1.15	0.3	**Yes**
	Peri	971	0.98	0.64,1.50	>0.9	
	Rural	938	**2.38**	**1.19,4.86**	**0.015**	
Tube well water	Urban	813	**2.53**	**1.45,4.54**	**0.001**	**Yes**
	Peri	971	**1.37**	**1.04,1.82**	**0.027**	
	Rural	938	1.31	0.93,1.86	0.12	
Animal owned by household	Urban	813	**1.55**	**1.17,2.06**	**0.002**	**Yes**
	Peri	971	0.99	0.75,1.30	>0.9	
	Rural	938	**0.54**	**0.37,0.78**	**0.001**	
Cattle or ruminant owned	Urban	813	NA	NA	NA	No
	Peri	971	2.33	1.64,1.11	0.2	
	Rural	938	0.89	0.68,1.17	0.4	
Poultry owned	Urban	813	**1.61**	**1.15,2.26**	**0.005**	**Yes**
	Peri	971	0.85	0.64,1.11	0.2	
	Rural	938	0.98	0.72,1.32	0.9	
Pet owned	Urban	813	1.24	0.90,1.69	0.2	**Yes**
	Peri	971	0.85	0.62,1.17	0.3	
	Rural	938	**0.66**	**0.48,0.91**	**0.012**	
Pig owned	Urban	813	NA	NA	NA	**Yes**
	Peri	971	**0.30**	**0.09,0.78**	**0.026**	
	Rural	938	0.93	0.66,1.31	0.7	
Animal kept inside house	Urban	813	**1.50**	**1.05,2.15**	**0.024**	**Yes**
	Peri	971	1.26	0.94,1.67	0.12	
	Rural	938	**1.16**	**1.18,2.19**	**0.003**	
Animal interacting with food	Urban	813	**1.69**	**1.21,2.36**	**0.002**	**Yes**
	Peri	971	**1.38**	**1.05,1.81**	**0.020**	
	Rural	938	**1.41**	**1.07,1.85**	**0.014**	
Animal faeces seen	Urban	813	1.02	0.77,1.34	>0.9	No
	Peri	971	1.08	0.77,1.52	0.7	
	Rural	938	NA	NA	NA	
River water exposure	Urban	813	0.89	0.65,1.21	0.5	**Yes**

	Peri	971	1.33	1.00,1.78	0.054	
	Rural	938	**1.61**	**1.18,2.19**	**0.003**	
Drain water exposure	Urban	813	0.76	0.44,1.29	0.3	**Yes**
	Peri	971	1.30	0.86,1.94	0.2	
	Rural	938	**0.49**	**0.27,0.84**	**0.013**	
Street food use	Urban	813	0.51	0.33,1.09	0.2	**Yes**
	Peri	971	1.56	1.01,2.49	0.053	
	Rural	938	**1.65**	**1.19,2.29**	**0.003**	
Shared plates	Urban	813	0.79	0.58,1.09	0.2	**Yes**
	Peri	971	1.12	0.85,1.47	0.4	
	Rural	938	**0.71**	**0.54,0.94**	**0.016**	
Market produce used	Urban	813	0.65	0.40,1.05	0.08	No
	Peri	971	0.69	0.45,1.05	0.078	
	Rural	938	0.95	0.72,1.26	0.7	

Table 7.3. Regional univariate analysis of key WASH and individual variables against ESBL *K. pneumoniae* colonisation

Characteristic	Region	n	OR	95% CI	p value	Model inclusion
Season (wet)	Urban	813	1.30	0.85,2.00	0.2	**Yes**
	Peri	971	**1.94**	**1.27,3.02**	**0.003**	
	Rural	938	**2.19**	**1.47,3.31**	**<0.001**	
Male sex	Urban	813	0.69	0.43,1.09	0.12	No
	Peri	971	1.12	0.74,1.68	0.6	
	Rural	938	0.84	0.56,1.23	0.4	
Age (log)	Urban	813	0.97	0.82,1.17	0.8	No
	Peri	971	0.98	0.82,2.60	0.2	
	Rural	938	1.02	0.86,1.21	0.8	
ABU *(Last 6 months)*	Urban	813	1.28	0.74,2.11	0.4	**Yes**
	Peri	971	0.75	0.34,1.46	0.4	
	Rural	938	**1.54**	**0.99,2.35**	**0.048**	
HIV reactive	Urban	813	1.12	0.45,2.41	0.8	**Yes**
	Peri	971	**2.29**	**1.15,4.24**	**0.012**	
	Rural	938	0.52	0.20,1.13	0.14	
Household density (log)	Urban	813	**2.29**	**1.32,3.99**	**0.003**	**Yes**
	Peri	971	0.95	0.57,1.60	0.8	
	Rural	938	**2.12**	**1.24,3.60**	**0.006**	
Income *(>40,000MK/month)*	Urban	813	1.16	0.76,1.78	0.5	No
	Peri	971	1.10	0.73,1.67	0.7	
	Rural	938	1.04	0.71,1.54	0.8	
Shared Toilet	Urban	813	1.39	0.91,2.16	0.13	No
	Peri	971	0.87	0.55, 1.34	0.5	
	Rural	938	1.32	0.85,2.00	0.2	
Drophole Present	Urban	813	1.07	0.60,1.81	0.8	No
	Peri	971	0.96	0.61, 1.47	0.9	
	Rural	938	1.33	0.89, 1.98	0.2	
Cleaning Materials available	Urban	813	1.07	0.64,1.73	0.8	No
	Peri	971	1.06	0.70,1.59	0.8	

	Rural	938	0.78	0.39,1.45	0.5	
Human Faeces visible	Urban	813	**1.58**	**1.03,2.44**	**0.039**	**Yes**
	Peri	971	1.44	0.90,2.25	0.12	
	Rural	938	1.01	0.68,1.49	>0.9	
Human defecation practiced	Urban	813	0.79	0.19,2.30	0.7	No
	Peri	971	0.75	0.36,1.41	0.4	
	Rural	938	0.64	0.31,1.21	0.2	
HWF present	Urban	813	1.55	1.00,2.42	0.053	No
	Peri	971	1.02	0.60,1.86	>0.9	
	Rural	938	1.29	0.88,1.93	0.2	
Soap present	Urban	813	1.19	0.56,2.30	0.6	No
	Peri	971	0.72	0.46,1.10	0.13	
	Rural	938	0.31	0.05,1.04	0.11	
Stored water covered	Urban	813	0.83	0.46,1.58	0.5	No
	Peri	971	0.73	0.48,1.12	0.14	
	Rural	938	1.08	0.73,1.60	0.7	
Stored water covered and tap	Urban	813	0.84	0.54,1.31	0.5	No
	Peri	971	0.68	0.28,1.42	0.3	
	Rural	938	1.06	0.71,1.58	0.8	
Utensil water	Urban	813	0.80	0.50,1.26	0.3	No
	Peri	971	0.62	0.25,1.28	0.2	
	Rural	938	0.88	0.49,1.48	0.6	
Piped water (i.e. kiosk)	Urban	813	1.53	1.00,2.34	0.052	No
	Peri	971	0.85	0.52,1.36	0.5	
	Rural	938	1.36	0.84,2.15	0.2	
Tap water (i.e. household tap)	Urban	813	0.66	0.43,1.02	0.061	No
	Peri	971	1.31	0.69,2.33	0.4	
	Rural	938	0.21	0.01, 1.00	0.13	
Tube well water	Urban	813	0.91	0.34,2.03	0.8	No
	Peri	971	1.08	0.71,1.67	0.7	
	Rural	938	0.80	0.51, 1.28	0.3	
Animal owned by household	Urban	813	1.39	0.91,2.13	0.13	No
	Peri	971	0.81	0.53,1.22	0.3	
	Rural	938	0.70	0.43, 1.20	0.2	
Cattle or ruminant owned	Urban	813	NA	NA	NA	No
	Peri	971	1.06	0.59,1.80	0.8	
	Rural	938	1.22	0.83,1.80	0.3	
Poultry owned	Urban	813	**1.46**	**1.12,2.87**	**0.013**	**Yes**
	Peri	971	1.12	0.74,1.69	0.6	
	Rural	938	1.37	0.88,2.21	0.2	
Pet owned	Urban	813	1.33	0.55,1.47	0.7	**Yes**
	Peri	971	1.01	0.61,1.62	>0.9	
	Rural	938	**1.59**	**1.05,2.39**	**0.027**	
Pig owned	Urban	813	NA	NA	NA	No
	Peri	971	NA	NA	NA	
	Rural	938	1.56	0.98,2.42	0.055	
Animal kept inside house	Urban	813	1.46	0.87,2.40	0.14	**Yes**
	Peri	971	0.52	0.30,0.86	**0.014**	
	Rural	938	**1.48**	**1.00,2.19**	**0.048**	
	Urban	813	1.33	0.80,2.15	0.3	No

Animal interacting with food	Peri	971	0.92	0.60,1.40	0.7	
	Rural	938	0.69	0.46,1.01	0.058	
Animal faeces seen	Urban	813	**0.63**	**0.40,0.96**	**0.036**	**Yes**
	Peri	971	1.42	0.82,2.60	0.2	
	Rural	938	NA	NA	NA	
River water exposure	Urban	813	1.48	0.94,2.30	0.088	No
	Peri	971	1.28	0.82,2.04	0.3	
	Rural	938	0.81	0.49,1.28	>0.9	
Drain water exposure	Urban	813	1.46	0.68,2.85	0.3	**Yes**
	Peri	971	0.91	0.45,1.68	0.8	
	Rural	938	**2.82**	**1.59,4.83**	**<0.001**	
Street food use	Urban	813	0.52	0.30,0.92	**0.036**	**Yes**
	Peri	971	0.86	0.48,1.67	0.6	
	Rural	938	0.90	0.59,1.41	0.6	
Shared plates	Urban	813	0.71	0.42,1.16	0.2	**Yes**
	Peri	971	0.84	0.54,1.27	0.4	
	Rural	938	**0.64**	**0.43,0.95**	**0.025**	
Market produce used	Urban	813	1.13	0.56,2.63	0.7	No
	Peri	971	0.80	0.44,1.55	0.5	
	Rural	938	1.13	0.75,1.71	0.6	

7.6. Multivariate models of risks associated with ESBL, ESBL-E and ESBL-K colonisation

To further explore the results of the univariate analysis, multivariate models were constructed to assess the risks associated with ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* colonisation. Variables were screened, and those which were significantly associated ($p<0.05$) with colonisation by univariate analysis in any region were considered for inclusion and those which were not significantly associated ($p<0.05$) with colonisation or where data was unavailable for at least one region were not included. Variables not used due missing regional data included the ownership of pigs or cattle, which were only present at households in the rural and peri-urban sites. The remaining covariates were then evaluated for regional effects using likelihood-test comparisons of model fit with and without regional effects for ESBL, ESBL *E. coli* and ESBL *K. pneumoniae* (**appendix 7i,7ii & 7iii**). Regionally adjusted and independent covariates were input into models with within household and within participant random effects, and these were fit with Stan v2.21.0 via the R *brms* v2.13.5 package with 4 chains per dataset each with 2000 iterations in total, with 1000 warm up iterations. Convergence was seen on the model trace plots (**appendix 7iv, 7v & 7vi**), and outputs were generated that expressed risk of colonisation as odds ratios (OR) with 95% CrI for ESBL (**Figure 7.5**), ESBL *E. coli* (**Figure 7.6**) and ESBL *K. pneumoniae* (**Figure 7.7**) colonisation.

The first model (**Figure 7.5**) highlighted higher odds of ESBL colonisation associated with increased household density (aOR =1.26, 95%CrI:0.85-1.86), or from households that used a shared toilet (aOR

=1.19, 95%CrI: 0.87-1.64), reported interacting with the local rivers (aOR =1.29, 95%CrI: 0.94-1.76) or where animal-food interactions were observed (aOR =1.42, 95%CrI: 1.03-1.97). Regionally specific ESBL colonisation risks were seen in households in the urban (aOR =1.71, 95%CrI: 0.77-4.06) and rural (aOR =1.55, 95%CrI: 0.68-3.54) regions who owned poultry, alongside most notably, the wet season in the peri-urban region (aOR =3.01, 95%CrI: 2.19-4.16).

Species-specific risks were noted for both regionally adjusted and unadjusted factors. From the unadjusted covariates, ESBL *E. coli* colonisation was associated with advanced age (aOR =1.15, 95%CrI: 1.05-1.26) and animal food interaction (aOR =1.56, 95%CrI: 1.10-2.20) (**Figure 7.6**). In contrast, ESBL *K. pneumoniae* colonisation was associated with the wet season (aOR =2.13, 95%CrI: 1.63-2.81) alongside households that had human faecal contamination seen in the environment (aOR =1.64, 95%CrI: 1.12-2.46) and in those owned poultry (aOR =1.49, 95%CrI: 0.85-2.60) (**Figure 7.7**). To a lesser extent, ESBL *K. pneumoniae* colonisation was also associated with ABU (aOR =1.22, 95%CrI: 0.86-1.71).

Key regionally-specific ESBL *E. coli* colonisation risks were seen with households that owned animals (aOR =2.57, 95%CrI: 0.62-10.89) in the urban region, used tap water (aOR =4.34, 95%CrI 0.95-20.08), owned poultry (aOR =1.35, 95%CrI: 0.54-3.39) or reported river water exposure (aOR =1.58, 95%CrI: 0.80-3.07) in the rural region, and in households that reported drain water exposure (aOR =1.73, 95%CrI: 0.73-4.01) in the peri-urban region (**Figure 7.6**). Lastly the wet season was a very high risk for ESBL *E. coli* colonisation in the wet season (aOR =2.66, 95%CrI: 1.93-3.67). Regionally-specific ESBL *K. pneumoniae* colonisation risks were seen in urban households that kept animals inside the house (aOR =1.54, 95%CrI: 0.61-3.97) or reported drain water exposure (aOR =2.29, 95%CrI: 0.81-6.23), in peri-urban households with HIV positive participants in (aOR =2.65, 95%CrI: 1.10-5.87) and within rural households that reported drain water exposure (aOR =3.44, 95%CrI: 1.11-10.75) (**Figure 7.7**). Finally, increased household density was associated with a higher risk of ESBL *K. pneumoniae* colonisation, and here the risk varied slightly between each region (**Figure 7.7**).

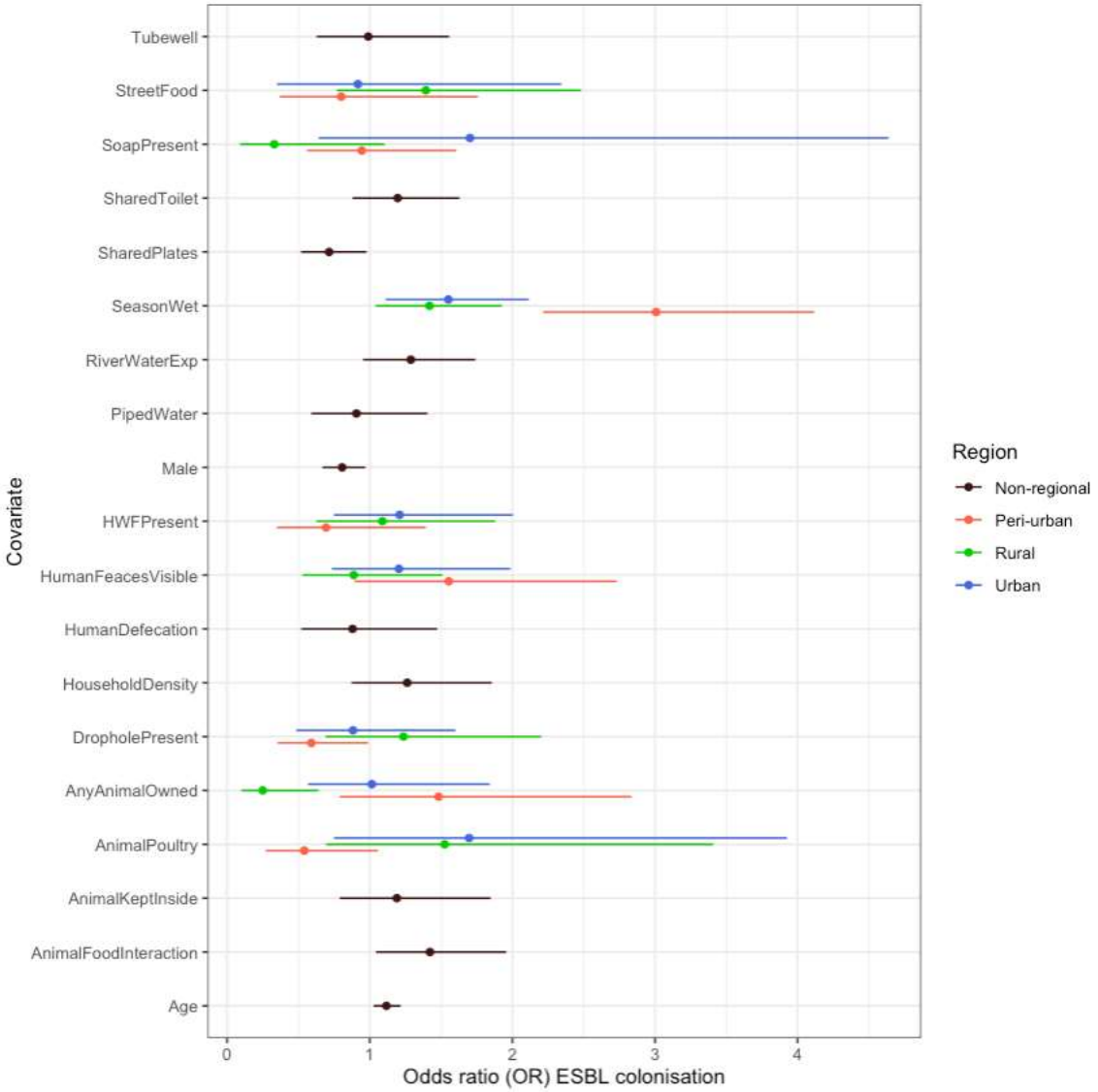


Figure 7.5. Parameter estimates for the fixed-effects used in a multivariate model of ESBL colonisation, expressed as odds ratios with 95% CrI. Covariates were either regionally adjusted (red=peri-urban, green=rural or blue=urban) or regionally unadjusted (black), dependant on likelihood ratio test results.

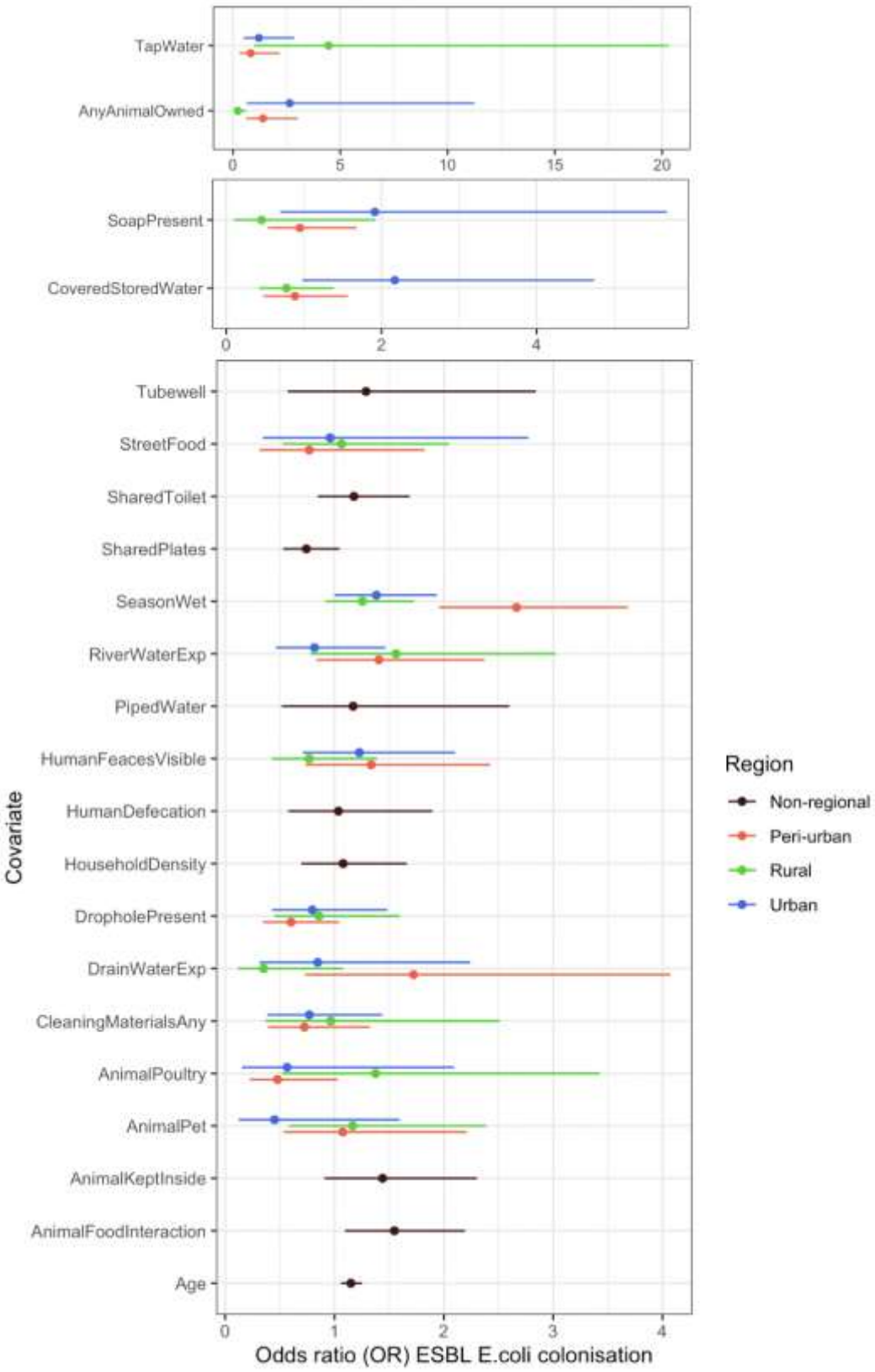


Figure 7.6. Parameter estimates for the fixed-effects used in a multivariate model of ESBL *E. coli* colonisation, expressed as odds ratios with 95% CrI. Covariates were either regionally adjusted (red=peri-urban, green=rural or blue=urban) or regionally unadjusted (black), dependant on likelihood ratio test results.

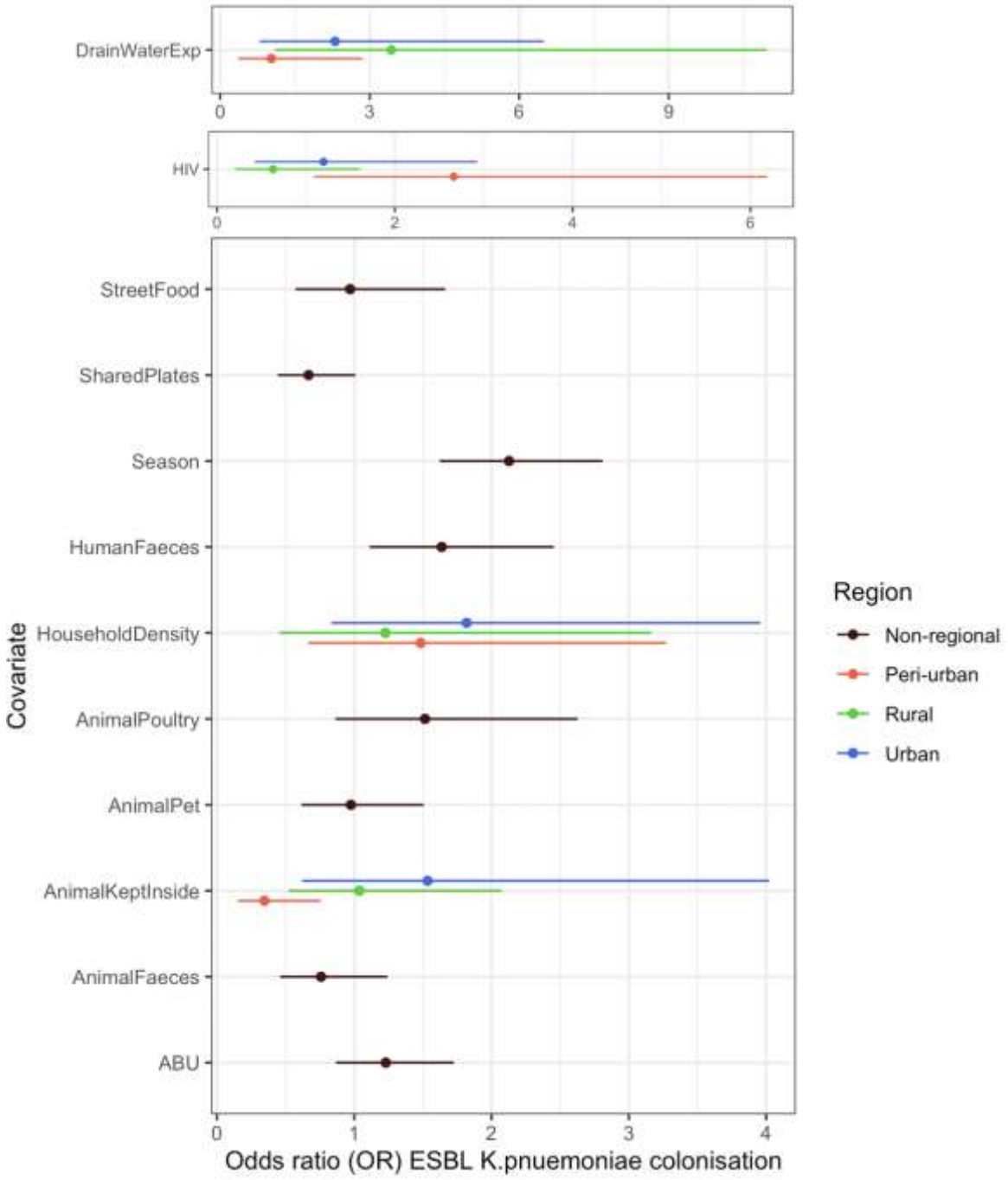


Figure 7.7. Parameter estimates for the fixed-effects used in a multivariate model of ESBL *K. pneumoniae* colonisation, expressed as odds ratios with 95% CrI. Covariates were either regionally adjusted (red=peri-urban, green=rural or blue=urban) or regionally unadjusted (black), dependant on likelihood ratio test results.

7.7. Discussion

Within this chapter, I first calculated the regional estimates from the Bayesian models in Chapter 6 illustrating key regional similarities and differences within the PCs. This was augmented by regional univariate analysis and mixed-effect multivariate models (MEMs) on individual variables, enabling the selection of factors permissible to targeted interventions and providing a greater interpretability from a WASH perspective. Individual factors such as age and sex were minimally seen to alter the chance of ESBL-E or ESBL-K colonisation within the cohort, with women and people of advancing age having a slightly higher risk of ESBL colonisation overall.

Wet season remained the largest risk factor for ESBL colonisation, however the analysis in this chapter illustrated that seasonal risk varied by setting, with peri-urban inhabitants more likely to be ESBL colonised in the wet season (aOR =3.01, 95%CrI: 2.19-4.16) compared with those in the urban (aOR =1.53, 95%CrI: 1.11-2.12) and rural (aOR = 1.41, 95%CrI: 1.06-1.92) regions. This was seen in both the analysis of the posterior estimates from mixed-effects models on PCs and the MEMs on individual variables. This finding suggests that the peri-urban areas of Malawi are more climate sensitive, and reasons for this may include differences in the geographic landscape or WASH infrastructure, variations in behavioural practices or distinct effects from key environmental or animal factors. Given that individual-level differences were small, and household densities and compositions were similar between the regions (Chapter 3) it is unlikely that household demographics or ABU are driving factors for seasonal risk.

Regional differences in the seasonal effect were seen to be greater with ESBL-E than ESBL-K, and this may be reflective of differences in the ecological niches of these bacteria. Non-human factors, such as the environment or animal co-habitation could be of less importance in ESBL-K colonisation compared to ESBL-E colonisation. *K. pneumoniae* is ubiquitous, but as a pathogen, is typically associated with hospital environments (414). However, environmental ESBL-K are very similar to clinical isolates (415) and this is thought to be as a result of mismanaged human effluent. Here, the data suggests asymptomatic community carriage may be driven primarily by human-human transmission rather than from environmental or animal sources, based on the association with household density and hand-hygiene factors (i.e. human factors) having increased odds of ESBL-K colonisation, but not ESBL-E colonisation. A recent large One-Health study from Italy evaluating AMR *K. pneumoniae* transmission found that less than 1% of clinical isolates were of non-human origins and this pointed to ecological

barriers limiting AMR transmission (138). In low-income settings this may be different, and it is anticipated that genomic analysis will resolve this question.

There was regional variation in risk of ESBL colonisation associated with animal ownership (**univariate analysis: Tables 7.1-7.3**). Given I have previously shown that the frequency and species of animal ownership, alongside animal management practices (i.e. waste management) differ by setting (**Chapter 1 & 2)**, these data suggest that animal-associated risks are related to regional differences in animal husbandry together with variations in the species present at households. Poultry are the species that provided the highest risk, especially in the urban site, and this may be associated with keeping them inside the house. It is well documented in other LMICs that individuals who have regular contact with poultry are at higher risk of ESBL-E colonisation (416–418). Rates of ESBL colonisation differ by species (**Chapter 5**) and variations in the use of antibiotics are dependent on the animal species and setting (**Chapter 3**). Allowing animals of any species to interact with food was a risk factor across all regions.

There was little difference in the proportion of ESBL colonisation in covered vs uncovered receptacles (**Chapter 5**) and I found no overall effect from coverage of stored water (Chapter 6). However, in this chapter I observed that coverage of drinking water in the rural setting provided a strong benefit (OR =0.69, 95%CrI: 0.53,0.90 *p =0.006*), and coverage of drinking water in the urban site led to increased risk (OR =1.79, 95%CrI: 1.17-2.78 *p =0.008*). This may indicate that regional fluctuations in hand-hygiene measures, the choice of the storage methods and the role of animal interactions are important in governing safe water management, and regional adaptations should be considered when implementing water management campaigns in southern Malawi. As set out in the SDGs, ideally an improved drinking water source should be used, whereby water is piped into the premises and free from contamination (345,419). In our setting household-controlled tap water had a varying effect on risk depending on the region and bacterial species, with tap water in the rural setting having a higher associated risk of ESBL-E colonisation (aOR =4.28, 95%CrI:0.96-19.72) than in the other settings. Together, these results illustrate that the risks of ESBL colonisation are related to choices of household water source and storage that are geographically specific and interlinked with other regionally-associated WASH factors, highlighting the complexity and importance of providing regional context when considering WASH risks.

Sanitation and hygiene practices vary by region (Chapter 4), and there are regional differences in risks associated with their implementation. In the MEMs, I found variations in the protective effects of

drophole covers and soap by region. This is likely to be related to the difference between access and usage. For example, soap is often prioritised for bathing, laundry or other purposes over hand washing, particularly in the urban setting (241). Therefore, having soap at a household does not always indicate that hand-hygiene is improved (240). Equally owning a drophole cover doesn't infer its use or an automatic improvement to household sanitation or fly reduction (360,420). In the MEMs, having a shared toilet increases your chance of being ESBL colonised across all regions (aOR =1.19, 95%CrI: 0.87-1.64), and this is consistent with the literature showing a reduced safety profile from the use of shared toilets (378,421,422). Open defecation is reportedly practiced at all 3 sites. However, the presence of visible human faeces was a hazard associated with ESBL-K only (aOR =1.64, 95%CrI: 1.12-2.46) and I did not find increased ESBL colonisation rates associated with households that report open defecation (aOR =0.88, 95%CrI: 0.51-1.53).

There was a trend in the peri-urban and rural regions towards a reduction in ESBL-E colonisation from access to hand-hygiene measures (such as cleansing materials, soap and HWF presence) although the effects from access to hand-hygiene measures overall were non-significant. Again, this can relate to the regional differences between access and usage. Nevertheless, taken into consideration alongside sanitation factors, the peri-urban region is most responsive to improvements in sanitation and hand-hygiene measures, and future research should be undertaken to assess the reasons for these regional differences and sanitation interventions at the peri-urban setting should be considered for greatest impact.

Household food-hygiene and eating practices vary by site, and regional fluctuations in ESBL colonisation associated with the use of shared plates and street food were identified. Street food was not a clear risk factor in any region, and interestingly the use of shared plates was protective overall (aOR =0.71, 95%CrI: 0.51-0.99), most notably in the rural region (OR =0.68, 95%CrI: 0.52-0.90 p =0.006). The reasons for shared plates being protective is unclear, as this is counter what we would expect to find, so further evaluation this finding is warranted in future studies.

Risks from environmental exposures were assessed, and here I found that households that interacted with the local rivers had higher odds of ESBL colonisation (aOR =1.29, 95%CrI: 0.95-1.75). This risk was present across all regions (when accounting for ESBL-E and ESBL-K), but most notably evident in the peri-urban (OR =1.38, 95%CrI: 1.04-1.83 p =0.024) and rural (OR =1.41, 95%CrI: 1.04-1.90 p =0.027) sites. Households that reported exposures to drain water also had higher odds of ESBL colonisation, but this was dependant on the bacteria-site combination, with higher risks of ESBL-E colonisation

associated with exposures at the peri-urban site (aOR =1.72, 95%CrI: 0.74-4.26) and higher risks of ESBL-K colonisation associated with exposures at the rural (aOR =3.44, 95%CrI: 1.05-11.19) and urban (aOR =2.27, 95%CrI: 0.82-6.25) sites. River and drains are likely to be of critical importance given the frequency of household interactions and high prevelance of ESBL bacteria. Specific drivers for the extraordinary levels of ESBL found in the river systems of southern Malawi are likely to relate to inadequate waste management and infrastructure to contain human effluent in conjunction with the presence and effects of resistance driving chemicals.

There are a number of limitations in the analysis undertaken in this chapter. Firstly, the PCs are non-quantifiable and while I broadly identified household-level and sample-level differences, independent logistic regressions for each level, including the top contributing covariates of each PC would better delineate the relative differences in the data. In relation to the MEMs, these were constructed by sensitivity screening of the univariates, which may introduce biases from inappropriate selection and deselection (423,424). Lastly, despite regional adjustments and considerations of colinearly, the interrelationship of AMR in a One-Health context is complex, and we should be cautious when drawing inference from these results alone.

In summary, within this chapter I identified that geographic location and associated variations in regional WASH infrastructure, practices and environmental exposures were shown to impact upon ESBL, ESBL-E and ESBL-K colonisation risk. Individual factors were less important than household related factors, and the wet season provided the greatest risk, most notably in the peri-urban site. Water management risks were dependant on the source and region, and across all sites there were increased risks associated with sharing toilets, river water exposure and with regards to ESBL-K in particular, increased household density. Animal-associated risks were dependant on the combination of the site, species and bacteria, with owning poultry being the animal associated with the highest risk to human gut colonisation, most notably in the urban setting. This exploratory work highlights crucial areas where future prospective research should be undertaken that evaluates the effect of WASH and environmental factors on community ESBL colonisation and takes into consideration nuances of the setting.

7.8. Appendix

Appendix 7.i. Table of parameter testing for regional adjustment of variables included in the ESBL mixed effects model

Variable	Likelihood ratio test	Adjust for Region*
Season	$\chi 2$ (2)= 9.01, p = **0.011**	**Yes**
Male	$\chi 2$ (2)= 0.84, p = 0.657	No
Age	$\chi 2$ (2)= 1.07, p = 0.583	No
ABU	*NA*	
HIV reactive	*NA*	
Household density	$\chi 2$ (2)= 2.35, p =0.309	No
Income >40,000MK/month	*NA*	
Shared Toilet	$\chi 2$ (2)= 1.76, p = 0.416	No
Drophole Present	$\chi 2$ (2)=7.52, p = **0.023**	**Yes**
Cleaning Materials available	$\chi 2$ (2)= 4.09, p = 0.129	No
Human Faeces visible	$\chi 2$ (2)= 6.25, p = **0.044**	**Yes**
Human defecation practiced	$\chi 2$ (2)= *6.10* p = 0.057	No
HWF present	$\chi 2$ (2)= 7.21, p = **0.027**	**Yes**
Soap present	$\chi 2$ (2)= 14.09, p = **<0.001**	**Yes**
Stored water covered	$\chi 2$ (2)= 17.1, p = **<0.001**	**Yes**
Stored water covered and tap	*NA*	
Utensil water	*NA*	
Piped water (i.e. kiosk)	$\chi 2$ (2)= 5.67, p = 0.059	No
Tap water (i.e. household tap)	*NA*	
Tube well water	$\chi 2$ (2)= 3.48, p = 0.175	No
Animal owned by household	$\chi 2$ (2)= 22.87, p = **<0.001**	**Yes**
Cattle or ruminant owned	$\chi 2$ (2)= 15.18, p = **<0.001**	No *(not in Urban region)*
Poultry owned	$\chi 2$ (2)= 13.71, p = **<0.001**	**Yes**
Pet owned	*NA*	
Pig owned	*NA*	
Animal kept inside house	$\chi 2$ (2)= 2.90, p = 0.234	No
Animal interacting with food	$\chi 2$ (2)= 0.69, p = 0.707	No
Animal faeces seen	*NA*	
River water exposure	$\chi 2$ (2)= 2.14, p = 0.344	No
Drain water exposure	*NA*	
Street food use	$\chi 2$ (2)= 21.57, p = **<0.001**	**Yes**
Shared plates	$\chi 2$ (2)= 5.37, p = 0.068	No
Market produce used	*NA*	

**An alpha level 0.05 has been used as a cut off for the decision to adjust for regional effects in the final mixed effect model.*

Appendix 7.ii. Table of parameter testing for regional adjustment of variables included in the ESBL *E. coli* mixed effects model

Characteristic	Likelihood ratio test	Adjust for Region*
Season	$\chi 2$ (2)= 8.33, p = 0.0155	**Yes**
Male	NA	
Age	$\chi 2$ (2)= 0.80, p =0.67	No
ABU	NA	
HIV reactive	NA	
Household density	$\chi 2$ (2)= 4.82, p =0.090	No
Income >40,000MK/month	NA	
Shared Toilet	$\chi 2$ (2)= 4.93, p = 0.085	No
Drophole Present	$\chi 2$ (2)= 8.51, p = 0.014	**Yes**
Cleaning Materials available	$\chi 2$ (2)= 7.66, p = 0.022	**Yes**
Human Faeces visible	$\chi 2$ (2)= 7.88, p = 0.019	**Yes**
Human defecation practiced	$\chi 2$ (2)= 5.64, p = 0.059	No
HWF present	NA	
Soap present	$\chi 2$ (2)= 14.32, p = <0.001	**Yes**
Stored water covered	$\chi 2$ (2)= 26.52, p = <0.001	**Yes**
Stored water covered and tap	NA	
Utensil water	NA	
Piped water (i.e. kiosk)	$\chi 2$ (2)= 4.39, p =0.111	No
Tap water (i.e. household tap)	$\chi 2$ (2)= 6.92, p =0.031	**Yes**
Tube well water	$\chi 2$ (2)= 4.28, p =0.117	No
Animal owned by household	$\chi 2$ (2)= 19.61, p = <0.001	**Yes**
Cattle or ruminant owned	NA	
Poultry owned	$\chi 2$ (2)= 8.91, p =0.011	**Yes**
Pet owned	$\chi 2$ (2)= 7.63, p =0.022	**Yes**
Pig owned	NA	
Animal kept inside house	$\chi 2$ (2)= 1.34, p = 0.510	No
Animal interacting with food	$\chi 2$ (2)= 0.94, p = 0.624	No
Animal faeces seen	NA	
River water exposure	$\chi 2$ (2)= 7.36, p = 0.025	**Yes**
Drain water exposure	$\chi 2$ (2)= 8.23, p = 0.016	**Yes**
Street food use	$\chi 2$ (2)= 20.84, p = <0.001	**Yes**
Shared plates	$\chi 2$ (2)= 5.72, p = 0.057	No
Market produce used	NA	

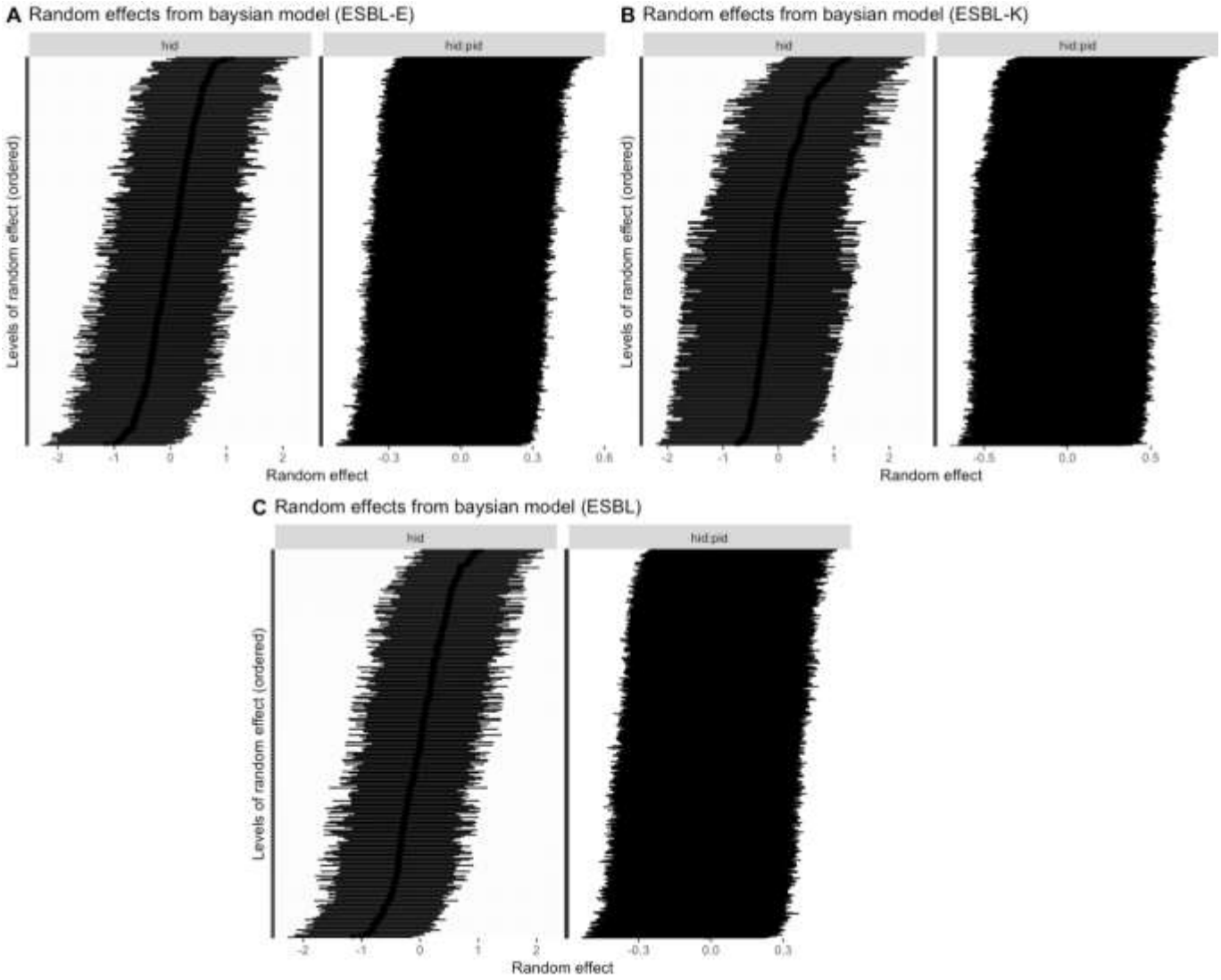
**An alpha level 0.05 has been used as a cut off for the decision to adjust for regional effects in the final mixed effect model.*

Appendix 7.iii. Table of parameter testing for regional adjustment of variables included in the ESBL *K. pneumoniae* mixed effects model

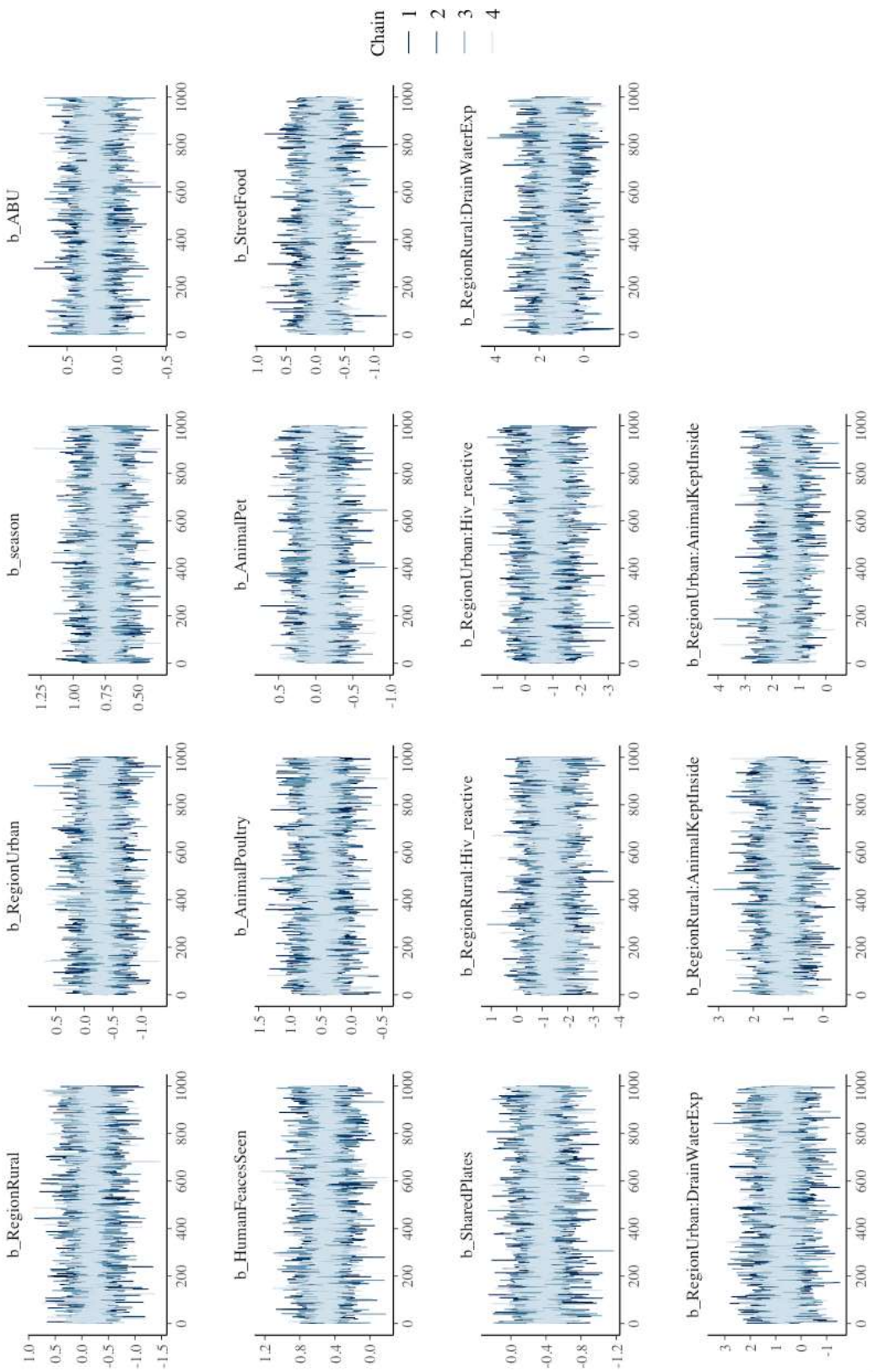
Variable	Likelihood ratio test	Adjust for Region*
Season	$\chi 2$ (2)= 3.25, p =0.197	No
Male	NA	
Age	NA	No
ABU	$\chi 2$ (2)= 3.08, p =0.215	No
HIV reactive	$\chi 2$ (2)= 7.87, p =0.020	**Yes**
Household density	$\chi 2$ (2)= 6.53 , p = 0.038	**Yes**
Income >40,000MK/month	NA	
Shared Toilet	NA	
Drophole Present	NA	
Cleaning Materials available	NA	
Human Faeces visible	$\chi 2$ (2)= 2.56, p =0.278	No
Human defecation practiced	NA	
HWF present	NA	
Soap present	NA	
Stored water covered	NA	
Stored water covered and tap	NA	
Utensil water	NA	
Piped water (i.e. kiosk)	NA	
Tap water (i.e. household tap)	NA	
Tube well water	NA	
Animal owned by household	NA	
Cattle or ruminant owned	NA	
Poultry owned	$\chi 2$ (2)= 2.24, p =0.327	No
Pet owned	$\chi 2$ (2)= 3.46, p =0.177	No
Pig owned	NA	
Animal kept inside house	$\chi 2$ (2)= 12.39, p =0.002	**Yes**
Animal interacting with food	NA	
Animal faeces seen	$\chi 2$ (2)= 5.56, p =0.062	No
River water exposure	NA	
Drain water exposure	$\chi 2$ (2)= 7.02, p =0.030	**Yes**
Street food use	$\chi 2$ (2)= 2.54, p =0.281	No
Shared plates	$\chi 2$ (2)= 0.81, p =0.665	No
Market produce used	NA	

**An alpha level 0.05 has been used as a cut off for the decision to adjust for regional effects in the final mixed effect model.*

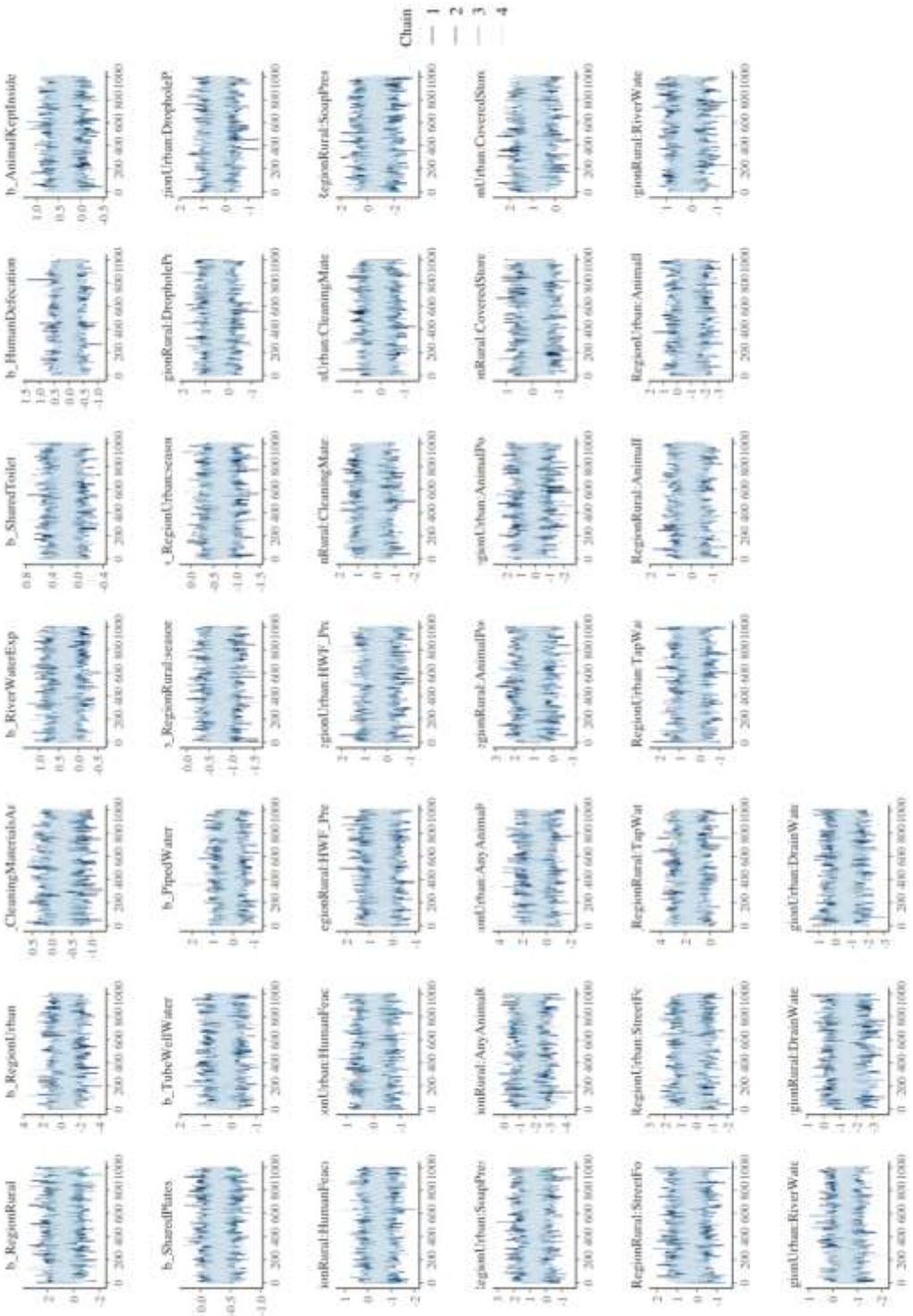
Appendix 7.iv. Random effects from Bayesian multivariate models of (a) ESBL-E, (b) ESBL-K and (c) ESBL colonisation.



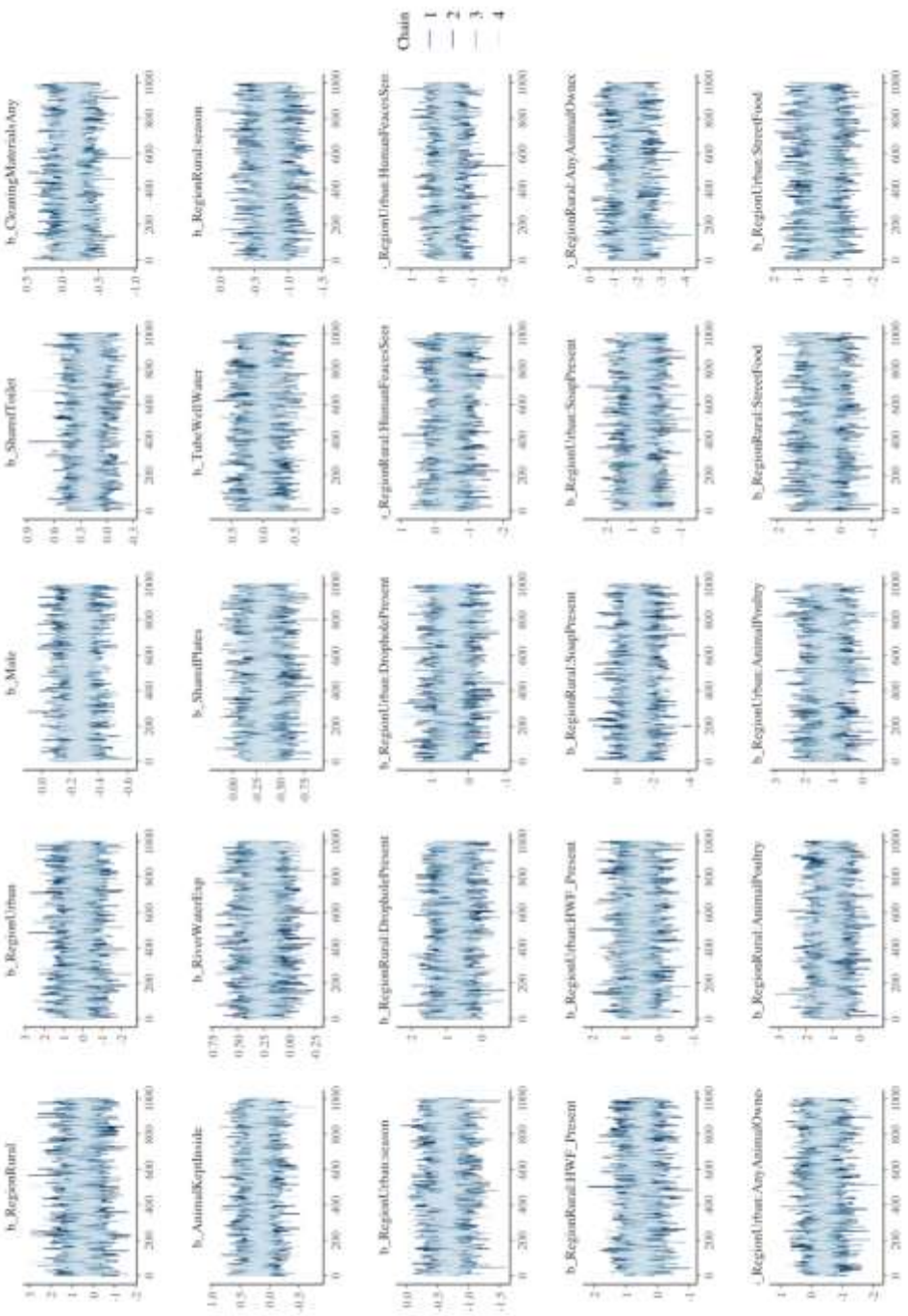
Appendix 7.v. Trace plots of ESBL-K model



Appendix 7.vi. Trace plots of ESBL-E model



Appendix 7.vii. Trace plots of ESBL model.



Chapter 8:

Prevelance and risks of antibiotics and resistance-driving chemicals in riverine networks of urban sub-Saharan Africa. A One-Health focussed case study from Blantyre, Malawi.

8.0. Chapter Summary

There is a paucity of evidence for the presence of antibiotics and resistance-driving chemicals (i.e. antibiotics, pesticides and heavy metals) in rivers from sub-Saharan African cities. These chemicals promote and maintain antimicrobial resistance in the environment and pose onward risks to human, animal and ecological health. In this chapter I describe the prevelance of key antibiotics and resistance-driving chemicals over a 15-month period in urban Blantyre, Malawi. Ecological risks have been quantified, based on chemical concentrations, and these illustrate that, in particular, antibiotic usage in the local population alongside waste management play a key role in the wider dissemination of resistance-driving chemicals into the aquatic environments within these settings. Future AMR research and surveillance strategies in LMICs should include assessments of antibiotics, pesticides, and heavy metals in the aquatic environment, and policymakers should adopt a One-Health approach to mitigation strategies that includes water sanitation and hygiene expertise.

8.1. Outline and contributions

This Chapter has been written in the format of a scientific manuscript, that is planned for submission to Lancet Planetary Health, which takes a One-Health approach to identifying the key resistance-driving chemicals in urban waterways in Malawi and evaluates the associated ecological risks. The chemical analyte, heavy metal and microbiological methods used have been published previously (425,426). Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC, LC-MS/MS), alongside non-targeted chemical residue identification was performed by Dr Grabic's team in the Czech Republic and inductively coupled plasma mass spectrometry (IPC-MS) was completed at the UK centre for ecology and hydrology (CEH), under the guidance of Dr Singer.

This manuscript summarises the environmental work undertaken within DRUM Workstrand 2, supported by Andrew Singer and Nicholas Feasey. I co-developed the study design, oversaw sampling and site selection, performed the statistical analysis and wrote the first draft of all sections of the manuscript. The identification of heavy metal and antibiotics, pesticides, herbicides and fungicides

from the samples collected in Malawi were completed by the teams at CEH (United Kingdom) and the University of South Bohemia (Czech Republic). Subsequent editing of the manuscript was undertaken by all authors. My contributions to this chapter and those of others are included in Table 8.0. References cited in the text of the manuscript have been placed at the end of the thesis.

Table 8.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter/paper were primarily drafted by the PhD candidate. All statistical analysis (other than the non-targeted chemical analysis) were performed by the PhD candidate. All graphicalisations (other than the non-targeted chemical analysis) were completed by the PhD candidate.
Contributions from external partners and DRUM consortium collaborators	Conceptualisation was a combination of Andrew Singer, Nicholas Feasey and the PhD candidate. The chemical analysis of water samples (UPLC, LC-MS/MS) was performed by Roman Grabic and Katerina Grabicova's team in the University of South Bohemia. The IPC-MS of water samples was performed by Andrew Singer and colleagues at CEH. Collection of samples in the field were primarily undertaken by Taonga Mwapasa and Gladys Namancha, with assistance from Tracy Morse, Kondwani Chidziwisano Witness Mtambo, Steria Chisesele, Dyson Rashid, Odetta Duwa, Lughano Ghambi, and Chiyembekeso Palije. No laboratory processing occurred in Malawi. Document review was provided by all authors.

Within this project, I had hoped to collect a range of data to augment that which has been discussed in the paper. This included a more extensive analysis of the physical properties of the river at each visit, in the form of (i) dissolved oxygen measurements, and (ii) pH measurements, alongside paralleled water sampling to identify the presence of ESBL bacteria, using the methodologies described in chapter 2. However, technical issues with the device that measured dissolved oxygen in combination with supply issues in pH strips precluded the inclusion of these measurements in this chapter. Furthermore, due to COVID-associated workflow prioritisation and a subsequent miss-communication led to a substantial period of data loss in the microbiological results. The absence of a continuous microbiological dataset meant that ESBL presence/absence was no longer permissible for inclusion in the thesis.

Prevelance and risks of antibiotics and resistance-driving chemicals in riverine networks of urban sub-Saharan Africa. A One-Health focused case study from Blantyre, Malawi.

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Abstract

Background:

Low and middle-income countries (LMICs) have high morbidity and mortality from drug-resistant infections and a high prevelance of carriage of antimicrobial resistant (AMR) bacteria amongst community members. These settings have high levels of human and animal antibiotic usage, limited waste-water treatment facilities and poor waste management systems to control excreta, leading to dispersal of AMR bacteria, AMR genes and antimicrobials into the local rivers. The ecological drivers of AMR in the aquatic environments of urban rivers have not been fully elucidated and limited evidence exists for the presence of antibiotics and resistance-driving chemicals in rivers from sub-Saharan African (sSA) cities. Evaluating the role of the riverine system in these sites will be important to determine the ecological niches and reservoirs of AMR within LMICs.

Methods:

River sites were longitudinally evaluated for a 15-month period between February 2020 and April 2021 in Blantyre, southern Malawi downstream of dense urban conurbations, light industry and a large tertiary hospital. Resistance-driving chemicals including antibiotics, antivirals, antifungals, pesticides, herbicides and fungicides were determined by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC, LC-MS/MS) and heavy metals were identified via inductively coupled plasma mass spectrometry (ICP-MS). Concentrations were compared to predicted no-effect concentrations (PNECs) from internationally agreed standards for antimicrobial resistance selection.

Findings:

A total of 25 antibiotics, 4 antiretrovirals, 3 antifungals and 2 antiparasitics commonly used in human medicine, alongside 30 pesticides, 7 herbicides and 8 fungicides used in agriculture were recovered from river water samplers in urban communities throughout the period. Twenty-five metals were also quantified, and were within allowable WHO limits; however, antibiotic concentrations of sulfamethoxazole, trimethoprim and metronidazole were consistently above PNECs.

Interpretation:

In urban sSA, antibiotics used in human health are found ubiquitously across time and space in our sample set. The levels present in excess of PNECs that are considered the lower threshold above which antimicrobial resistance selection is expected to occur. This is likely to result from a combination of inadequate WASH infrastructure in densely populated urban environments and human antimicrobial usage in HIV, TB, gastrointestinal and respiratory disease; highlighting that the riverine network may be an important ecological niche for the acquisition, maintenance, and transmission of AMR in LMIC community settings.

Funding:

Medical research council and Wellcome Trust

Research in context

Evidence before this study

There is a paucity of evidence for the presence of antibiotics and resistance-driving chemicals (i.e. antibiotics, pesticides and heavy metals) in rivers from sub-Saharan African cities. These chemicals

promote and maintain antimicrobial resistance (AMR) in the environment and pose onward risks to human, animal and ecological health.

Added value of this study

This One-Health study describes the prevelance of key antibiotics and resistance-driving chemicals continuously over a 6-month period from a dense urban city in Malawi. Ecological risks have been quantified, based on chemical concentrations, and illustrated that antibiotic usage in the local population alongside waste management play a key role in the wider dissemination of antibiotics into the aquatic environments within these settings.

Implications of all the available evidence

Within urban sSA communities it is critical to preserve good waste management of human and animal faeces to curb the spread of antibiotics and resistance-driving chemicals into the riverine environment. Future AMR research and surveillance strategies in LMICs should include assessments of antibiotics, pesticides, and heavy metals in the aquatic environment, and policymakers should adopt a One-Health approach to mitigation strategies that includes water sanitation and hygiene (WASH) expertise.

Introduction

Antibiotics are primarily used in the treatment and prevention of disease in humans and animals, alongside the promotion of growth within the animal sector (32). Antibiotic resistance (AMR) is annually associated with 3.57 million human deaths and will lead to an economic loss of $100 trillion every year by 2050 if urgent action is not taken (13,90). Global health inequities and the absence of access to reserve antibiotics means that the greatest burden of AMR will be felt in low and middle-income countries (LMICs) (90,91). Furthermore, in these settings, AMR is also a threat to the livestock sector and thus to the livelihoods of millions who raise animals for subsistence (427).

The role of the environment as a reservoir for AMR is growing with a growing evidence base for its relevance to human health. As such, it is critical to adopt a One-Health approach when considering interventions that tackle AMR on a global scale (153,194,428). Around 40-90% of antibiotics consumed by humans and animals are excreted in an active form, and these can be dispersed into groundwater and the wider riverine network (35,194). The presence of antibiotics, alongside other key resistance-driving chemicals (i.e. pesticides and heavy metals) in these aquatic environments promotes

horizontal gene transfer (HGT) and alters microbial communities, contributing to the dissemination of antibiotic resistance genes (ARGs) and subsequently poses downstream risks to human health (194–196). In certain settings, this is compounded by pollution from inadequate treatment of industrial, domestic, and agricultural waste, enhancing the resistome in the environment (197).

Within LMICs, there is a paucity of adequate water, sanitation and hygiene (WASH) infrastructure and the adoption of WASH behavioural practices that lead to high levels of faecal contamination of local rivers (209,343,429). Increasing urbanisation within LMICs additionally compromises ecological health via the pooling of domestic sewerage and agricultural run-off from subsistence and small-scale farming (209,430). Furthermore, a high proportion of LMICs are located in sub-tropical areas of sub-Saharan Africa (sSA) or Asia, which are frequently prone to seasonal changes in rainfall and temperature. These settings permit hydrological and growth conditions that both promote the development of AMR bacteria in sewerage and provide seasonal variations in the concentrations of antibiotic residues in waterways; which in turn contributes to dynamics in AMR selection pressures within the riverine environment (431). Research on the distribution and ecological risks of resistance-driving chemicals in urban rivers from these settings is scarce, particularly in sSA (404). Therefore, it is important to establish a baseline for the presence of antibiotic residues and co-selecting agents (e.g., pesticides, metals), from waterways. This understanding could be used to gauge the success of future interventions/stewardship efforts to reduce the AMR burden in LMICs.

Within this study we establish the presence of antimicrobial resistance-driving chemicals at key sites within the riverine network of Blantyre, Malawi. Blantyre has a population of ~830,000 people, is served by a single 1350-bed tertiary hospital, and has basic citywide sanitation infrastructure, with only 1 operational wastewater treatment plant (WWTP). The waterways selected are fed by dense urban and peri-urban communities and are included alongside a city centre site downstream of the hospital. Longitudinal sampling over a 1-year period permitted assessment of the fluctuations in chemical concentrations, and ecological risks were determined in line with predicted no-effect concentration (PNEC) limits that are putative targets agreed by the AMR Industry Alliance for antibiotic discharge to the river environment (195).

Methods

Site selection, study design and sampling methods

This study was embedded within the drivers of resistance in Uganda and Malawi (DRUM) research portfolio, and river water sites were selected from within polygons of the urban and peri-urban boundaries of Blantyre, southern Malawi (**Figure 1**) (432). Site selection was informed by transect walks undertaken in the urban (Ndirande) and peri-urban (Chileka) districts, and a pragmatic approach was taken to site selection which accounted for logistical challenges, staff safety and local permissions (**appendix i**). 5 sites were identified which demarcated upper and lower sections of the riverine networks of Ndirande and Chileka (**appendix ii & iii**).

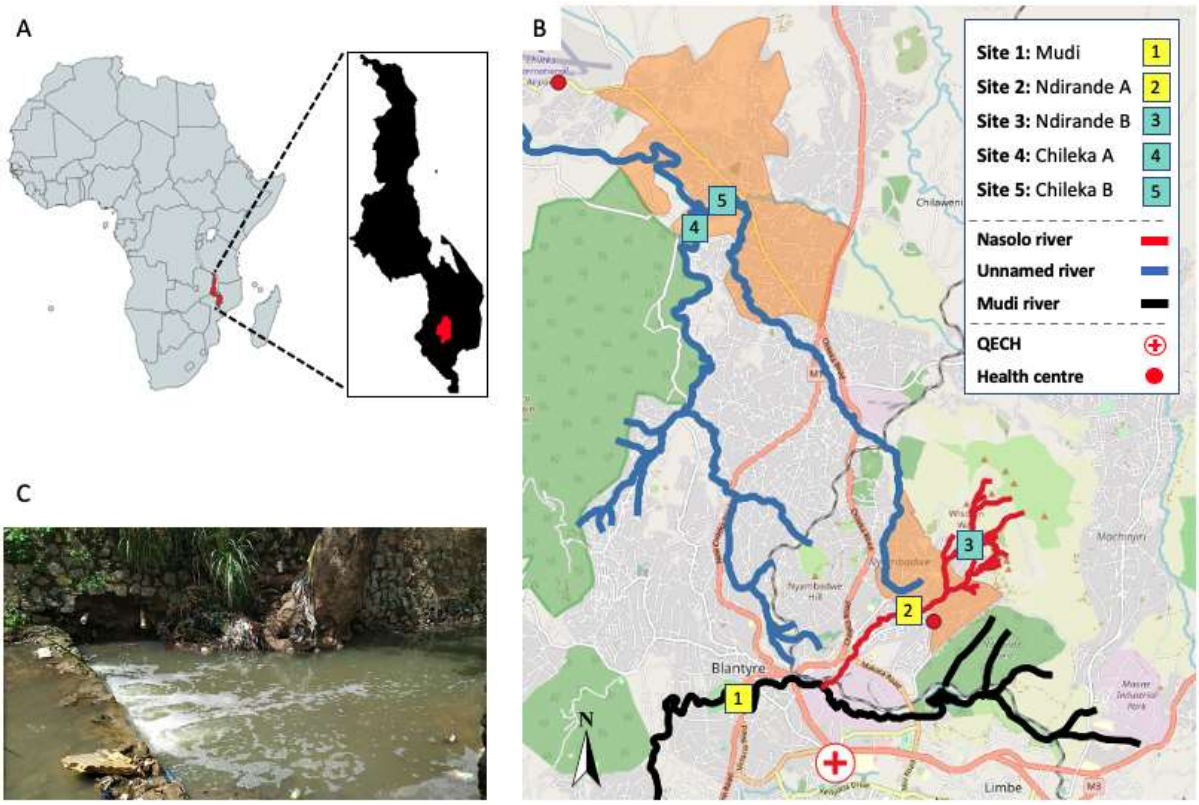


Figure 1. *Study setting, riverine network and sampling sites. Water was obtained from rivers in Blantyre city in southern Malawi (a), across 5 sites within urban (1,2 & 3) and peri-urban (4,5) wards (orange) within the city boundaries (b). All sites were sampled during the pilot phase, and 2 sites (1 & 2) were enrolled into the continuation phase (b). Decisions on sites included in the continuation phase were made based on consistent year-round flow, logistics and safety profiling. A typical sampling site (site 2) has been shown in panel C.*

River water sampling for chemical analytes was undertaken between February 2020 and November 2021, separated into a 9-month pilot phase (between February 2020 and October 2020) and a 6-month continuous phase (between November 2020 - April 2021). River water sampling for heavy metals was completed between May and November 2021. During the pilot phase, river water was purposively collected, and the utility of each site was assessed. Given logistical challenges, primarily due to theft and mechanical loss encountered in the pilot phase (**appendix i**), we focussed on 2 key urban sites (1&2) for the continuous phase, and these underwent uninterrupted sampling over a 6-month period.

Polar organic chemical integrative samplers (POCIS) [Nya Exposmeter AB, Trehorningen 34, SE-92266 Tavelsjo, Sweden, www.exposmeter.com] were sited in the urban rivers and replaced at 2 weekly intervals (**appendix i**). POCIS consist of a sorbent sandwiched between two polyethersulfone (PES) membranes, fixed into a porous metal cage, and the membrane allows for the passage of analytes such as antibiotics onto the sorbent, where they become sequestered (433–436). Samplers were placed at a depth of 20-100cm at the fastest portion of the river and attached via metal wire to a stake on the riverbank, hidden from view. On removal, the sampler cage was detached, and the membrane was washed with deionised water to remove any heavy soiling, before being placed in an aluminium bag, sealed, and transported to the laboratory within 2hrs, whereupon it was stored at -80^{o}C. Longitudinal metadata of river water parameters were collected alongside citing of the samplers.

A grab sample of river water was collected in a 30ml universal container for metal analysis. Samples were transported to the laboratory within 2 hours, stored at ambient temperature in the dark.

Chemical and heavy metal analysis

A suite of antimicrobials, metabolites, pesticides and metals were analysed on the basis of evidence in the literature for their role in the selection or co-selection of antibiotic resistance genes and to examine *a priori* assumptions about antimicrobial use in Blantyre (**Tables 1, 2 & 3**). POCIS samplers were extracted using standard procedures as described previously (425), and chemical analysis was performed using ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC, LC-MS/MS; TSQ Quantum Ultra or Quantiva mass spectrometers, Accela 1250 pump, both Thermo Fisher Scientific; PAL autosampler, CTC Switzerland) (426,436). Limits of quantification (LOQ) were calculated from the instrumental limit of quantification by correcting to the internal standard response, for the matrix effect, for internal standard response, and aliquot/volume of individual

samples (436). Non-targeted screening of LC-MS/MS results was performed using compound discoverer 3.3 software to permit identification of chemical compounds that were present, but not included in the initial selection, including antiretrovirals (ARVs), antibiotics, antifungals and antiprotozoals. Principle component analysis (PCA) of POCIS LC-MS/MS compounds determined site-based differences in chemical compositions. Metals were identified through inductively coupled plasma mass spectrometry (IPC-MS).

Table.1. List of antibiotics and their metabolites screened in river water samples.

Antibiotic Class	Antibiotic Name	Acronym
β-lactams (β-Ls)	Amoxicillin	AMX
	Ampicillin	AMP
	Cloxacillin	CLX
	Flucloxacillin	FLX
	Penicillin G	PENG
	Penicillin V	PENV
	Cefalexin	CEF
	Cefixime	CFX
	Cefotaxime	CTX
	Cefuroxime	CXM
Quinolones (QNs)	Ciprofloxacin	CIP
	Difloxacin	DIF
	Enoxacin	ENX
	Enrofloxacin	EFX
	Levofloxacin + Ofloxacin	LEV
	Lomefloxacin	LOM
	Norfloxacin	NOR
	Perfloxacin	PER
	Roxithromycin	ROX
MLS drugs *(MLS)*	Azithromycin	AZM
	Clarithromycin	CLR
	Clindamycin	CLI
	Clindamycin sulfoxide	CLS
	Erythromycin	ERY
	Tylosin	TYL
Sulphonamides *(SAs)*	Sulfadiazine	SFD
	Sulfamerazine	SFD
	Sulfamethazine	SFT
	Sulfamethizole	SFZ
	Sulfamethoxazole	SMX
	Sulfamethoxine	SMI
	Sulfamethoxypyridine	SMP
	Sulfamoxole	SML
	Sulfaphenazole	SPZ
	Sulfapyridine	SPY
	Sulfaquinoxaline	SFQ
	N1 Acetyl SMX	NA1
	N4 Acetyl SMX	NA4
	Sulfathiazole	STZ
Tetracyclines *(TCs)*	Chlortetracycline	CLT
	Doxycycline	DOX
	Oxytetracycline	OXY
	Tetracycline	TET

Other antibiotics *(Other)*	Chloramphenicol Metronidazole Rifampicin Trimethoprim	CHL MET RIF TRI
Antifungals and Antiprotozoals *(Fung)*	Ornidazole	ORN
	Miconazole Terbinafine	MIC TER

Table 2. List of pesticides, herbicides and fungicides screened in river water samples.

	Class	Acronym	Chemical names
Pesticides	Chloroacetanilides	CHLA	Acetoclor, Acetoclor_ESA, Metazachlor, Metalachlor, Metalachlor_ESA
	Organochlorine	ORGC	Chloridazon_methyl_desphenyl, Chloridazon_desphenyl, Chloridazon
	Neonicotinoid	NEON	Thiamethoxam, Imidacloprid
	ß-methoxyacrylates	ß-MET	Azoxystrobin
	Carbamate	CARB	Pirimicarb, Carbofuran-3-hydroxy
	Benzonitrile	BENZ	Ioxynil
	Urea	UREA	1-(3.4-Dichlorophenyl)_urea, Atraton, Bensulfuron_methyl, Chlorotoluron, Chlorotoluron_desmethyl, Fenuron, Foramsulfuron, Isoproturon, Isoproturon_didemethyl, Isoproturon_monodemethyl, Linuron, Methabenzthiazuron,Metobromuron, Metoxuron, Metsulfuron_methyl, Monolinuron.
	Triazine	TRIZ	Terbuthylazine_hydroxy, Terbuthylazine_desethyl-2-hydroxy, Terbuthylazine_desethyl, Terbuthylazine, Simazine_hydroxy, Simazine, Sebuthylazine, Propazine_hydroxy, Propazine, Prometryn, Metribuzin_desamino, Metribuzin, Atrazine_desisopropyl, Atrazine_desethyl-desisopropyl, Atrazine_desethyl-2-hydroxy, Atrazine_desethyl, Atrazine_2-hydroxy, Atrazine, Ametryn
	Organophosphate	ORGP	Chlorpyrifos, Diazinon, Dimethoate, Malathion, Pirimiphos_ethyl, Pirimiphos_methyl
	DEET	DEET	Imazamox, Imidazolinone
	Starlicide	STAR	3-chloro-4-methylaniline
	Ryanoid	RYAN	Chlorantraniliprole
Herbicides			2.4.5-trichlorophenoxyacetic_acid, 2.4-D, 2.4-Dichlorphenoxypropionic_acid, 4-Isopropylaniline, Bentazone, Clomazone, Desmetryn, Dimethenamid_ESA, Diuron, Diuron_desmethyl, Florasulam, Hexazinone, Imazamethabenz_methyl, Lenacil, MCPA, MCPP, Picloram, Terbutryn, Triallat
Fungicides			Carbendazim, Cyproconazole, Dimethomorph, Epoxiconazole, Flusilazole, Metalaxyl, Metconazole, Propiconazole, Pyrimethanil, Tebuconazole, Triadimenol, Triticonazole

Table 3. List of metals screened in river water samples.

Metal	Acronym	Metal	Acronym
Aluminium	Al	Molybdenum	Mo
Arsenic	As	Nickel	Ni
Barium	Ba	Lead	Pb
Beryllium	Be	Rubidium	Rb
Cadmium	Cd	Antimony	Sb
Cerium	Ce	Selenium	Se
Cobalt	Co	Tin	Sn
Chromium	Cr	Strontium	Sr
Caesium	Cs	Titanium	Ti
Copper	Cu	Uranium	U
Iron	Fe	Vanadium	V
Lanthanum	La	Tungsten	W
Lithium	Li	Zinc	Zn
Manganese	Mn		

Statistical analysis

Predicted no-effect concentrations (PNECs) were obtained from international guidance (**appendix iv**) (195). Statistical analysis and graphic visualisations including means +/- standard deviation (SD), boxplots, Pearson's coefficient matrix, PCA and PNEC tables were performed using R studio (Version 1.4.11). Sampling site maps were drawn using QGIS (Version 3.4). Wet season was classified as samples obtained between Nov-Apr and dry season was classified as samples obtained between May-Oct.

Results

A total of 25 antibiotics, 4 antiretrovirals (ARVs), 3 antifungals, 2 antiparasitics used in human and animal medicine, alongside 30 unique pesticides, 7 herbicides and 8 fungicides used in agriculture were recovered from 54 river water samplers in urban communities throughout the period between February 2020 and April 2021 (**Table 4** & **Figure 2**). Antibiotics were found in every river sample (100%, n=54), and we identified the presence of 8 sulphonamides (SFD, SFT, SMX, SMI, SPY, SFQ, STZ, NA4), 6 macrolide 5(AZM, CLR, CLI, CLS, ERY, TYL), 5 β-lactam, including 3 cephalosporin (PENG, CLX, CEF, CFX, CXM) and 4 unclassified (CHL, MET, RIF, TRI) antibiotics, alongside 1 antifungal (MIC) and 1 antiprotozoal (ORN) through the selected analysis (**Table 4**). Results from non-targeted screening also identified the presence of ARVs (Lopinavir, Efavirenz, Atazanavir, Nevirapine), antifungals (Griseofulvin) antiparasitics (Praziquantel) and the carbapenem antibiotic Imipenem (IMI) (**appendix v**). SMX, NA1, TRI, MET, ERY, SFT, SPY, IMI and AVRs were found in all locations and CXM, CHL, CLI, SFD, STZ and ORN were found in 4/5 locations (**appendix vi**). The concentrations of antibiotics ranged

from 0.19-15,000 (ng/L), and sulfamethoxazole (it's metabolite NA1), trimethoprim, metronidazole and erythromycin were the dominant compounds found in river water, constituting 83.1%, 10.5%, 3.4% and 2.0% of the total analytes respectively (**appendix vi**). In addition to this, we consistently identified the macrolides AZM (n=28, 51.9%), CLR (n=23, 42.6%), CLI (n=27, 50.0%), CLS (n=11, 20.4%), ERY (n=51, 94.4%), TYL (n=1, 1.9%) and 3GCs, such as CXM (n=24, 44.4%) in river water samples (**appendix vi**).

Variations in antibiotic concentrations were identified, and these were dependant on antibiotic class and river site (**appendix vii**). In the dense urban community (sites 2 &3) we typically found higher levels of sulphonamides and metronidazole (**Figure 3a & 3d**), in the city centre (site 1) we found higher levels of macrolides (**Figure 3b**), and in the peri-urban region (sites 4 & 5) we found increased concentrations of the injectable cephalosporins (**Figure 3c**).

The relationship between recovered antibiotic concentrations illustrated that sulfamethoxazole, its metabolite NA1, and trimethoprim were closely associated (**Figure 4**). This reflects the similarity in chemical structures, in conjunction with widespread reliance on co-trimoxazole (CTX) for the treatment of bacterial disease in the local population and co-trimoxazole preventive therapy (CPT) in the context of HIV. CTX was in turn associated with rifampacin, pointing towards a role in tuberculosis therapy, and other related antibiotics included metronidazole and cefixime, and erythromycin and azithromycin, which are used as broad-spectrum treatments in a range of gastrointestinal and respiratory infections in humans and animals.

In the continuation phase we assessed the flux of antibiotic presence and concentrations at 2 sites (1 & 2) over the wet season (**Figure 5**). Within this period, we regularly recovered TRI, SMX, MET and RIF residues from the river water of both sites. Furthermore, antibiotics were either consistently present or absent, with 62.2% (n= 28/45) of the antibiotics discovered at least once, found for ≥4 consecutive months. There were, however, marked fluctuations in the concentrations of antibiotics seen on a month-month basis at both sites, and this in turn impacts upon the selection pressures within the riverine environment (**Figure 5 & Table 5**). PCA highlighted that chemical composition differed substantially between sites, and this is likely to reflect differences in the geography upstream (light industry and tertiary hospital effluent vs dense conurbation) (**appendix vi**).

To determine whether antibiotic residues in the urban sites were likely to impact on antimicrobial selection in the aquatic environment, we compared measured concentrations to PNECs set out in the

in guidance from the AMR industry alliance (**Figure 6**). Using this approach, we identified that antibiotic concentrations in urban rivers within Blantyre are typically lower than the PNEC values, and only SMX, TRI and MET were at levels above the upper limit of PNEC. TRI and MET were often <2 times the limit of advised PNECs, however SMX was frequently seen at much higher levels, and was recovered up to >10 times the PNEC.

Other than Be and Sn, all metals were detected in the water samples. Metal concentrations varied by site and element, with neither site showing an increased presence of all metals tested (**Table 6**). Heavy metal concentrations for Cu, Cr, Fe, Ni, S and Zn were higher in the central urban river system downstream of light industry (site 1) and metal concentrations of As, Li, Mn, Rb and Sr were higher in the river system downstream of a dense urban conurbation (site 2) (**appendix viii**). None of the mean metal concentrations were above the WHO or United States Environmental Protection Agency (USEPA) reference standards (437), although individually, high levels of Ni (>20µg/L) Mg (>100µg/L) and Fe (>300µg/L) were found in excess of WHO reference standards (**appendix ix**).

Antibiotic	Mean	SD	Antibiotic	Mean	SD
AZM	1.22	2.64	PENG	0.02	0.13
CEF	0.06	0.25	RIF	4.67	6.39
CFX	0.04	0.12	SFD	1.80	3.48
CXM	34.78	64.63	SFT	1.79	2.19
CHL	2.79	5.23	SMX	3167.56	2978.90
CLR	1.05	1.80	NA4	1636.70	1497.58
CLI	0.28	0.40	SMI	0.01	0.07
CLS	0.13	0.33	SPY	3.59	3.56
CLX	2.05	2.90	SFQ	0.02	0.12
ERY	118.10	189.30	STZ	2.12	2.75
MET	198.08	471.83	TRI	604.23	540.29
MIC	0.01	0.05	TYL	0.01	0.07
ORN	0.88	1.59			

Table 4. Antibiotics (and their metabolites) antifungals and antiparasitics identified at river sites, presented with their mean (SD) concentrations (ng/L).

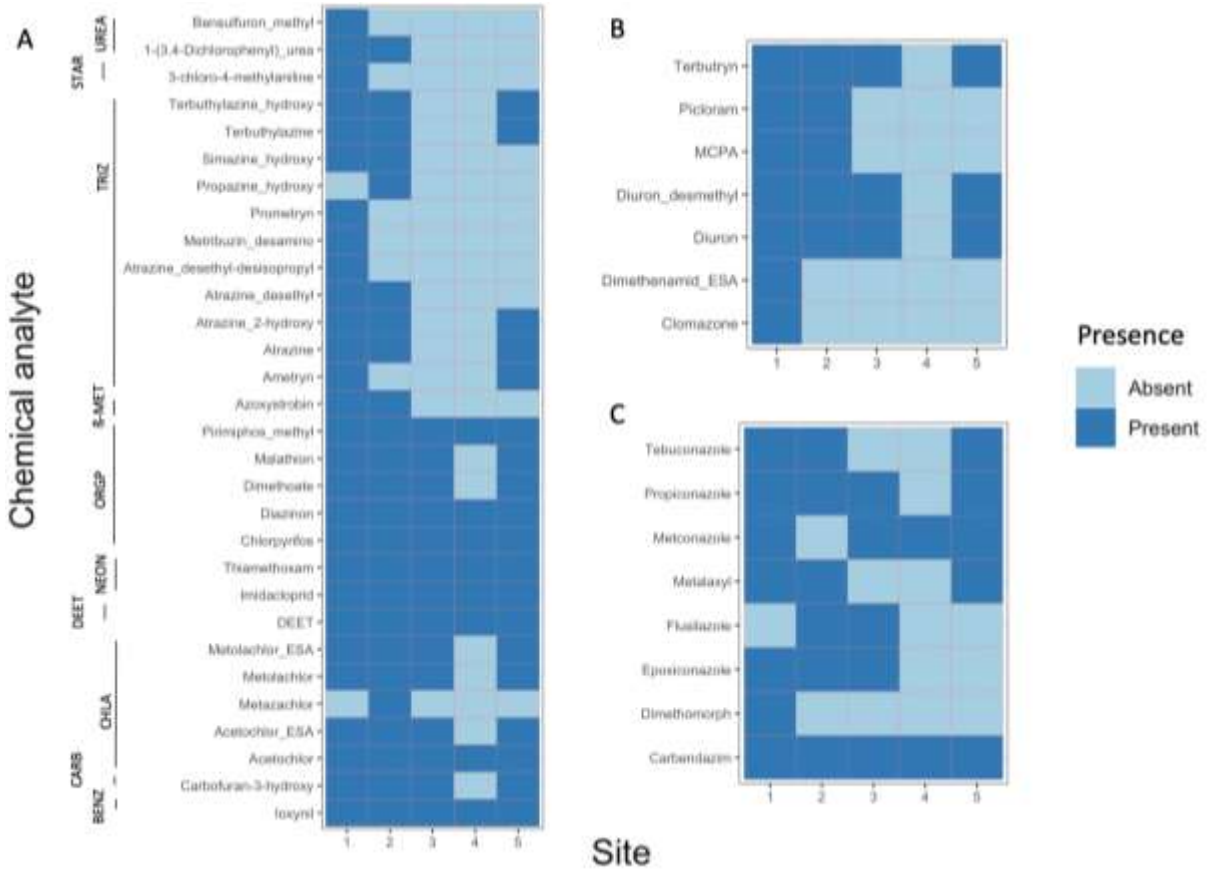


Figure 2. *Panel plots of the presence and absence of pesticides (a), herbicides (b) and fungicides (c) identified at river sites over the study period. Pesticides have been grouped by class, and presence has been coloured as absent (at all timepoints) or present (at ≥ 1 timepoint).*

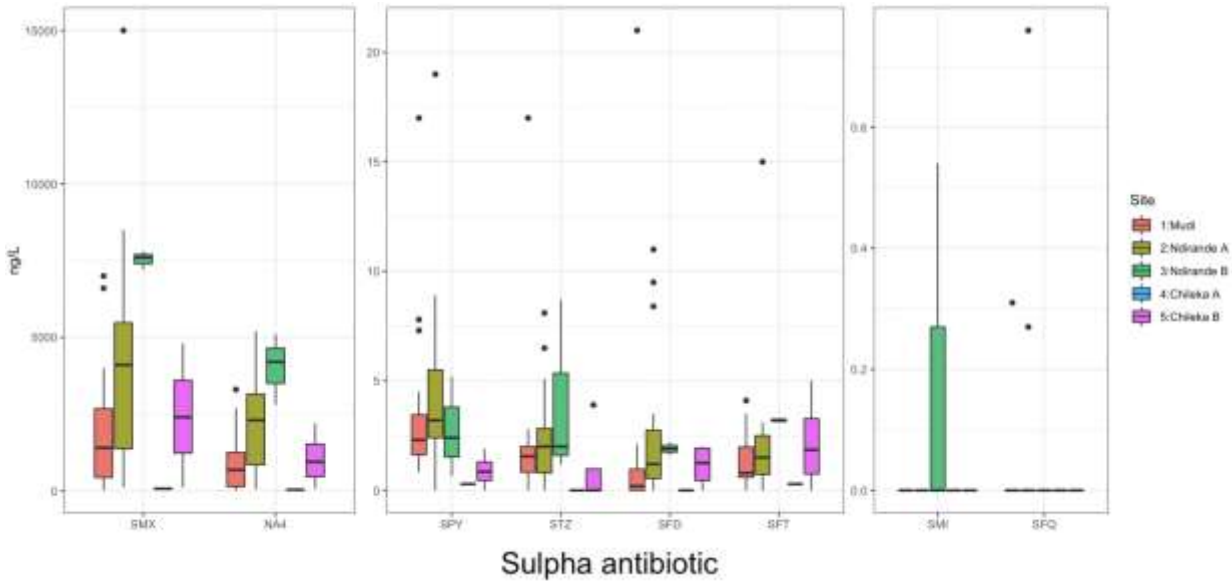


Figure 3a. *Boxplots of sulpha antibiotic concentrations (ng/L) at each site over the total study period, inclusive of the pilot and continuation phase, separated by antibiotic class.*

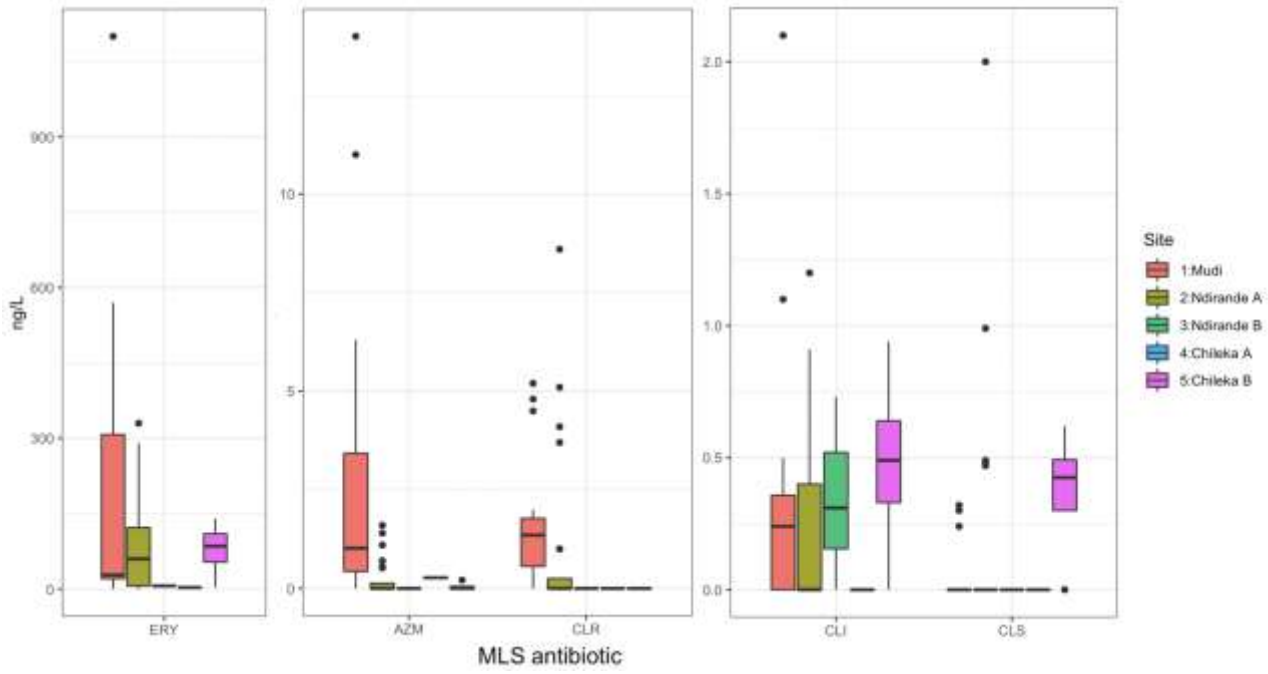


Figure 3b. *Boxplots of MLS antibiotic concentrations (ng/L) at each site over the total study period, inclusive of the pilot and continuation phase, separated by antibiotic class.*

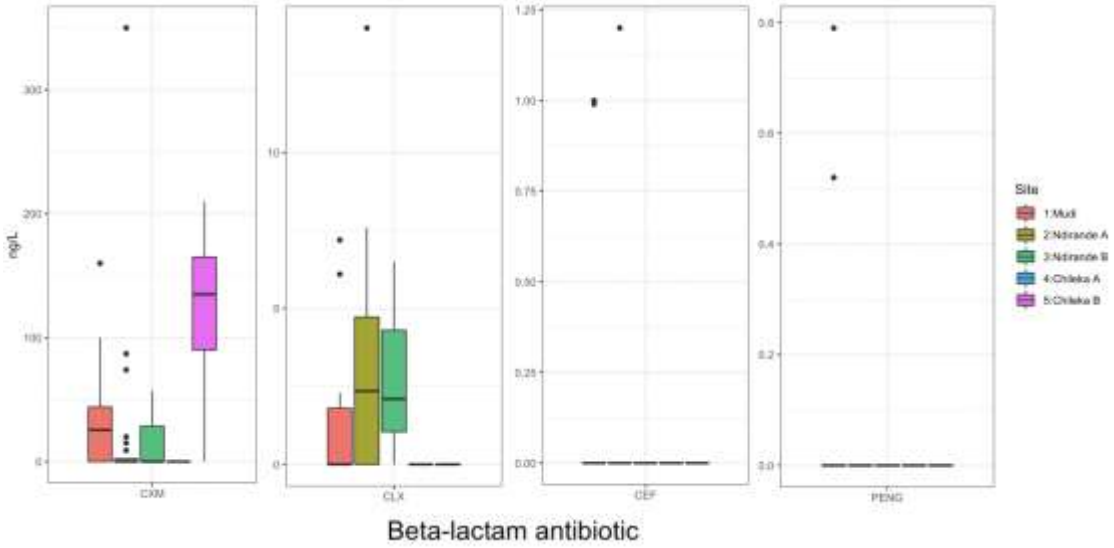


Figure 3c. *Boxplots of ß-lactam antibiotic concentrations (ng/L) at each site over the total study period, inclusive of the pilot and continuation phase, separated by antibiotic class.*

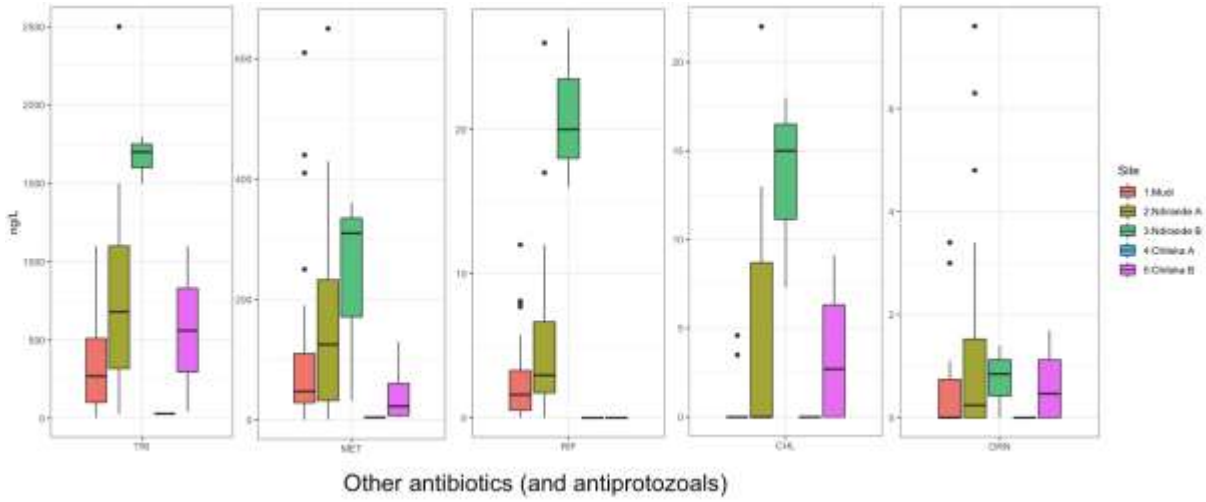


Figure 3d. *Boxplots of antibiotic (unattached class) and antiprotozoal concentrations (ng/L) at each site over the total study period, inclusive of the pilot and continuation phase, separated by antibiotic class.*

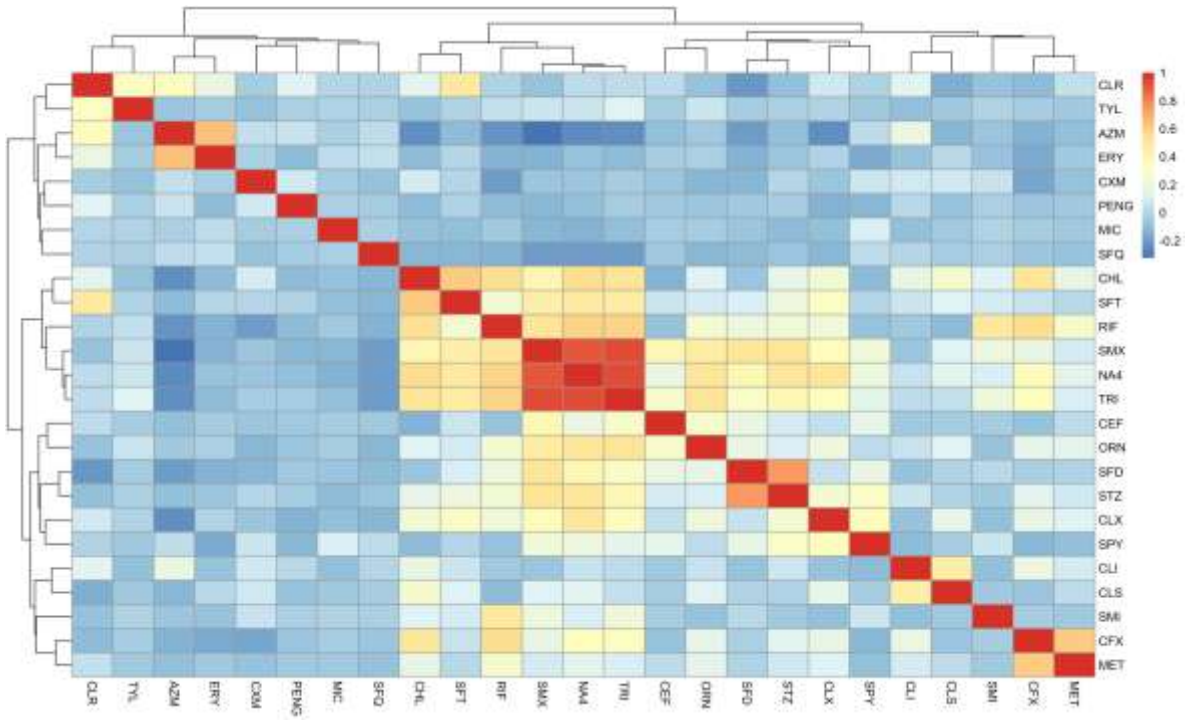


Figure 4. *Pearson's correlation matrix of antibiotics in river water across all 5 study sites. Correlation coefficients are illustrated on a colour spectrum, with those in red and orange showing the highest degree of relationship.*

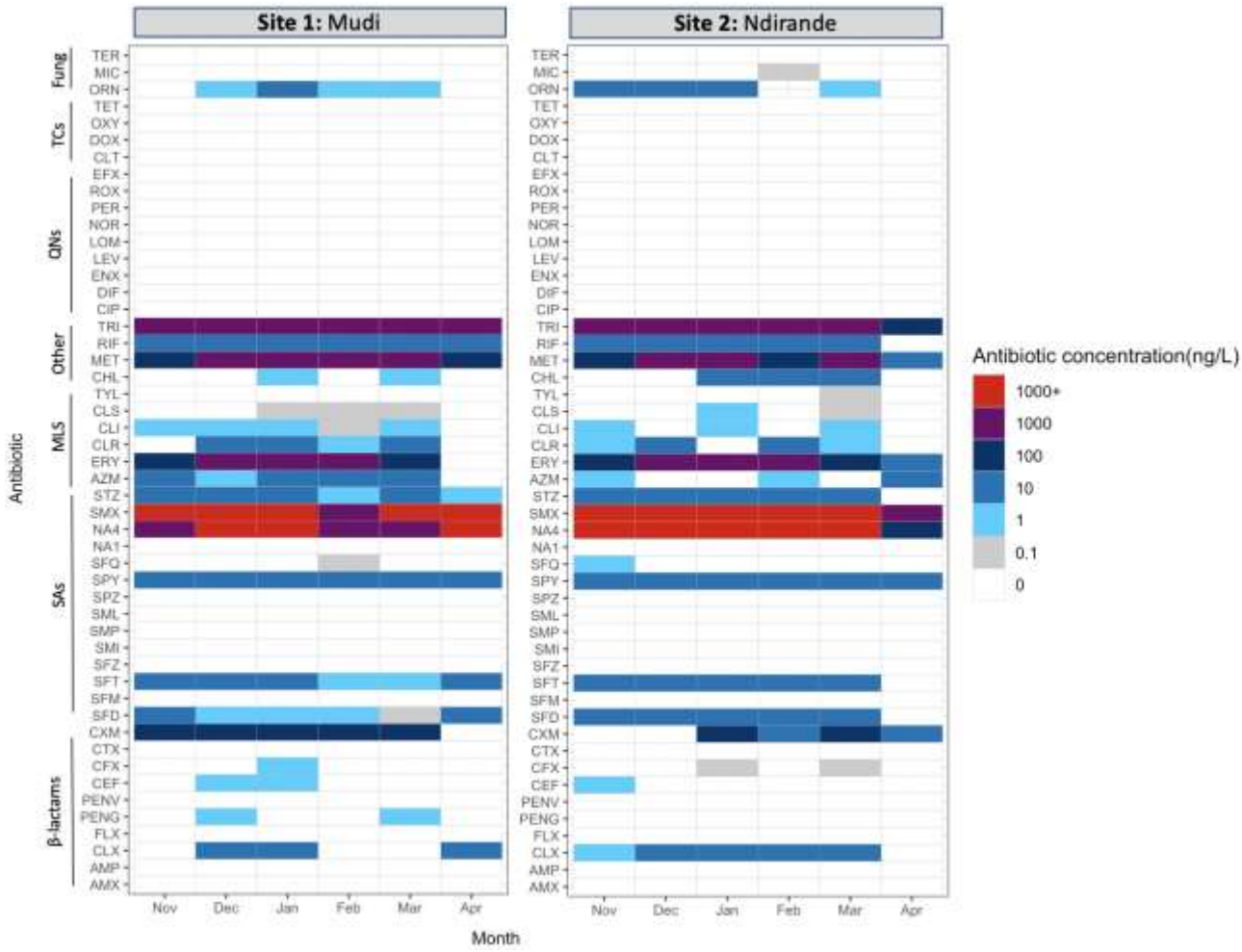


Figure 5. *Monthly presence and concentration (ng/L) of antibiotics in river water from sites 1 and 2 during the continuous phase, stratified by antibiotic class. Antibiotics below the LOQ and are not detected are highlighted in yellow.*

Figure 6. *Monthly presence of antibiotics in river water from sites 1 and 2 during the continuous phase, stratified into values that are within safe (green, <PNEC) and unsafe (red, >PNEC) PNEC levels. Values inside the cells describe the ratio of analyte:PNEC, and a value of 0 illustrates where an antibiotic was identified above the LOQ but below 0.01% of the agreed PNEC target. Where antibiotics were identified but no agreed PENC definitions exist, these have been coloured in light green and where no antibiotic was found these have been coloured white.*

Table 5. Antibiotic mean (SD) concentrations at the 5 sites sampled.

Antibiotic	Site 1 *n=22*	Site 2 *n=24*	Site 3 *n=3*	Site 4 *n=1*	Site5 *n=4*
AMX					
AMP					
AZM	2.70 (3.66)	0.24 (0.48)		0.27	0.05 (0.10)
CEF	0.09 (0.29)	0.05 (0.24)			
CFX	0.02 (0.11)	0.02 (0.08)	0.29 (0.24)		
CTX					
CXM	35.73 (43.61)	23.13 (73.25)	19.00 (32.90)		120.00 (88.31)
CHL	0.37 (1.20)	3.64 (5.91)	13.43 (5.52)		3.63 (4.45)
CIP					
CLR	1.51 (1.52)	0.98 (2.18)			
CLI	0.31 (0.48)	0.23 (0.35)	0.35 (0.37)		0.48 (0.39)
CLS	0.04 (0.10)	0.18 (0.46)			0.37 (0.26)
CLX	1.05 (2.00)	3.29 (3.33)	2.86 (3.31)		
DIF					
DOX					
ENX					
EFX					
ERY	188.68 (267.59)	78.70 (89.20)	6.86 (1.17)	3.10	78.75 (57.78)
FLX					
LEV					
LOM					
MET	268.96 (718.45)	162.96 (162.76)	234.00 (176.71)	3.00	44.65 (58.79)
MIC		0.02 (0.07)			
NOR					
ORN	0.51 (0.95)	1.30 (2.11)	0.75 (0.71)		0.66 (0.82)
OXY					
PENG	0.06 (0.20)				
PENV					
PER					
RIF	2.75 (3.35)	5.36 (6.13)	21.00 (5.57)		
ROX					
SFD	1.39 (4.42)	2.35 (3.03)	1.90 (0.30)		1.12 (0.95)
SFM					
SFT	1.29 (1.16)	2.05 (2.91)	3.2 (0.10)	0.29	2.18 (2.19)
SFZ					
SMX	1892.64 (1926)	4042.91 (3341)	7533.33 (305)	7.00	2427.50 (2023)
NA1					
NA4	876.13 (898)	2200.54 (1547)	4033.33 (1159)	33.00	1040.25 (922)
SMI			0.18 (0.31)		
SMP					
SML					
SPY	3.37 (3.55)	4.47 (3.80)	2.75 (2.29)	0.29	0.90 (0.80)
SFQ	0.01 (0.07)	0.04 (0.16)			
STZ	2.09 (3.41)	2.18 (2.00)	3.97 (4.11)		0.98 (1.95)
TER					
TET					
TRI	344.20 (290.02)	740.12 (566.54)	1666.67 (152.75)	30.00	656.75 (455.90)
TYL		0.02			

**Blacked out boxes indicate antibiotic presence undetected (below limit of detection).*

Table 6. *Mean (SD) concentrations (µg/L) of antibiotics identified from sites 1 and 2 in urban Blantyre.*

Metal	Site1, N = 27[1]	Site2, N = 28[1]
Al	24.00 (20.71)	21.19 (20.83)
As	0.80 (0.58)	2.13 (1.70)
Ba	111.33 (34.73)	139.46 (40.36)
Cd	0.01 (0.01)	0.00 (0.00)
Ce	0.01 (0.01)	0.01 (0.01)
Co	0.44 (0.54)	0.49 (0.71)
Cr	5.22 (3.37)	0.88 (0.64)
Cs	0.01 (0.01)	0.04 (0.01)
Cu	7.29 (2.99)	4.72 (1.80)
Fe	50.62 (74.63)	13.09 (9.78)
La	0.01 (0.00)	0.00 (0.00)
Li	1.12 (0.16)	3.13 (0.43)
Mn	40.58 (123.12)	97.12 (244.44)
Mo	1.51 (2.05)	1.47 (1.99)
Ni	11.78 (6.00)	0.70 (1.12)
Pb	0.06 (0.14)	0.01 (0.01)
Rb	14.31 (7.97)	44.26 (16.53)
Sb	17.16 (9.51)	0.56 (0.23)
Se	0.45 (0.13)	0.59 (0.12)
Sr	515.44 (71.99)	970.46 (165.24)
Ti	11.05 (9.00)	12.49 (10.33)
U	0.10 (0.06)	0.04 (0.06)
V	2.75 (2.02)	1.88 (0.91)
W	0.01 (0.04)	0.00 (0.00)
Zn	35.11 (39.98)	7.62 (5.62)

[1] Mean (SD)

Discussion

Within this study we illustrate that resistance-driving chemicals are consistently recovered from urban waterways in a large sSA city, posing risks to human, animal, and ecological health. We identified higher levels of sulfamethoxazole than any other antibiotic, followed by metronidazole, rifampacin and macrolides. This spectrum of antibiotics reflects those that are typically used locally in human medicine to treat a broad range of bacterial diseases (SMX), including TB (Rif), gastrointestinal and respiratory infections (MET/macrolides), and the prevention of opportunistic infections in HIV (CTX). Evidence for the presence of antibiotic residues in urban rivers from LMICs, particularly sSA is limited (438). Where data exists, sulphonamides predominate, and are ubiquitous to tropical rivers across Asia (439) and sulfamethoxazole is the most commonly detected antibiotic in African surface waters; frequently reported at concentrations ranging between 0.00027 – 39 $\mu g/L^{-1}$ (404,438). Given the absence of antibiotic manufacturing plants, and no functioning WWTP upstream of any of the included river sites, along with the high reported levels of faecal contamination of urban rivers in these settings (213,440), these results suggest that ineffectual waste management of human effluent leads to the widespread dissemination of antibiotics in the urban riverine environment.

Human antibiotic usage in Malawi is complex, and influenced by vulnerabilities of access and cost, alongside intrinsic health system constraints (441). This leads to a narrow spectrum of typically oral antibiotics used (116), and these are the same compounds that we frequently encountered in urban surface waters. While campaigns to reduce community antibiotic prescribing are ongoing, we should remain cognisant that antibiotics provide a large positive benefit to population health, and a priority focus should be on improvements of waste management and environmental removal of antibiotics, instead of a reduction in potentially life-saving antimicrobial therapy.

Within animal health, fewer antibiotics are used in low-income settings compared to high-income settings, but there is limited accurate data on specific ABU metrics from LMICs. Here, the antibiotics we found at the highest concentrations are not those routinely used by local veterinarian services or purchased over-the counter for treating sick animals. Nevertheless, without accurate prescription data, it is unclear what percentage of antibiotic in the river system are present as a direct result of animal health.

The selection risk from antibiotics were derived from PNECs proposed for use by the AMR Industry Alliance as discharge limits from manufacturing facilities, and these have been endorsed by several

industrial partners and countries, (195). When we compared antibiotic concentrations found in urban Malawian rivers to these targets, we see that SMX, TRI and MET are consistently reported above recommended limits, over extended periods of time. To survive in toxic conditions, bacteria, in particular Enterobacteriaceae develop resistance via intrinsic mutations or through acquisition of genetic determinates (6). The elevated presence of antibiotics in the environment is believed to increase the rate of selection for antibiotic resistance, which may allow the environment to form a key niche for the maintenance and evolution of AMR (194,430).

We frequently also found pesticides, herbicides and fungicides in the rivers sampled. Due to the absence of available eco-toxicology data, there are currently no internationally agreed targets for PNEC of these chemicals in surface waters. Nevertheless, their role has been widely reported to influence selection pressures on bacteria in the aquatic environment (442–444), and agrochemicals are frequently used by households and subsistence farmers in LMICs, including Malawi, and have been found in local surface waters previously (445–447). Therefore their role in this setting may be uniquely placed, given pressures on farmers to maintain crop growth (448) alongside inadequate resources to effectively regulate the importation, production, sale and use of these chemicals (449).

Metals have previously been identified in the river systems (450) and drinking water (234) of Blantyre through point prevelance studies. Longitudinal data within this study illustrates a consistent presence of metals in urban waterways, with occasional concentrations above recommended WHO or USEFA limits. The continued presence of antibiotics, pesticides and heavy metals in these sites may well serve as an important driving factor in the high levels of reported antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in urban sSA rivers including those found in Blantyre waterways (451).

The prevelance of ARB in sub-tropical rivers, like those seen in Malawi, are likely to be influenced by seasonal trends in rainfall. Increased rainfall leads to widespread flooding and overflowing of pit latrines into local rivers and groundwater. Floodwaters elsewhere have been shown to have higher amounts of *E. coli* and ARGs [sul1, IntI1], leading to increased exposure to pathogens and AMR risks in these scenarios (206). The paucity of adequate sanitation infrastructure in urban settings intensified the effects of these events and could lead to seasonal fluctuations in effluent and antibiotics in local river systems.

There are several limitations to this study. Firstly, there are multiple factors that alter the levels of antibiotics in the aquatic environment including photodegradation, biodegradation and river flow rates. Photodegradation and biodegradation differ depending on the chemical structure and river flow rates fluctuate substantially over small timescales and between the wet and dry season. Furthermore, although rivers were sampled continuously, the frequency of 2-weekly sampling does not permit the assessment of risks on a daily or weekly basis, and alterations in the concentrations on these timescales cannot be determined. Logistical challenges and financial constraints meant that we sampled for one year at 2 sites and citywide and seasonal risks have been made as extrapolations from these timescales. Ideally surveillance would be continuous at a greater number of river sites, over a number of seasons, alongside the collection of population-level and meteorological metadata. Lastly, given the high morbidity and mortality from drug-resistant bloodstream infections in sSA, alongside the relationship we found to human health prescribing, ideally, we would have targeted screening for chemical residues including ceftriaxone, carbapenems and colistin to determine antibiotic residues that may be of greatest local concern.

While the risks of resistance-driving chemicals in the aquatic environment have not been quantified for human or animal health, in urban Blantyre we find that antibiotics in excess of PNECs that are considered safe for ecological health. This is directly linked to inadequate WASH infrastructure in densely populated urban environments and human antimicrobial usage, and highlights that the riverine network may be an important ecological niche for the acquisition, maintenance, and transmission of AMR in LMIC settings.

Contributions

DC, AS, and NF conceived the study. DC and AS devised the analysis plan. DC and RG and KG analysed the data. AS, NF and NE verified and interpreted. DC drafted the initial manuscript. All authors contributed to revision of the manuscript. DC and NF acquired the study funding and DC, ToM, KC, TrM, RG and KG verified the underlying data. All authors take responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Acknowledgements

This study was conducted within the DRUM consortium. Ethical approval was obtained for this study from the College of Medicine Research and Ethics Committee, Malawi (P.11/18/2541) and the Liverpool School of Tropical Medicine, UK (18-090). In addition, permissions were granted from village leaders, and support obtained from local community advisory groups. Sensitizations of study areas were conducted prior to study initiation.

Appendices

Appendix i. Logistical challenges at sampler sites, and community engagement activities.

A pilot phase between February 2020 and April 2020 to assess for logistical challenges. The majority of the sites faced significant challenges from theft and the unsuccessful recovery of filters after either a 7 day or 14-day period. In downstream sites, high fluctuations in rainfall during the "rainy" season led to flash flooding and mechanical destruction of samplers, while sites in dense urban environments had high levels of theft. An important component of the success of this project was as a result of ongoing local engagement activities. Local chiefs and community leaders were surveyed for the acceptance of samplers and verbal permissions were granted. Where sites fell on private property, verbal agreements were drafted for placement of samplers prior to siting. However, theft in particular was hampering any meaningful longitudinal sampling in the urban community sites and mechanical loss was problematic in the central city site, situated at the golf course.

At the golf course, working in partnership with the head groundsman the team found a suitable site on the river which was away from public walkways (reduced theft), in a wider part of the river (less prone to mechanical loss) and safe to access by the field team. At the urban site in Ndirande, local leaders suggested discussing with businesses and households that worked nearby or lived next to the river whether they could become guardians or local champions, assisting with the success of the project. The field teams then undertook a survey alongside the river edge, and 3 individuals who lived nearby highlighted that they would like to take part. After consultations with these individuals, a number of recommendations were implemented. Firstly, 2 suitable areas were identified where filters could be positioned away from people. Secondly, filters would be sited at less busy times of the day by members of the local population (i.e. not MLW field teams) so as to not raise suspicion. Lastly, regular checks (by local residents) were made every 2-3 days to ensure filters were not removed or had become visible. All of these measures were successfully employed, and the 3 individuals became

guardians who assisted us throughout the study, in return for remuneration of their time. This led to no further loss of the filters via theft or mechanical destruction at the urban site. Within Chileka, we attempted siting filters at the local school, however this was unsuccessful due to mechanical destruction and no local champions were identified.

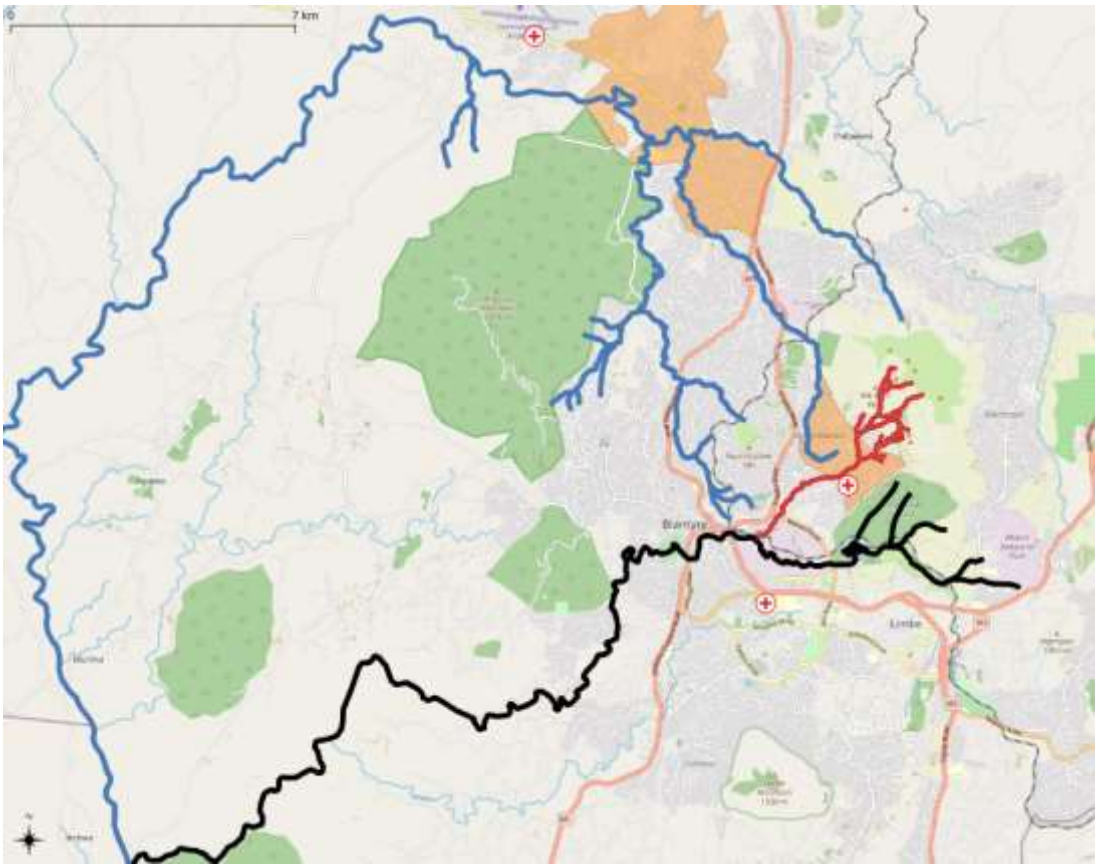
The intention of the study was to be operational for the period of 1 year, and have continuous sampling, to allow for the assessment of antibiotic presence monthly, accounting for seasonality. Before efforts were implemented to address the logistical challenges identified in the pilot phase, the study was required to stop due to COVID-19 restrictions. The study was not active from the period of April 2020 to October 2020 due to Malawi Ministry of Health and MLW community based COVID-19 restrictions. Due to logistical and financial constraints, once re-operational in November 2020 a rational approach to sampling was undertaken, with a reduction of sites to 2, which focussed on the presence of antibiotics in urban riverine environments alone (**see Figure 1**).

For the continuation phase, the samplers were placed into a small surrounding cage to reduce the risk of mechanical destruction (picture below, adapted from "Instillation of POCIS samplers" by R Grabic, University of South Bohemia), and then submerged into the river. Samplers were attached via a wire and secondary rope to metal posts drilled into the edge of the riverbank at points out of view from the public. The length of wire was ~20cm long to enable continuous submersal and limited movement. Collection and replacement of samplers were undertaken at times of reduced footfall, by members of the community known to the study team, in an effort to reduce the chance of filter discovery and theft.

a) outside cage b) internal PES membrane. c) completed POCIS with wire

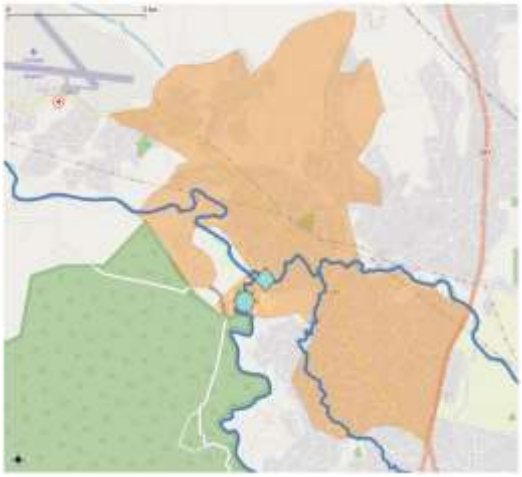
Appendix ii. Detailed maps of the riverine network of Blantyre, including (a) Blantyre city (b) Ndirande and (c) Chileka. DRUM study polygons have been demarcated in orange. Sampling sites have been geolocated (site 1: star, site 2: triangle, site 3: square, site 4: circle, site 5: diamond) alongside the key rivers (black = Mudi river, red = Nasolo river, blue = unnamed river).

(a)



(b)

(c)

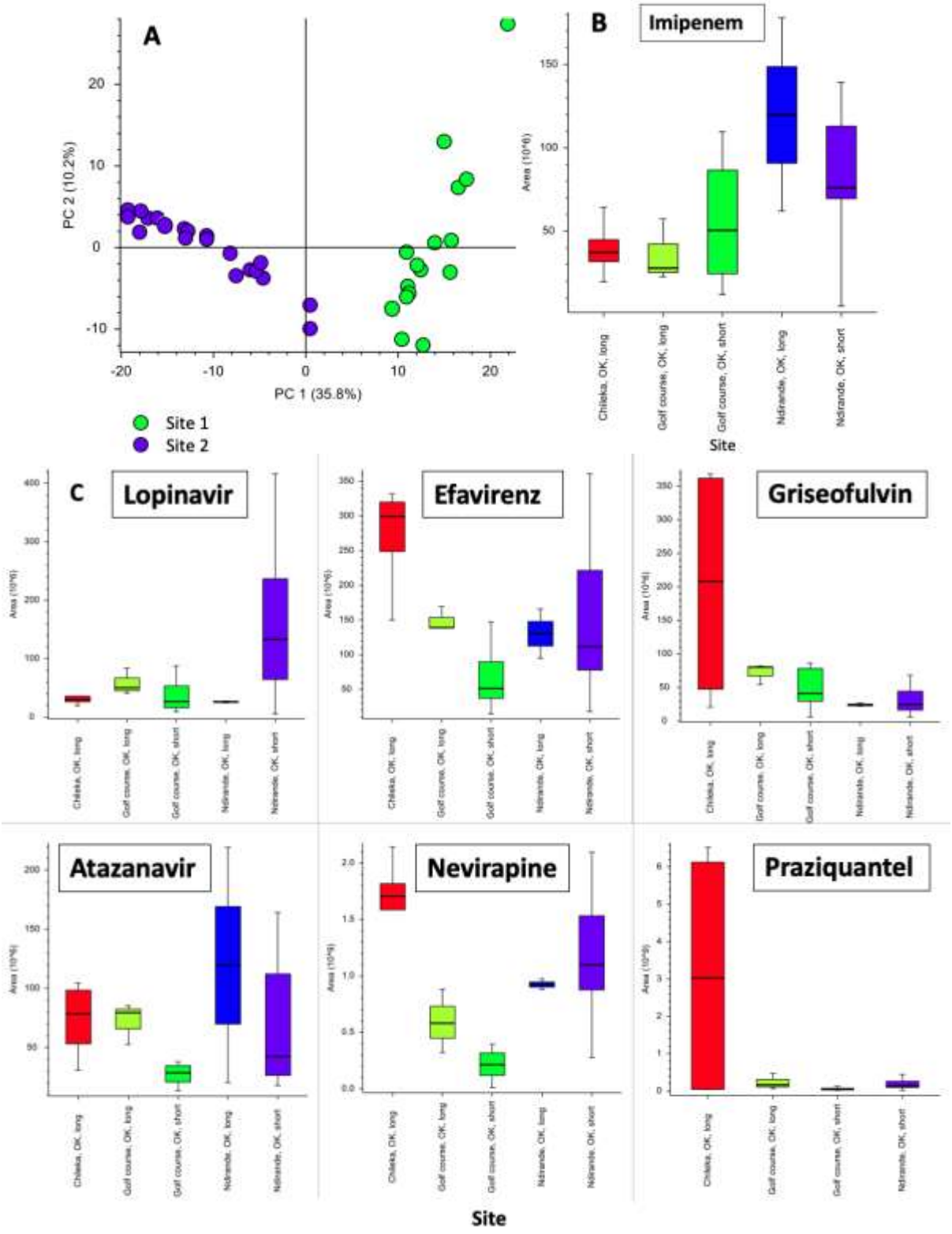


Appendix iii. Photos of the sampling sites at study initiation. Local approvals and permissions were granted.

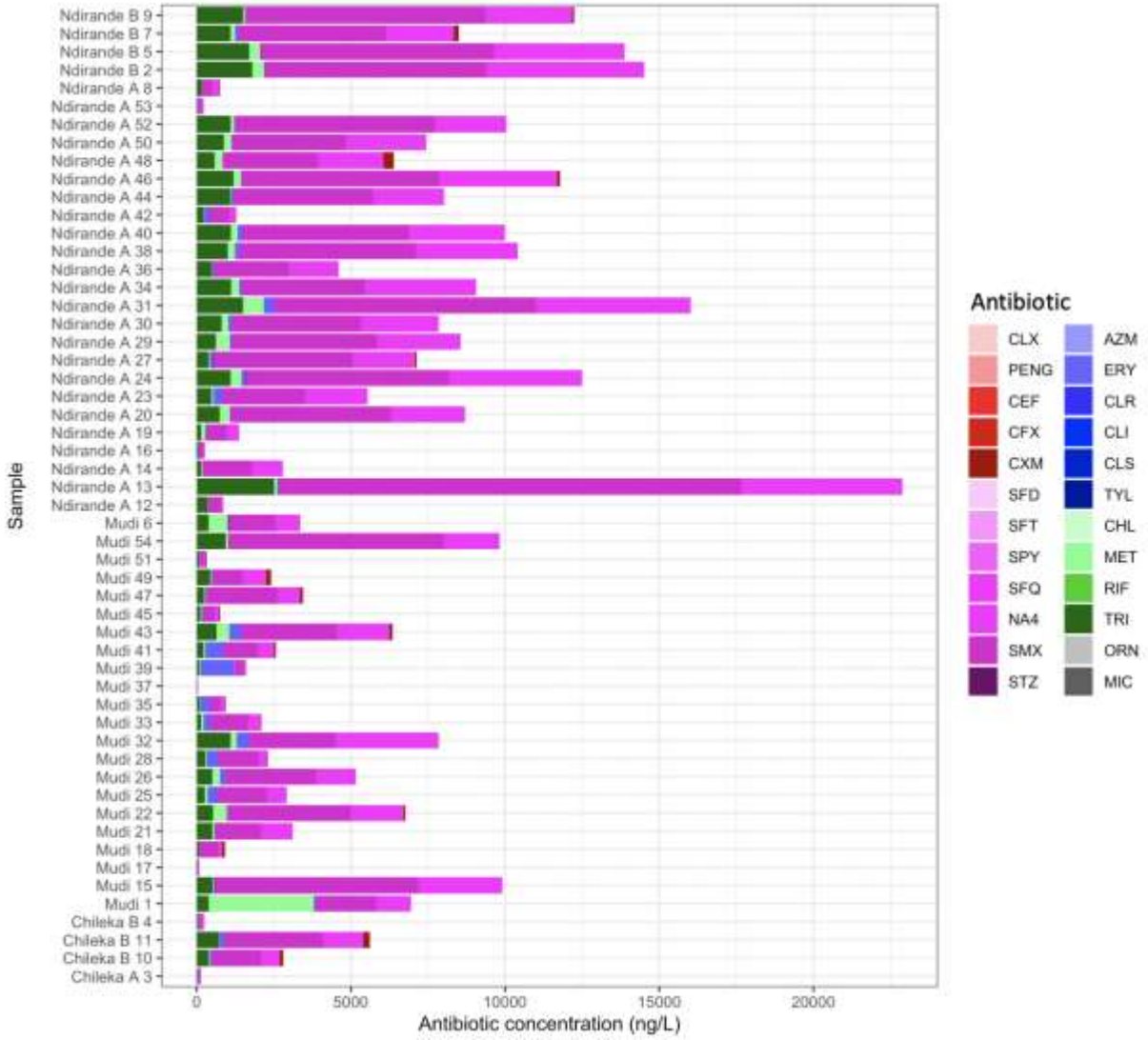
Appendix iv. List of PNEC values adapted from the AMR industrial alliance discharge targets.

Antibiotic	PNEC	Antibiotic	PNEC	Antibiotic	PNEC
Amikacin	16	Cloxacillin	0.13	Oxytetracycline	0.5
Amoxicillin	0.25	Colistin	2.0	Pefloxacin	8.0
Amphotericin B	0.02	Daptomycin	1.0	Phenoxymethylpenicillin	0.06
Ampicillin	0.25	Delamanid	0.03	Piperacillin	0.5
Anidulafungin	0.02	Doripenem	0.11	Polymixin B	0.06
Avibactam	200	Doxycycline	2.0	Retapamulin	0.06
Avilamycin	8.0	Enramycin	4.8	Rifampicin	0.06
Azithromycin	0.02	Enrofloxacin	0.06	Roxithromycin	1.0
Aztreonam	0.5	Ertapenem	0.13	Secnidazole	1.0
Bacitracin	8.0	Erythromycin	0.5	Sparfloxacin	0.06
Bedaquiline	0.08	Ethambutol	2.0	Spectinomycin	32
Benzylpenicillin	0.25	Faropenem	0.02	Spiramycin	0.5
Capreomycin	2.0	Fidaxomicin	0.02	Streptomycin	16
Cefaclor	0.50	Florfenicol	2.0	Sulbactam	16
Cefadroxil	2.0	Fluconazole	0.25	Sulfadiazine	13
Cefalonium	21	Flumequine	0.25	Sulfamethoxazole	0.6
Cefaloridine	4.0	Fosfomycin	2.0	Tedizolid	3.2
Cefalothin	2.0	Fusidic acid	0.5	Teicoplanin	0.5
Cefazolin	1.0	Gatifloxacin	0.13	Telithromycin	0.06
Cefdinir	0.25	Gemifloxacin	0.06	Tetracycline	1.0
Cefepime	0.5	Gentamicin	0.15	Thiamphenicol	1.0
Cefixime	0.06	Imipenem	0.13	Tiamulin	1.0
Cefoperazone	0.5	Isoniazid	0.13	Ticarcillin	8.0
Cefotaxime	0.1	Itraconazole	0.01	Tigecycline	1.0
Cefoxitin	8.0	Kanamycin	1.0	Tildipirosin	0.42
Cefpirome	0.06	Levofloxacin	0.25	Tilmicosin	1.0
Cefpodoxime	0.25	Lincomycin	0.81	Tobramycin	1.0
Cefquinome	1.6	Linezolid	6.7	Trimethoprim	0.5
Ceftaroline	0.06	Loracarbef	2.0	Trovafloxacin	0.03
Ceftazidime	0.5	Mecillinam	1.0	Tylosin	1.0
Ceftibuten	0.25	Meropenem	0.06	Vancomycin	8.0
Ceftiofur	0.06	Metronidazole	0.13	Viomycin	2.0
Ceftobiprole	0.23	Minocycline	1.0	Virginiamycin	2.0
Ceftolozane	1.9	Moxifloxacin	0.13		
Ceftriaxone	0.03	Mupirocin	0.25		
Cefuroxime	0.5	Nalidixic acid	16		
Cephalexin	0.08	Narasin	0.5		
Cephradine	N/A	Neomycin	0.03		
Chloramphenicol	8.0	Netilmicin	0.5		
Ciprofloxacin	0.06	Nitrofurantoin	64		
Clarithromycin	0.08	Norfloxacin	0.5		
Clinafloxacin	0.5	Ofloxacin	0.5		
Clindamycin	0.1	Oxacillin	1.0		

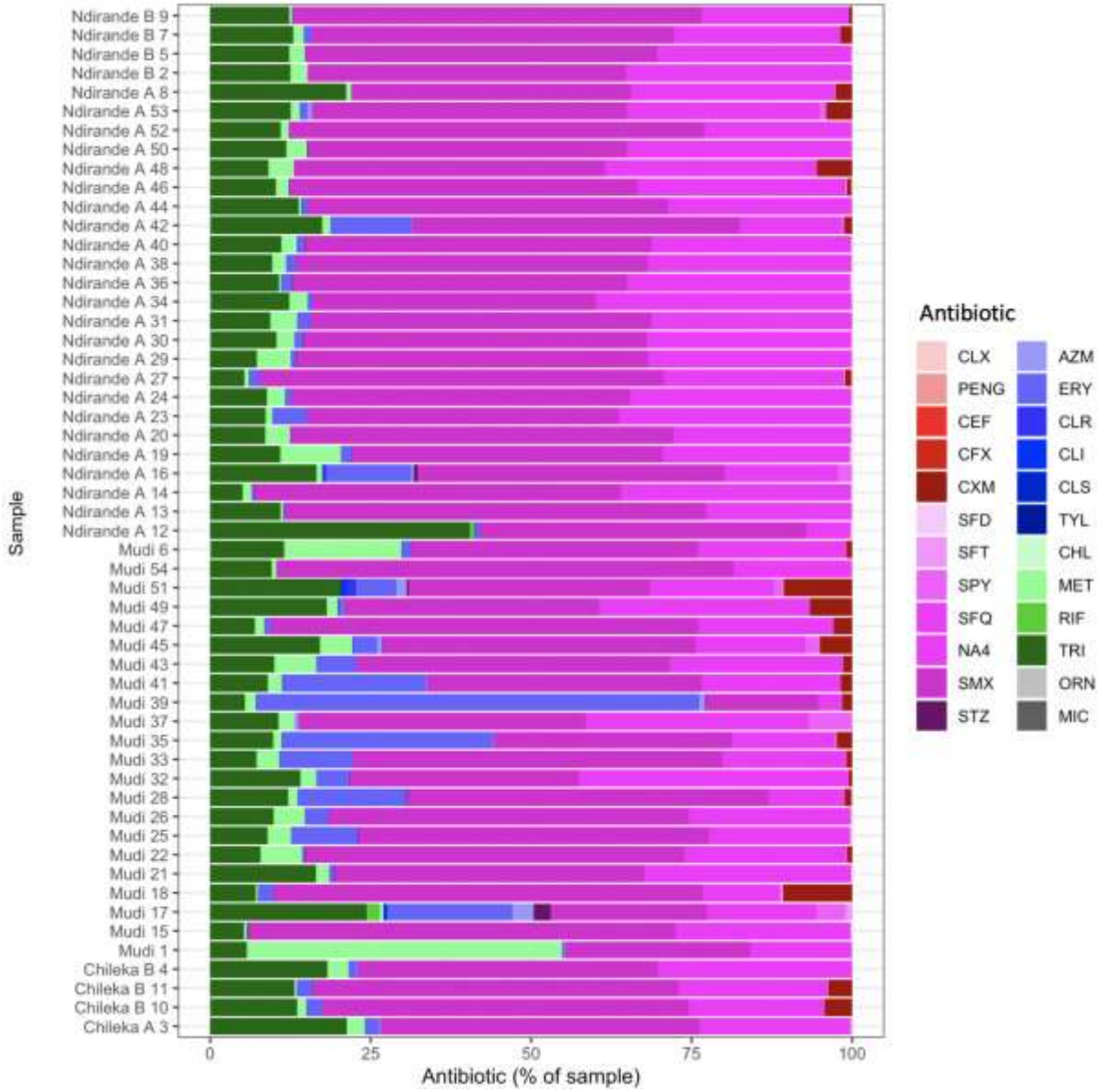
Appendix v. Non-targeted chemical analysis results. (a) PCA analysis of chemical compounds in site 1 and site 2, (b) concentration of *novel* antibiotics identified through non-targeted method, (c) concentration of ARVs, antiprotozoals and antifungals identified.



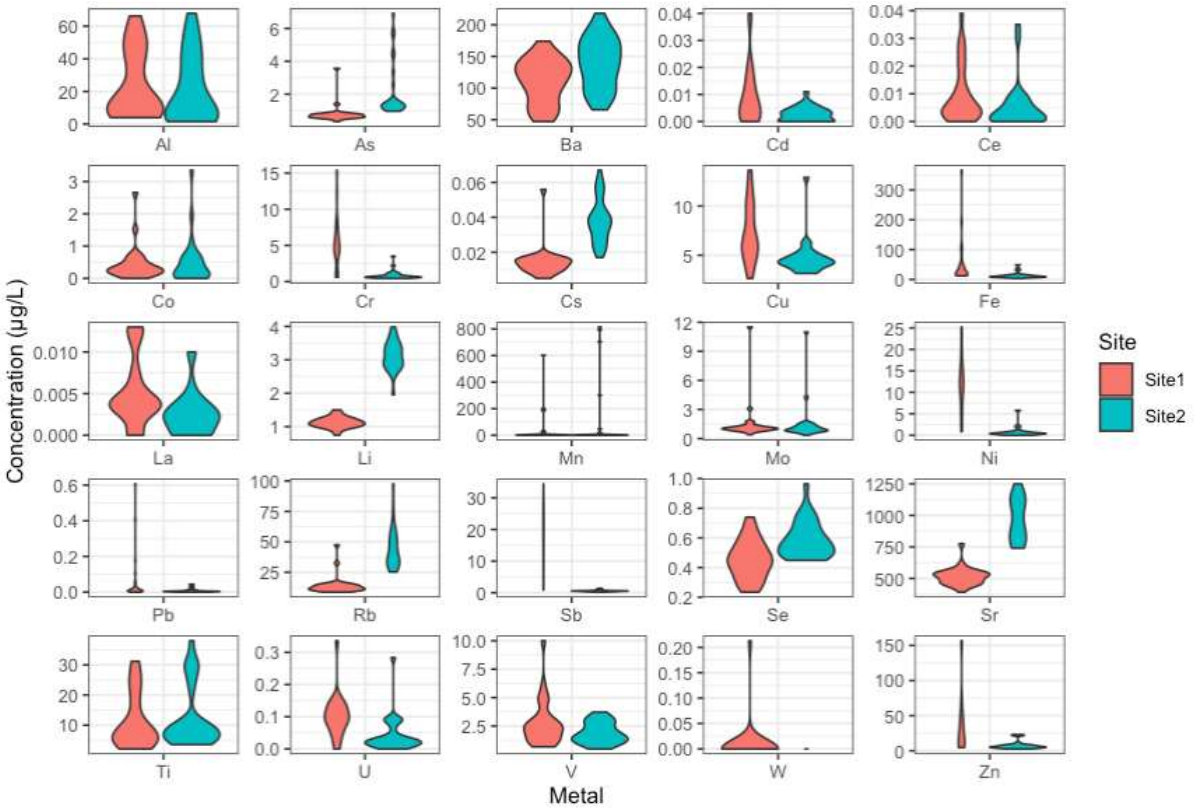
Appendix vi. Cumulative total of antibiotics identified from each sample, stratified by site and coloured by antibiotic class.



Appendix vii. Spatiotemporal variations in antibiotic compositions in the 54 samples collected, stratified by site and coloured by antibiotic class.



Appendix viii Violin plots of metal concentrations (µg/L) by site



Appendix ix. Maximum concentrations (*µg/L*) of metals identified at site 1 & 2.

Heavy metal	Maximum concentration µg/L (site 1)	Maximum concentration µg/L (site 2)	WHO reference values	USEFA reference values
Al	66.32	67.90	200	200
As	3.57	6.92	10	10
Ba	174.00	218.00	-	-
Be	Not found	Not found	-	-
Cd	0.04	0.01	3	5
Ce	0.04	0.04	-	-
Co	2.66	3.35	-	-
Cr	15.40	3.52	50	100
Cs	0.06	0.07	-	-
Cu	13.64	12.91	2000	1300
Fe	**366.00**	49.00	300	300
La	0.01	0.01	-	-
Li	1.50	3.98	-	-
Mn	**814.00**	**602.00**	100	50
Mo	11.50	11.00	-	-
Ni	**25.20**	5.78	20	-
Pb	0.61	0.04	10	15
Rb	47.60	97.60	-	-
Sb	19.70	1.38	-	-
Se	0.74	0.96	40	-
Sr	776.00	1250.00	-	-
Sn	Not found	Not found	-	-
Ti	30.60	38.00	-	-
U	0.34	0.28	30	-
V	10.00	3.72	-	-
W	0.21	Not found	-	-
Zn	156.62	23.15	1000	1000

Chapter 9:

Conclusions and future directions

9.0 Introduction

In this thesis I have presented the results from two observational studies undertaken in southern Malawi, which were designed to broadly assess key risks for carriage of ESBL-E and ESBL-K in Malawian communities. In Chapter 1 I presented a hypothesis that within low-income settings, ineffectual household WASH practices and a paucity of WASH infrastructure contribute to ESBL contamination of the household environment and pollution of the riverine and community environment via inadequate management of faecal sludge. Interactions between humans, animals and environmental reservoirs of ESBL bacteria in these settings promote the acquisition, maintenance and spread of ESBL-E and ESBL-K, ultimately resulting in increased levels of gut carriage of these drug resistant organisms. In this chapter I summarise my findings and suggest future research priorities and important next steps.

My contributions to this chapter and those of others are included in Table 9.0.

Table 9.0. Chapter contributions made by the PhD candidate, alongside those from external partners and DRUM consortium collaborators

	Listed chapter contributions
Personal contribution	All sections of this chapter were drafted by the PhD candidate
Contributions from external partners and DRUM consortium collaborators	Guidance and document review was provided by the PhD supervisory team and DRUM collaborator, Tracy Morse.

9.1. Summary of findings

A large, longitudinal, household-centred study was undertaken in urban, peri-urban and rural communities of southern Malawi, which collected demographic, WASH and microbiological data from humans, co-located animals and the household environment. The methods for this study were described in Chapter 2. In Chapter 3 I outlined the approach to random household selection and describe similarities between the baseline metrics in households from this study compared with other

studies in our setting. This illustrated that the households recruited are likely to be representative of urban, peri-urban and rural sites within Malawi, allowing us to make generalisable estimates from the findings obtained, and determine regionally-related differences in ESBL-E and ESBL-K risks.

In Chapters 3 and 4 I found a similar household density between the regions (mean 4.5), with households in the rural setting on average poorer than those in the urban or peri-urban setting. The median age of household members was 18yrs, and participants were invariably in good health with few co-morbidities or recent hospital admissions; with an adjusted HIV prevalence of 14.0% across the study cohort. Antibiotic exposure in the study cohort was predominantly limited to oral amoxicillin, co-trimoxazole and metronidazole and associated with episodes of illness, irrespective of diagnosis. ABU was higher in the rural site compared to other regions, and in children under 5. Animal ownership was commonplace (58.7% households) and highest in the rural site, with poultry the most frequently owned animal type. The animal species present at households varied by setting, with larger livestock animals more often seen in the rural area, and domestic animals seen in the urban and peri-urban sites. Preventative measures were employed to reduce episodes of animal illness, and when animals became unwell households would only occasionally seek specialist advice or give medication, and therefore there was limited ABU exposure seen in household-owned animals.

There was a paucity of household WASH infrastructure and access to materials that enable safe toileting, adequate sanitation or effectual hand-hygiene and waste management was limited across all sites. This was paralleled by behavioural proxies that may increase the risk of bacterial transmission, such as household attitudes to water usage, food-hygiene, open defaecation, and handwashing. Finally, I noted interactions between household participants and key environmental sites including rivers and drains, which were found in Chapter 5 to be heavily contaminated with ESBL bacteria, particularly within the urban setting.

In Chapter 5, I made a detailed microbiological description of the landscape of ESBL-E and ESBL-K at the study households and in the broader environments that these households interact with. The phenotypic results illustrated a very high level of ESBL colonisation in humans, animals and key environments, especially those with inadequate WASH infrastructure or poorly governed waste management systems (i.e. dumping of waste in rivers and open drains). This fits with the hypothesis that WASH inadequacies contributes to widespread human and animal faecal contamination of the environment, and the high levels of ESBL-E found in humans, animals and the environment demonstrate that these compartments are interconnected. I also identified that there are bacterial

species-specific differences, with the ratio of ESBL-E: ESBL-K differing by sample type. In human and animal stool, there was a higher proportion of ESBL-E compared to ESBL-K, whereas in environmental samples there was a similar proportion of ESBL-E and ESBL-K, excepting food and stored water where ESBL-K were more common, indicating different ecological niches for the respective bacteria. Lastly in Chapter 5, I identified a higher rate of ESBL colonisation in the urban setting compared to the other regions, for both human and animal stool, but also in food, household surfaces, floors and the external environment.

In Chapters 6 and 7, I explored how the individual-level, household-level and sample-level information broadly explained the microbiological findings described in Chapter 5 through PCA and mixed-effect models designed to assess for associations with human ESBL, ESBL-E and ESBL-K gut colonisation and evaluate regional similarities and differences in risk. The outputs of the modelling illustrated that there was a trend towards an increased risk from household contamination, piped-water usage and in the case of ESBL-K, poor hand-hygiene, increased household density and drain-water exposure. Site-dependant water management, sanitation and hand-hygiene practices influenced ESBL colonisation status and across all regions there were risks associated with sharing toilets and river water exposure. Alongside this, there was a strong seasonal association with ESBL colonisation, likely to be consequent upon the ability of neither the environment nor WASH infrastructure to cope with seasonal heavy rainfall, flushing human and animal waste through the environment. Predictions made from these models therefore suggested that future WASH interventions to curb ESBL transmission should consider integrating water management, hand-hygiene and environmental-hygiene measures as part of their strategy for maximal effect.

There were regional differences in risks, with the peri-urban site being the most climate sensitive and having the highest odds of ESBL colonisation in the wet season. Animal-associated risks were dependant on the combination of the site, animal species and bacterial species, and individual-level differences were minimal between the regions. These results indicate that geographic location and associated variations in regional WASH infrastructure, practices and environmental exposures, does impact upon ESBL, ESBL-E and ESBL-K colonisation risks differentially.

Lastly, in Chapter 8 I assessed the prevalence of key antibiotics and resistance-driving chemicals in urban waterways of Blantyre that could be contributing to the creation of an ecological niche supportive of ESBL-E. Here, a total of 25 antibiotics, 4 antiretrovirals, 3 antifungals and 2 antiparasitics commonly used in human medicine were identified, alongside 30 pesticides, 7 herbicides and 8

fungicides used in agriculture, and 25 heavy metals were recovered from river water samplers in urban communities. The heavy metals were all within allowable WHO limits; however, antibiotic concentrations of sulfamethoxazole, trimethoprim and metronidazole were consistently above PNECs; highlighting that in urban Malawian rivers, antibiotics used in human health are consistently found across time and space, at levels that can drive and sustain the emergence of AMR-harbouring bacteria.

9.2. Conclusions and future research priorities

I found a staggeringly high prevalence of ESBL colonisation in humans and animals, alongside ESBL contamination of the households and broader environment (i.e. rivers and drains) in southern Malawi. I have highlighted the key role that WASH infrastructure and behavioural proxies have on driving human community carriage of ESBL bacteria in southern Malawi and propose that without adequate efforts to reduce ESBL contamination of the shared environment, both at a household level and community level, we are unlikely to control ESBL transmission in this setting. Furthermore, future interventions and policy designed to interrupt AMR transmission should be cognisant of regional differences in AMR-prevalence that are likely consequent upon different WASH infrastructure, and adaptions made wherever possible which are tailored to the local population for maximal effect.

9.2.1. Next steps in the data analysis

9.2.1.1. Short-read sequencing of isolates

The AMR data presented in this thesis are solely phenotypic. To accurately determine the relationship between isolates cultured from humans, animals and the environmental and assess the flux between these compartments, bacterial typing based on whole genome sequence data will be imperative. This approach will also enable us to go beyond the associations identified in this thesis and infer directionality of ESBL transmission. Lastly, WGS will allow us to track if clones of local or global clinical importance (i.e. ST131 *E. coli* containing *bla*CTX-M-15) are present in healthy community members, animals or community environments within this study, and compare these findings to those obtained from other settings.

This work has been held up for nearly 2 years by issues arising from the need for a Nagoya Protocol compliant contract. This has now been issued by the government of Malawi. DNA has been extracted

from all ESBL-E and ESBL-K isolates obtained in the study (1 pick per plate for each ESBL-E and ESBL-K). These will be whole genome sequenced on the Illumina X10 platform (Illumina Inc, California, USA) at the Wellcome Sanger Centre (UK) to produce 150bp paired end short reads as part of the DRUM consortium and will be augmented with long read sequencing on the MinION platform (Oxford Nanopore Technologies, UK) for a select number of isolates; enabling the characterisation of MGEs and evidencing the extent to which HGT is occurring.

9.2.1.2. Metagenomic genomic analysis to determine the diversity of ESBL bacteria and better identify the ecological niche of ESBL AMR.

Shotgun metagenomic analysis of enriched samples not under antibiotic selection pressures (i.e. BPW) will be undertaken to identify the relative abundance of bacterial species and spread of ARGs present in human, animal and environmental samples. This will allow us to better understand the biology of within-host and within-compartment ESBL-E and ESBL-K diversity and investigate the human, animal and environmental resistomes in an effort to more accurately determine where the ecological niche of ESBL-E and ESBL-K lies.

Total DNA had been extracted from human and animal stool and river water samples obtained from households in the study, alongside DNA from plate sweeps of the ESBL ChromAgar media from stool and environmental samples. These will be sequenced on the Illumina HiSeq 4000 platform (Illumina Inc, California, USA) and Illumina X10 platform (Illumina Inc, California, USA) respectively, at the Wellcome Trust Sanger Centre (UK) and will be complimented by the sequencing outlined above.

9.2.1.3. Incorporation of observational and genomic datasets into models of ESBL risk within our setting, to better refine potential interventions and areas for future research.

Here, I use self-reported WASH data by household participants. Detailed observations of WASH practices were undertaken in parallel by DRUM, and these will provide more accurate behavioural insights. This data will be important if we are to propose future WASH interventions, as observational data is frequently missing from studies, hampering the intended effects of interventions on outcomes of interest (i.e. hand-washing interventions on frequency of diarrheal episodes). The frequency and nature of these interactions may be contributors to the acquisition, maintenance and transmission of ESBL bacteria in humans and animals within our setting.

Therefore, to develop our understanding of the key drivers of ESBL AMR, the DRUM consortium will take an agent-based modelling approach which permits the incorporation of qualitative (i.e. observational) and quantitative information alongside genomic data into models that describes AMR movement between humans, animals, and the environment. 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; ultimately enabling us to inform the design of interventions aimed at interrupting ESBL transmission in our setting.

9.2.2. Ongoing research within DRUM

9.2.2.1. Analysis of sub-studies designed to evaluate the role of the local environment and food chain on community ESBL colonisation

Given the high levels of ESBL present in the local drain and river environments, a better evaluation of the risk pathways alongside environmental mapping within the urban settings is required. Within the wider DRUM consortium we have completed transect walks of the regional polygons alongside year-long longitudinal microbiological sampling of areas of key risks, as determined by the SaniPath tool. The methods for this sub-study have been broadly outlined in Chapter 2, and from this we will be able to identify the urban sites where ESBL bacteria are most prevalent alongside key human and animal interactions. The aim of this will be to refine the urban hotspots and behavioural risks that may be permissible to educational or interventional campaigns.

Secondly, considering the high level of food contamination, it will be important to consider the risks of AMR transmission along the food chain, from farm to fork. A large number of households rely on local vendors for daily food supplies, and therefore, determining the risk pathways and prevelance of AMR bacteria in local markets will allow us to better understand food-hygiene factors that drive local AMR. Here, within the DRUM consortium I have been involved with the WASH team on development of a market project in urban Blantyre that focuses on a combination of observational and microbiological data collection, to highlight the role that local marketplace plays on ESBL transmission.

Both of these sub-studies have completed baseline data collection and are undergoing initial statistical analysis by members of the DRUM consortium. Total DNA has been extracted from pre-enriched media (buffered peptone water) of ESBL positive samples obtained from these projects and shotgun

metagenomic sequencing of these samples will detail the local environmental resistomes and add to our understanding of environmental ESBL diversity.

9.2.2.2. Clinical blood-stream infection study, assessing the relationship between the diversity of ESBL Enterobacteriaceae seen in BSIs compared with those obtained from patient stool and households of patients with BSIs.

To understand the biology of within-host and within-household ESBL diversity, we have developed a clinical cohort study recruiting ESBL Enterobacteriaceae BSI patients from the local hospital (QECH). Patients who are blood culture positive for *Enterobacteriaceae* are separated into community acquired infections (CAIs) and hospital acquired infections (HAIs) and a household follow-up is undertaken at patients with community-acquired ESBL BSIs. The microbiological sampling strategy and CRFs parallel those undertaken in the community study within this thesis, and via a mixture of short-read sequencing, mSweep and shotgun metagenomics we will be able to compare the diversity of AMR genes found in the microbiome of BSI patients alongside their family members and household environments, contextualising them within the broader community and environment of Blantyre. Ultimately this study might identify household ESBL transmission risks within BSI individuals and provide a platform for future research priorities and the development of targeted interventions to interrupt transmission of AMR-pathogens that are tailored to Malawi's needs.

9.2.3. Future research priorities

9.2.3.1. Evaluate effects of climate change on ESBL transmission

Analysis of temporal and spatial AMR data obtained in this study alongside available meteorological, hydrographic and sanitation (shit-flow) data may provide insights into the role of different components of seasonality in driving the seasonal effects on ESBL colonisation reported here. Modelling of climate forecasts might then be integrated with AMR data to determine whether climate change will lead to increased dissemination of ARB and ARGs into the broader environment, or enhance the transmission of AMR, and subsequent burden of AMR disease.

9.2.3.2. *Understand the local environmental and animal drivers of antibiotic and biocide use, alongside continued microbiological surveillance of ESBL ARG and ARB*

High levels of antibiotics above PNECs and faecal contamination of the riverine network may highlight why the river environment is an important ecological niche for the acquisition, maintenance, and transmission of AMR in LMIC community settings. While I postulate that this is a result of the combination of densely populated urban environments with inadequate WASH infrastructure to control excreta and routine antimicrobial use in the local population (i.e. HIV, TB, gastrointestinal and respiratory infections), more information is required on the use of antibiotics and resistance-driving chemicals in local agricultural and animal practices. Research in this area will allow us to discern the key sources responsible for the dissemination of antibiotics and biocides into the riverine network and illuminate areas for future policy development.

Furthermore, the high levels of antibiotics and ESBL bacteria found in the rivers described within this thesis promotes the integration of riverine surveillance of resistance-driving chemicals, ARGs and ARB within future AMR research undertaken in LIC settings. This would also ideally be accompanied by inclusion of broader environmental surveillance into national action plans and international surveillance campaigns to better enable the evaluation of One-Health drivers of ESBL AMR.

9.2.3.3. A trial of complex WASH interventions to interrupt household ESBL transmission.

Modelling ESBL carriage in our communities predicted reductions in ESBL-carriage following the adoption of improved WASH practices. We should therefore consider a trial of complex WASH interventions aimed at water management, hand-hygiene and environmental-hygiene infrastructure and practices to reduce household ESBL transmission, and ultimately the outcome of human ESBL colonisation. This would be a novel method for reducing AMR in LIC settings, in that it is not reliant solely on restriction of antibiotic consumption and is likely to have added health benefits for the local population.

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