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Key environmental exposure pathways to antimicrobial resistant bacteria in southern Malawi: A SaniPath approach

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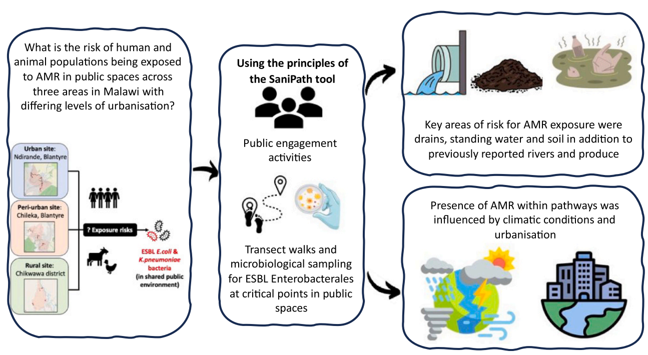
HIGHLIGHTS

- Drains and standing water were key exposure pathways for ESBLs in public spaces
- ESBL prevalence was positively associated with urbanisation.
- Climatic conditions play a significant role on environmental ESBL contamination
- Whole system improvements in environmental health practices and infrastructure needed

GRAPHICAL ABSTRACT



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ABSTRACT

Antimicrobial resistance (AMR) poses a severe global health threat, yet the transmission pathways of AMR within communal public environments, where humans and animals interact, remain poorly explored. This study investigated AMR risk pathways, prevalence, and seasonality of extended-spectrum β-lactamase (ESBL) producing *E. coli* and *K. pneumoniae*, and observed practices contributing to environmental contamination within urban, peri-urban, and rural Malawi.

Using the SaniPath tool, in August 2020, transect walks across three Malawian study sites identified potential AMR exposure pathways, including drains, standing water, soil, and areas of communal hand contact. Subsequently, from September-2020 to August-2021, 1440 environmental samples were collected at critical points along exposure routes (n = 40/month from each site). These underwent microbiological analysis using chromogenic agar techniques to detect the presence of ESBL *E. coli* and ESBL *K. pneumoniae*.

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Results showed the highest ESBL prevalence in urban environments (68.1 %, 95%CI = 0.64–0.72, $p < 0.001$) with a higher ESBL presence seen in drains (58.8 %, 95%CI = 055–0.62, $p < 0.001$) and soil (54.1 %, 95%CI = 0.46–0.62, $p < 0.001$) compared to other pathways. Environmental contamination was attributed to unavailability and poor condition of sanitation and hygiene infrastructure based on key informant interviews with community leaders ($n = 9$) and confirmed by independent observation. ESBL prevalence varied between seasons (χ^2 (2,$N = 1440$) = 10.89, $p = 0.004$), with the highest in the hot-dry period (55.8 % ($n = 201$)). Prevalence also increased with increased rainfall (for ESBL *E.coli*).

We highlight that community environments are likely to be a crucial component in AMR transmission, evident in the abundance of ESBL bacteria in identified exposure pathways. Additionally, poor sanitation infrastructure and practices coupled with seasonal dynamics further affect the presence of ESBLs in communal environments. Therefore, a context appropriate whole system approach that tackles infrastructure and behavioural factors, supported by effective surveillance is required to impact AMR and a range of aligned development challenges in these settings.

1. Introduction

Antimicrobial resistance (AMR) is currently one of the greatest threats to global health and development, causing up to 1.27 million annual deaths globally with significant impacts in sub-Saharan Africa (sSA) (Lester et al., 2020; Murray et al., 2022). Efforts to reduce the prevalence of AMR have previously focused on the healthcare sector through antibiotic stewardship and surveillance (Cueni, 2020; Godman et al., 2017; Katale et al., 2020; Lee et al., 2013; Pokharel et al., 2019; Yau et al., 2021). However, recent studies from both high and low-income countries, have shown a lack of correlation between antibiotic use and AMR, with prevalence being associated with indicators of transmission such as; poor hygiene, poor WASH infrastructure, access to clean water and open defecation (Caudell et al., 2018; Collignon et al., 2018; Fuhrmeister et al., 2023; Hendriksen et al., 2019; Ramay et al., 2020).

This is unsurprising, as poor environmental health practices and infrastructure are known to contribute to the transmission of pathogens including enteric bacteria such as *E. coli*, which are frequently resistant to antimicrobials (Iskandar et al., 2020; Katale et al., 2020). Preventing the transmission of enteric bacteria in the community through improved environmental health is therefore a critical element in the control of AMR. Improving the safety of the environment in sub-Saharan African countries such as Malawi is a high priority, but remains a public health challenge, (Cassivi et al., 2020; Fuhrmeister et al., 2023). This is perpetuated by the growing rate of urbanisation, which has increased population density and unplanned settlements in urban environments resulting in pressure on the already limited environmental health resources, leading to contamination of the wider environment (Cocker et al., 2023; Nadimpalli et al., 2020; Spuhler and Senn, 2020).

A study by Musicha et al. (2017) found rising rates of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *K. pneumoniae* spp. among patients with blood stream infections in Malawi, which are associated with increased mortality (45 % case fatality proportion), and longer hospital stays for patients (Lester et al., 2020). Furthermore, the rise in ESBL *E.coli* and ESBL *K. pneumoniae* in patients has coincided with high human colonisation rates of ESBL bacteria in both community and household settings (Cocker et al., 2023; Doi et al., 2017; Mahmud et al., 2020), which can lead to onward infections (Willems et al., 2023). In Malawian household environments, human colonisation with ESBL producing *E. coli* and *K. pneumoniae* has been associated with poor WASH infrastructure, co-location of animals inside the house, drinking water sources, poor WASH behaviours and increased rainfall (i.e. seasonality) (Cocker et al., 2023; Sammarro et al., 2022). However, there is a gap in our understanding of the prevalence and exposure risk to resistant microbes in public spaces (Ahammad et al., 2018). This gap is reflected in the Malawi National Action Plan for AMR which does not tackle all the relevant potential pathways and drivers of AMR in the environment (Singer et al., 2016). Thus, there is a need to understand the risk associated with relevant pathways of transmissions in the wider community spaces, where both people and animals interact with the environment.

This study examined human and animal exposure to ESBL *E. coli* and *K. pneumoniae* in public spaces in both urban and rural settings in Southern Malawi. Using the principles of the SaniPath tool (Raj et al., 2020), we aimed to understand the practices contributing to environmental contamination and exposure in community settings, the prevalence of ESBLs in community environments and the effects of seasonality.

2. Materials and methods

2.1. Study setting

This study was undertaken across three study sites in Blantyre and Chikwawa districts in southern Malawi, as part of the Drivers of Resistance in Uganda and Malawi (DRUM) research consortium (Cocker et al., 2022b). In Blantyre, the study was conducted in Ndirande (urban) which is characterised by high population density and informal settlements, and Chileka (peri-urban) characterised by moderate population density with both formal and informal settlements. Chikwawa (rural) is characterised by low population density with dispersed and clustered settlements. The different geographical dynamics provide an overview of the level of risk to AMR exposure within these different community based public spaces. Due to the large scale of the study sites (Cocker et al., 2022b) each site was further fractionated into three sections (nine in total) to provide a suitable sample area while maintaining representation of the three locations (Fig. 1). The fractions targeted were under the responsibility of different community leaders within the study sites.

2.2. Study design

To assess the relevant pathways of ESBL-enterobacteriaceae (ESBL-E) in the communities, this longitudinal study used a triangulated mixed-methods approach (qualitative and quantitative techniques) in two phases:

1. Preliminary assessment (September 2020);
   - (a) Key informant interviews.
   - (b) Transect walks.
2. Monthly assessments (September 2020–August 2021)
   - (a) Transect walks.
   - (b) Environmental sample collection.

Environmental sample collection focused on two key AMR human pathogens in our exposure pathways; ESBL *E. coli* and ESBL *K. pneumoniae* as these bacteria are resistant to third generation cephalosporins, key first line agents in the empiric management of suspected severe bacterial infections in these settings (Lester et al., 2020). Monthly assessments of ESBL presence from September 2020 to August 2021 enabled us to determine how seasonality potentially affected the presence of ESBL *E. coli* and ESBL *K. pneumoniae* in exposure pathways. Data collection tools and procedures were pre-tested in the urban study site. Data related to household practices in these study sites has been published elsewhere (Cocker et al., 2023; Sammarro et al., 2022).

2.3. Data collection

2.3.1. Phase 1: preliminary assessment

As per the SaniPath preliminary assessment (Raj et al., 2020), Phase 1 involved (i) choosing target neighbourhoods (as described above) (ii) interviewing key informants (iii) performing transect walks (iv) selecting relevant pathways and (v) preparing the preliminary assessment report.

2.3.1.1. Key informant interviews. Key informant interviews (KIIs) were conducted over three days in each study site to identify perceived areas of potential environmental contamination associated with both infrastructure and practices.

Nine community leaders (one from each area section) (Group Village Heads (GVH) or Village Heads (VH)) were purposively sampled for the KIIs with guidance from the Community Advisory Groups (CAG); a local committee that facilitates research mobilisation at a local level.

Open ended questions were used to understand how sanitation practices, and available facilities and infrastructure, potentially contribute to contamination of public spaces in the each setting. Each interview took 30–50 min at the participant's residence. With permission from the participants, the interviews were audio recorded in Samsung Galaxy Tab A7.0 2016 tablets and notes were taken for translation and transcription.

2.3.1.2. Transect walks. Preliminary transect walks followed the KIIs, to identify potential exposure pathways to AMR. Transect walks in each fractionated area section took three to four hours to complete and it took three days to complete each study site. We used the principles of the SaniPath tool (https://sites.google.com/view/sanipathwiki) (Raj et al., 2020), although researchers were not accompanied by community representatives to reduce social desirability bias where leaders might consciously and unconsciously guide the transect walks away from problematic areas to present a more favourable view of the sanitation conditions than what actually exist. To qualify as an exposure pathway, we observed if there was a likelihood of either people or animals coming into contact with identified area. We used the SaniPath ten potential exposure pathways (for example, surface water, open drains, raw produce, ocean water and shared public latrines) as a basis, and compared the standard list with the pathways identified in the DRUM household study (e.g., animal stool, drains, and other contact surfaces) (Cocker et al., 2022b). Our finalised list of pathways was used during sample collection in the monthly assessments. The walking track was recorded through the Map My Tracks application (https://www.mapmytracks.com/outfront) and recorded on video camera for easy identification, and replication of walking routes in the consecutive months. GPS locations for identified risk pathways and sample sites were recorded for subsequent sampling.

2.3.2. Phase 2: monthly assessments

Following the initial transect walks, the subsequent monthly assessments utilised the recorded walking tracks and GPS locations for easy identification of risk pathways. Samples were collected from critical points on the identified exposure pathways and were examined to determine the presence/absence of ESBL *E. coli* and ESBL *K. pneumoniae*. In this study, critical points of sample collection were described as points of exposure for people and/or animals within a transect walk e.g., a point that is used as a footpath along the drain. Due to resource availability and as informed by initial assessments, a total of 120 samples per month ($n = 40$ in each site/one sample per pathway) were collected, for a period of 12 months.

Drain and standing water samples were obtained via sterile Thermo Scientific™ Sterilin™ Polypropylene 30 mL Universal Containers. Two bottles were used, one for collection and the other for storing the sample. Soil samples were collected using two sterile boot socks which were worn over a sample collector's shoe, one boot sock was used to prevent the collector's shoe from contaminating the sample and the outer boot sock was used to collect the sample. The sample collector then randomly walked over the critical point of an exposure pathway to capture traces

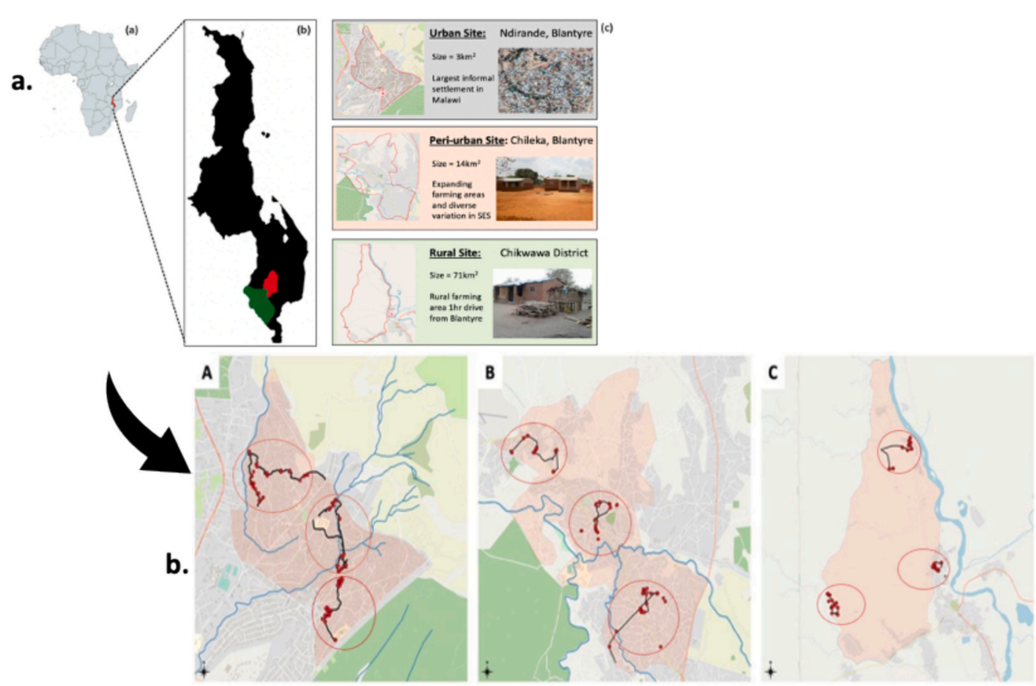

Fig. 1. **a.** Malawi study sites in the DRUM consortium (Cocker et al., 2022b) **b.** Study sites further fractionated into area sections in the (a) Urban (b) Peri-urban and (c) Rural study sites.

of potential contamination. The outer boot sock was then removed and carefully stored in a Whirl-Pak® sampling bag (Cocker et al., 2022a). Lastly, areas of frequent contact such as borehole handles were sampled by swabbing using 3 M™ Sponge-Stick swabs (Cocker et al., 2022a).

All sample forms were completed electronically using the KoBo-Collect application (https://www.kobotoolbox.org/) with the geo-locations of the sample sites also saved. Samples were then transported to the laboratory in a cooler box at 2–8 °C, and processed within four hours.

2.3.3. Rainfall and temperature data

The rainfall and temperature data utilised in this study were requested from the Department of climate change and meteorological services in Malawi.

2.4. Data management and analysis

2.4.1. Qualitative analysis

The research team manually transcribed, translated (from local language (Chichewa) to English), and analysed the qualitative data in Microsoft Word 2016 using thematic analysis (Downe-Wamboldt, 1992). The research team reviewed the transcripts recurrently and thematic areas of interest were identified. The qualitative results were integrated with the quantitative findings at interpretation stage to expose practices which may be contributing to contamination of identified exposure pathways.

2.4.2. Quantitative analysis

Microbiological analysis of the samples was used to determine the phenotypic presence of ESBL *E. coli* and ESBL *K. pneumoniae*. In brief, all microbiological samples collected were pre-enriched in buffered peptone water (BPW) and placed in an aerobic incubator for 18-24 h at 37 ± 1 °C to improve recovery of gram-negative organisms. In addition, water samples underwent membrane filtration prior to the addition of BPW. All samples were then plated onto ESBL CHROMagar™ and placed in an aerobic incubator for 24 h at 37 ± 1 °C before being examined. ESBL *E. coli* were speciated with CHROMagar™ methods alone and PCR was undertaken on blue colonies to determine the presence/absence of ESBL *K. pneumoniae.* Details of these techniques can be found in the protocol paper and associated SOPs (Cocker et al., 2022a, 2022b).

Transcribed sample results were entered using Microsoft Excel 2016 and descriptive univariate and bivariate analysis were conducted using SPSS 28.0 (IBM Statistics for Windows, Armonk, NY: IBM Corp) to describe ESBL presence in the exposure pathways across the three study sites. Results were presented in graphs and tables as percentages and frequencies. Then, One-proportion *Z*-test was performed to test the statistical significance of ESBL presence across study sites, using 95 % confidence intervals (95 %-CI). Chi-square goodness of fit test was used to compare the distribution of ESBL between different groups of risk pathways, study sites and ESBL species. A logistic regression study was then run to infer the effect of: study site, exposure pathway, rainfall, monthly minimum temperature, monthly maximum temperature with the covariates adjusted to account for scaling (log) and categorical values, on ESBL presence. An independent logistic regression was run for each bacteria species, and the Logistic regression Coefficients are reported as LC. The rainfall and temperature data used in this study were obtained from the Department of Climate Change and Meteorological Services.

2.5. Ethical consideration

Ethical approval was obtained from Kamuzu University of Health Sciences Research Committee (#P. 11/18/2541). Written informed consent for adult participants in the study was obtained from community leaders. Furthermore, written informed consent for photographs was obtained from community members who were captured in photographs or video material.

3. Results and findings

3.1. Preliminary assessment/contributing practices to environmental contamination

Outcomes from the preliminary assessment highlighted the status of sanitation and hygiene from the community leader's perspective in their respective settings (S1). A total of nine interviews were conducted with community leaders, one interview in each section, of which 56 % ($n = 5$) were male.

3.1.1. Availability and access to WASH facilities

All community leaders reported adequate access to safe water in their respective communities. Safe water sources reported consisted of piped household water and public taps (kiosks) in the urban setting, and piped household water, public taps (kiosks) and boreholes in peri-urban and rural settings. Leaders also reported availability of sanitation facilities in the form of pit latrines (which were the majority across all sites) and flush toilets in urban and peri-urban settings. However, there were concerns raised over the poor condition of both the sanitation and hygiene infrastructure in all settings, particularly pit latrines, which did not fully contain faecal matter due to overflow and leakages, especially in the rainy season, and bathing shelters which did not have effective drainage systems resulting in standing water within household and public environments. Shared sanitation facilities were common in all study settings, attributed to inadequate financial and technical resources, and specifically a lack of available land for toilet construction due to high population density and unplanned housing in the urban area. Households without toilet facilities were said to use neighbour's toilets or those of close family members in the urban setting. Multiple tenants within a compound were commonly found using one sanitation facility.

> "*We could say every household has a latrine, however, there are some less privileged households that do not have a latrine, but these are usually within a compound with close relatives or neighbours who let them use their latrines.*"
>
> -Community leader, Chikwawa

3.1.2. Environmental health practices

Poor environmental health practices such as improper disposal of both solid and faecal (animal and human) waste was reported to contribute to environmental contamination in the different study sites. Domestic solid waste in urban Ndirande was said to be disposed of in open dumping sites and in rivers. This was attributed to insufficient land for rubbish pits and lack of waste collection services from the local authority, caused by poor road networks resulting from unplanned housing in the informal areas.

> "*Households usually dispose of their waste in Nasolo River, or in Mudi Forest owned by Blantyre Water Board who came to encourage household members to dig rubbish pits. But if you look at the type of housing in this community, there is no space left for rubbish pits in household compounds, so people are still compelled go to the forest or Nasolo.*"
>
> -Community Leader, Ndirande

In *peri* urban Chileka, leaders highlighted that some households have rubbish pits, others use private waste collection services, while there were also households that disposed of their domestic waste in the river. In rural Chikwawa, households had rubbish pits and most waste was burnt within the household yard or in the pit. However, community leaders reported poor animal waste management in the region as most households owned multiple animals that roam around the community, and whose faecal waste contaminated both the household and wider community environment. These animals were also kept indoors overnight for security purposes. Consistent with household level data from the wider DRUM study (Cocker et al., 2023), when asked about the community member's contact with rivers, leaders in all three study sites highlighted that their community members had regular contact with river water for bathing/playing (mostly children) washing clothes, sourcing sand and water collection for household laundry, cleaning, and bathing.

3.1.3. Perceived risk of pathogen transmission

Community leaders indicated a high degree of perceived risk for disease transmission (e.g., cholera, schistosomiasis, diarrhoea) from contact with open water sources, and animals in all study sites. There was no perceived risk of AMR transmission from contact with open water sources.

> "*Yes, children usually play and swim in the rivers when their parents are washing clothes, but the problem is when they play with the river water for two-to-three times, they end up getting Schistosomiasis...*"
>
> -Community Leader Chileka

However, keeping animals indoors (inside a family house) was mainly for security reasons and there was no perceived risk of disease or AMR transmission related to this practice.

3.2. Community risk and exposure pathways

To identify the location of potential exposure pathways (for humans and animals), a risk profile was developed for each region as observed during the preliminary transect walks. Eight potential pathways for risk exposure were identified (Table 1), with exposure being through either (i) contact (i.e., touching or stepping on a pathway), and/or (ii) ingestion (i.e., direct eating or drinking from a risk pathway).

The identified exposure pathways were then grouped into four types of samples as described in Table 2 by study site. Animals and river water were not sampled in this research work as these have been reported elsewhere; which illustrated very high levels of ESBL contamination (Cocker et al., 2023; Sammarro et al., 2022). Fig. 2 shows examples of exposure pathways in a community setting.

3.3. Microbiological sampling

Risks associated with potential exposure pathways were validated through a total of 1440 samples collected between September 2020 and August 2021. These comprised of drains (45.4 %, $n = 654$), standing water (34.8 %, $n = 501$), environmental swabs (9 %, $n = 130$) and soil samples (10.8 %, $n = 155$), spread across 40 sampling points in each study site (Fig. 1).

3.3.1. Distribution of samples across exposure pathways

The distribution of exposure pathways from which samples were collected varied across study sites. Open drains were more prevalent in the urban location (46.3 %) and were associated with visible latrine leakage, standing water was more frequently seen in the rural location (41.5 %), while soil (51.0 %) and high-risk environmental areas (47.7 %) were most prevalent in *peri* urban Chileka (Table 3).

Sampling frequency of each exposure pathway was iterative and dictated by the prevalence of specific risks during the monthly sampling period. For example, the rainy season would see higher levels of standing water compared to the dry season and could be associated with a higher risk of faecal contamination due to flooding of latrines.

Table 1
Exposure routes for observed exposure pathways.

Pathway	Type of exposure			
	Contact		Ingestion	
	Human	Animal	Human	Animal
Drain water	x	x		x
Standing water	x	x	x	x
Public taps/boreholes (hand contact)	x			
Waste disposal sites	x	x		x
Soil	x	x	x	x
Animals	x	x		
Bathing shelter wastewater	x	x		x
River water	x	x		x

Table 2
Contributing sources of exposure pathways.

Exposure pathway	Sources per location		
	Urban	Peri-urban	Rural
Drain water 	Greywater Storm water run-off Water source run-off Latrine leakage	Greywater Storm water run-off Water source run-off	Grey water Storm water run-off Water source run-off
 Standing water	Unprotected well Grey water (e.g., bathing shelters) Flooded storm water Clogged drains Latrine leakage	Unprotected wells Flooded storm water Clogged drains Broken water pipes	Grey water (e.g., bathing shelters) Flooded storm water Clogged drains Animal drinking place
Soil 	Public play areas Waste dumping sites Footpaths (human)	Public play areas Waste dumping sites	Footpaths (animal and human)
 Environmental high-risk areas	Grocery shop serving areas	Borehole handles	Borehole handles

3.3.2. Presence of ESBL E. coli and ESBL K. pneumoniae in the local environment

Overall, 50.6 % (CI: (47.2 %, 53.9 %), $p < 0.001$) of the total samples contained ESBL *E. coli* or ESBL *K. pneumoniae*. Of these, 28.5 % (CI: (25.4 %, 31.5 %), p < 0.001) contained ESBL *E. coli only,* 10.5 % (CI: (8.4 %, 12.6 %), p < 0.001) contained ESBL *K. pneumoniae only,* and 11.6 % (CI: (9.4 %, 13.8 %), p < 0.001) had both ESBL *E. coli* and ESBL *K. pneumoniae* present. The proportion of ESBL varied across study sites (X^2, $p = 0.004$), with the majority of samples in the urban setting culturing ESBL bacteria (68.1 %, CI: (64.0 %,72.3 %)) compared to the peri-urban (42.1 %, CI: (37.7 %, 46.5 %)) and rural locations (41.4 %, CI: (37.1 %, 45.9 %)). ESBL *E. coli* was the most prevalent bacteria across all study sites (Fig. 3).

Prevalence of ESBL bacteria also varied between exposure pathways (X^2, p < 0.001), with the highest presence in drain samples (58.8 %, 95 %-CI: (55.1 %,62.6 %), p < 0.001) and lowest presence in environmental swabs (9.2 %, 95 %-CI: (4.3 %, 14.2 %), p < 0.001). ESBL *E. coli* was the predominant species found in all sample types, while ESBL *K. pneumoniae* was more prevalent in soil samples compared to the other sample types. Detailed variations in ESBL presence in the exposure pathways across the three study sites has been shown in Fig. 4.

Fig. 2. (a) community member accessing water from an open well (standing water) (b) pigs drinking from an open drain, (c) children drinking and playing with standing water (d) animal at a dumping site(soil) and (e) A community member accessing water from a borehole.

Table 3
Distribution of sample types per site.

Site	Sample Type (n (%))				Total (n)
	Drain	Standing water	Soil	High risk Environments	
Rural	183 (37.5)	208 (43.3)	30 (6.3)	59 (12.3)	480
Peri-urban	168 (35.0)	171(35.6)	79 (16.5)	62 (12.9)	480
Urban	303 (63.1)	122 (25.4)	46 (9.6)	9 (1.90)	480
Total	654 (45.4)	501(34.8)	155 (10.8)	130 (9.0)	1440

3.3.3. Seasonal relationship of ESBL prevalence in the environment

We studied ESBL prevalence across the three seasons (hot-dry, hot-wet, and cool-dry season) over the 12-month period (S2). The ESBL prevalence varied across the seasons (X^2, p = 0.004), with more ESBL contamination observed in the two hot seasons, [hot-dry season = 55.8 % (CI: (50.7 %,61.0 %)) and hot-wet season = 52.0 % (CI: (48.0 %,56.0 %))], compared to the cool season [44.8 % (CI: (40.3 %,49.2 %))] (Fig. 5).

When comparing exposure pathways across the three seasons we found that only standing water ($p = 0.01$) was associated with a statistically significant difference, with the hot seasons generally associated with a higher prevalence (S3).

Effects of the study site, exposure pathway, rainfall, monthly minimum, and maximum temperatures on the prevalence of ESBL *E. coli* and ESBL *K. pneumoniae* were examined. Here, we found that urbanisation has a positive effect on both ESBL *E. coli* (LC: +0.70) and ESBL *K. pneumoniae* prevalence (LC: +0.63) (Table 4). Conversely, sample type has a negative effect on both ESBL *E. coli* (LC:−0.42) and ESBL *K. pneumoniae* prevalence (LC:−0.28), with drier samples, i.e. environmental swabs and soil being more likely to test negative for ESBL than those from moist environments such as drains and standing water. Rainfall had a positive effect on *E. coli* prevalence (LC:+0.19), but was not associated with *K. pneumoniae*, and could be attributed to rain promoting the expanded dispersion of human faecal waste within exposure pathways. However, maximum temperature had a positive effect on

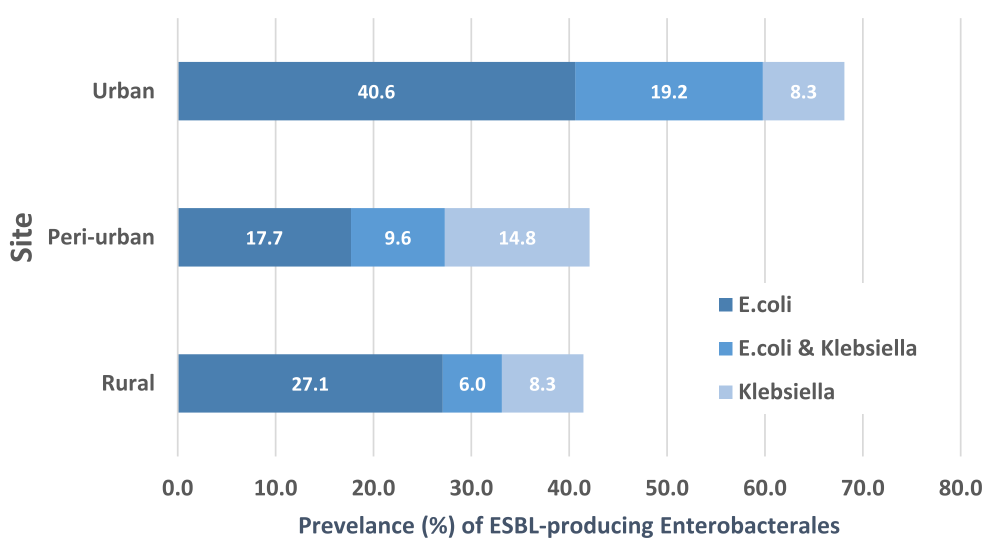

Fig. 3. Prevalence of ESBL Enterobacterales (*E. coli* & ESBL *K. pneumoniae*) identified in the study sites, subcategorised by bacterial species.

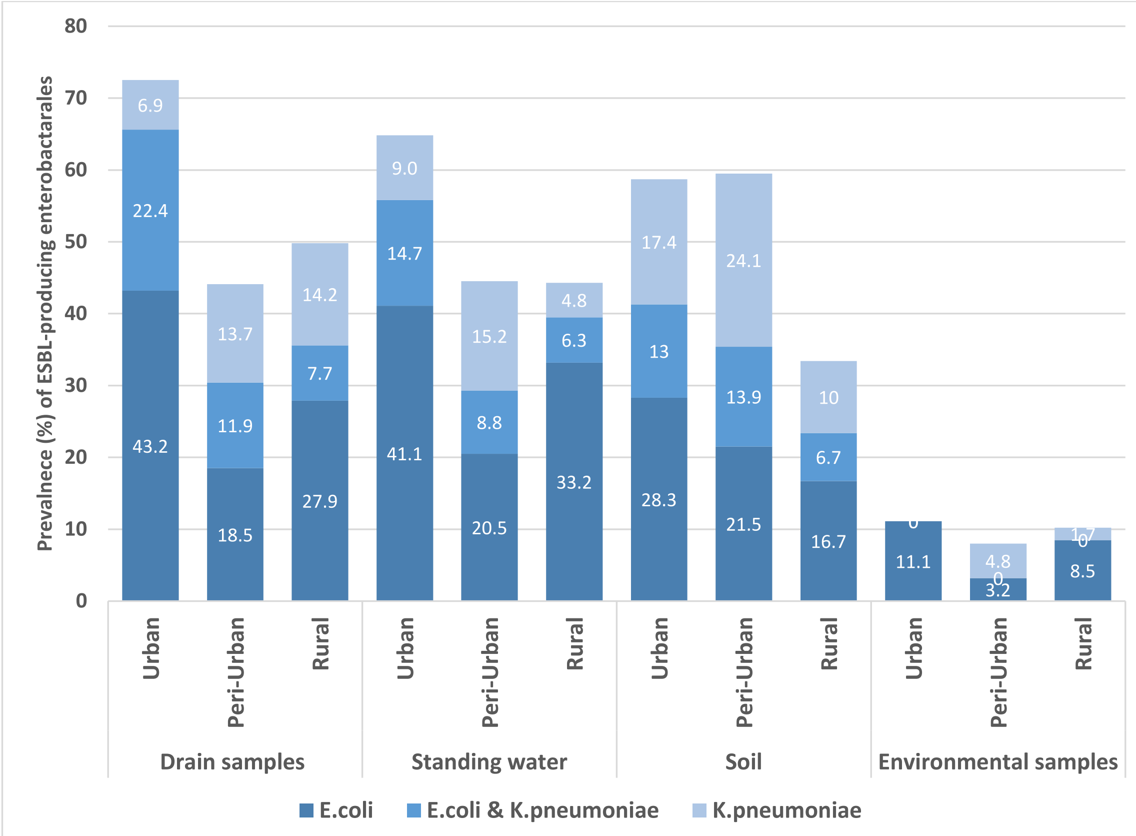

Fig. 4. Prevalence of ESBL Enterobacterales (E.coli & ESBL K.pneumoniae) subcategorised by bacterial species and stratified by exposure pathway and setting.

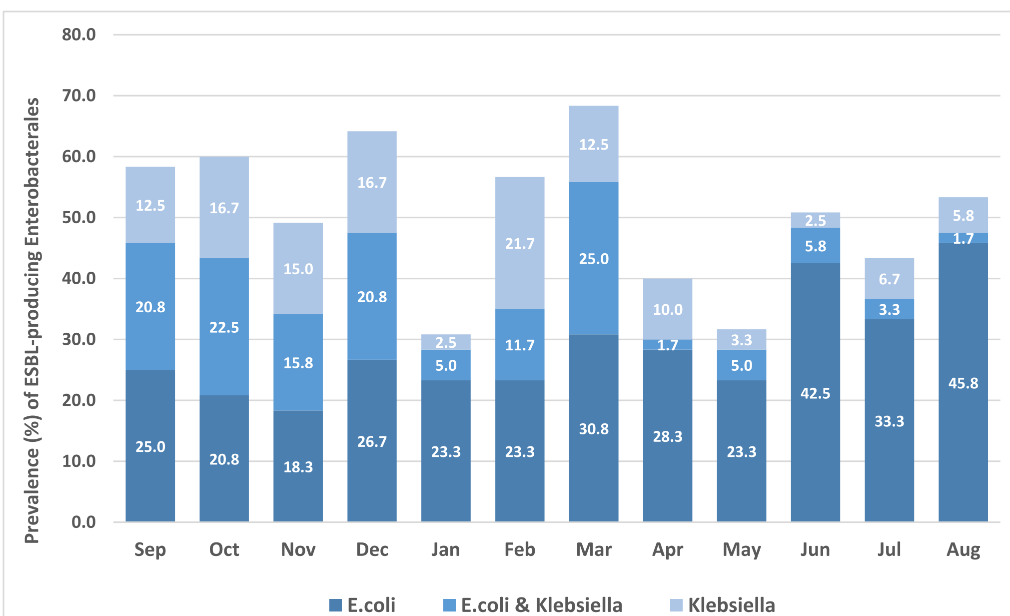

Fig. 5. ESBL positivity across 12 months of sampling (September 2020 – August 2021).

both *E. coli* (LC:+2.34) and *K. pneumoniae* (LC:+2.85), and the average minimum temperature had a negative effect on *E.coli* (LC:−2.23) and positive effect on *K. pneumoniae* (LC:+2.85). These results indicate that *E. coli* and *K. pneumoniae* prevalence is greater on hot days, with *E. coli* prevalence also susceptible to broad swings in temperature variations, highlighting that environmental AMR is a climate sensitive issue in this setting.

4. Discussion

The SaniPath tool has been recommended for assessing the risk of exposure to faecal contamination in public spaces in low-resource urban

Table 4
ESBL prevalence as affected by different factors using logistic regression models for *E. coli* and *K. pnuemoniae*. (LC = Logistic regression Coefficients).

Variable	*E. coli*		*K. pneumoniae*	
	LC	*p*-value	LC	*p*-value
Study site	+0.70	<0.001	+0.63	<0.001
Sample type	-0.42	<0.001	-0.28	=0.001
Rainfall	+0.19	<0.001	+0.04	=0.437
Min Temp	-2.23	<0.001	+1.79	=0.003
Max Temp	+2.34	<0.001	+2.85	<0.001

settings, and within this study, we have extended that application to assess the potential risk of exposure to ESBL *E. coli* and *K. pneumoniae* in urban, peri-urban, and rural settings. We identified open drains, standing water, soil, and areas of frequent communal contact as the key potential exposure pathways to AMR across all study sites. Microbiological evaluation of these pathways was focussed on open drains, standing water, soil, and areas of frequent communal contact as other key pathways were assessed separately (Cocker et al., 2023). Our longitudinal assessment over 12-months identified extensive ESBL contamination of drains, standing water and soil in public spaces, and increased ESBL prevalence associated with both urbanisation and seasonal effects of increased rainfall and temperature. This aligns with other reported findings from a household study conducted in the same settings (Cocker et al., 2023) where high levels of human ESBL-producing *E.coli* and *K pneumoniae* colonisation were associated with urbanisation and the wet season. Our findings highlight the key ESBL exposure routes to both people and animals, alongside risks posed by high levels of AMR bacteria in the different exposure pathways across each site throughout the year.

These exposure pathways are in line with other reported AMR and faecal exposure pathways across Africa, Asia and some high-income countries (Banu et al., 2021; Ogbonna et al., 2018; Okaali et al., 2022; Raj et al., 2020; Robb et al., 2017; Ronoh et al., 2020; Wang et al., 2022). Alongside rivers, animal faeces and food produce (Cocker et al., 2023) drains and standing water were the predominant risk pathways in urban and rural settings respectively, linked to poor infrastructural design and practices, as reported by community leaders. Community leaders in Malawi associated contact with river water and drain water to other diseases such as cholera, schistosomiasis, and diarrhoea but not as transmission routes of AMR. This lack of association between general environmental exposure and the risk of contracting resistant infections emphasises the need for increased community education that targets behavioural determinants associated with AMR exposure and indicates a potential key area for intervention (Tull et al., 2023).

Overall, ESBL-E pathogens were identified in all risk pathways in the community environment with ESBL *E. coli* more prevalent than *K. pneumoniae*, suggesting that Enterobacteriaceae species have different ecological niches. Therefore, a holistic approach to environmental hygiene is required, which is able to target different drivers of bacterial contamination within key exposure pathways. In this study, the high prevalence of ESBL *E.coli* identified could be associated with increased faecal contamination arising from latrine and bathing shelter leakages (Table 2), which are sources of most drains and standing water pathways. Furthermore, the higher AMR burden in the urban setting is likely to be contingent on the informal nature of urban settlements in low-income countries, which have limited access to sanitation infrastructure and services, as highlighted by the community leaders in this study in line with other studies in similar settings (Allcock et al., 2017; Nadimpalli et al., 2020; Zhu et al., 2022). This can result in unsafe containment of wastewater and faecal sludge that leaks and/or overflows into drains and other surface water bodies that are in close proximity with homes and highly frequented public spaces in urban settings (Collignon et al., 2018; Larsson and Flach, 2021; Macintyre et al., 2017; Musoke et al., 2021; Woolhouse et al., 2015). These results indicate that environmental AMR is associated with the state of water, sanitation and waste infrastructure and management in low-income settings (Cocker et al., 2023; Collignon et al., 2018; Fuhrmeister et al., 2023), and could contribute to the local burden of human AMR in Malawi (Lester et al., 2022).

Higher levels of *K. pneumoniae* were detected in soil samples than in other sample types. Soil can serve as a reservoir for various microorganisms, including *K. pneumoniae*, due to its ability to provide nutrients and favourable conditions for their survival (Stanley et al., 2022). The higher levels of *K. pneumoniae* in these samples suggest that human-and animal-effluent contaminated soil may have a role in the persistence and distribution of AMR in a community environment (Musoke et al., 2021; Samreen et al., 2021) and should be considered as a potential reservoir and target for AMR surveillance.

Different reasons were attributed to the cause of poor sanitation infrastructure across community settings, and it is important to understand context-specific factors that influence environmental health in low-income countries. Community leaders cited a lack of land for proper latrine construction in the urban site, while inadequate financial and technical resources were highlighted in the rural area, both of which translate into poor sanitation access and standards, which limits households ability to move up the sanitation ladder (UNICEF, 2020). This emphasises the need for context appropriate sanitation standards if we are to eliminate the AMR burden in Malawi, and similar settings. It is now generally accepted that "*toilets alone are not the solution*", but rather the safe containment and disposal of faecal waste for treatment, to avoid re-entry of faecal waste into the domestic environment (Ercumen et al., 2017; Russel et al., 2019; United Nations, 2016). Therefore urban communities could adopt innovations such as the container based sanitation and the communal sanitation systems which have been implemented in Kenya and South Africa; whose associated benefits are high household/population coverage and safe management of faecal sludge, alongside other added benefits such as cost-effectiveness (Beukes et al., 2017; CBSA, 2019; Navarro, 1994; Roma et al., 2010; Russel et al., 2019; Wilburn, 2016).

There is also a need ensure that sanitation and hygiene solutions are resilient to environmental impacts and climate change in both the urban and rural communities (UNICEF, 2020). We observed a significant seasonal impact on the presence of ESBLs in all study sites and the identified risk pathways. Our seasonal analysis found higher ESBL contamination in the hot seasons and the regression analysis showed that increased rainfall was associated with increased ESBL *E.coli* contamination. The analysis further reveals an increased prevalence of *K. pneumoniae* in hotter days, while ESBL *E. coli* is more prevalent in cooler days, supporting observed seasonal trends (S4). The findings suggest that ESBL prevalence is a climate sensitive issue as different climatic factors such as rainfall and temperature have different impacts on the survival, spread and persistence of ESBL-producing bacteria in a community environment (Bashawri et al., 2020; Bock et al., 2022). We also conclude that differences in ESBL prevalence across seasons may be attributed to study specific factors such as type of exposure pathways, sample collection frequency, and geographical location.

The risk pathways in this study were sampled once in a month, however, other researchers should consider assessing ESBL presence at short intervals considering the variability of ESBLs in the sampled environments. Additionally, the study did not include other identified pathways such as food, drinking water, and river water, as these are more linked to household environments, nevertheless, we captured these pathways in another study in the same context but at a different time point (Cocker et al., 2023; Sammarro et al., 2022). We believe the risk pathways highlighted in this study offer valuable insights into communal risk pathways of AMR.

5. Conclusion and recommendation

Our study provides valuable insights into the risk pathways of AMR within community environments of urban, peri-urban, and rural settings of Malawi using the principles of the SaniPath tool in conjunction with supporting evidence from longitudinal microbiological analysis. A paucity of WASH infrastructure and practice was shown across the study areas, particularly in the urban setting. Drains, standing water, soil and areas of frequent communal contact were highlighted as significant risk pathways. High rates of ESBL Enterobacteriaceae were identified in all risk pathways, and the level of environmental ESBL contamination was impacted by climatic conditions and the degree of urbanisation. These results highlight the need to prioritise investments in WASH through improved environmental health standards that target a whole system One-Health approach which tackles infrastructure, behaviour, and is context specific in nature as stipulated in the Malawi National Action Plan on AMR (WHO, 2022). Only by addressing these issues can we effectively mitigate the transmission of AMR in these settings, and hope to contribute to better public health, social and environmental outcomes as well as broader health systems strengthening.

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CRediT authorship contribution statement

Taonga Mwapasa: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Kondwani Chidziwisano:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Madalitso Mphasa:** Writing – review & editing, Formal analysis. **Derek Cocker:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Lorenzo Rimella:** Writing – review & editing, Formal analysis, Data curation. **Stevie Amos:** Writing – review & editing, Formal analysis. **Nicholas Feasey:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Tracy Morse:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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