MAJOR ARTICLE

Risk factors, temporal dependence, and seasonality of human ESBL-producing *E. Coli* and *K. Pneumoniae* colonisation in Malawi: a longitudinal model-based approach.

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Background: Sub-Saharan Africa has the highest estimated death rate attributable to antimicrobial resistance (AMR), especially due to Extended-Spectrum Beta-Lactamase-producing Enterobacterales (ESBL-E). However, the dynamics of human colonisation in the community with ESBL-E are not well described. Inadequate water, sanitation and hygiene (WASH) infrastructure and associated behaviours are thought to play an important role in

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transmission of ESBL-E, and an improved understanding of the temporal dynamics of within-household transmission could help inform the design of future policies.

Methods : In this 18-month study, using microbiological data and household surveys, we built a multivariable hierarchical harmonic logistic regression model to identify risk factors for colonisation with ESBL-producing *E. coli* and *K. pneumoniae*, reflecting household structure and temporal correlation of colonisation status.

Results: Being male was associated with a lower risk of colonisation with ESBL-producing *E. coli* (OR 0.786 CrI[0.678-0.910]) whilst the use of a tube well or a borehole was associated with an increased risk (OR 1.550 CrI[1.003-2.394]). For ESBL-producing *K. pneumoniae*, recent antibiotic exposure increased risk of colonisation (OR 1.281 CrI[1.049-1.565]) while sharing plates decreased that risk (OR 0.672 CrI[0.460-0.980]). Finally, the temporal correlation range of eight to eleven weeks provided evidence that within-household transmission occurs within this time frame.

Conclusions: We describe different risks for colonisation with different enteric bacterial species. Our findings suggest interventions to reduce transmission targeted at the household-level need to focus on improving WASH infrastructure and associated behaviours, whilst at the community level they should focus on both environmental hygiene and antibiotic stewardship.

Keywords: antimicrobial resistance, Africa, community

INTRODUCTION

In 2015, the World Health Organisation declared antimicrobial resistance (AMR) as a priority public health threat [1, 2]. The latest estimates showed that 4.95 million deaths were associated with bacterial AMR in 2019 [3]. The attributable death rate was highest in sub-Saharan Africa (sSA), where leading pathogens included *Klebsiella pneumoniae* and *Escherichia coli*, however large gaps in data availability were noted [3]. Of particular note has been the rapid emergence of Extended-Spectrum beta-Lactamases (ESBL) in gram-negative bacteria [4]. Studies have shown that the prevalence of infections caused by ESBL-producing Enterobacterales (ESBL-E) in sSA is high, with prevalence varying between 5 and 84%, with a relationship to setting [5, 6]. A separate 2019 review found a median prevalence of resistance of 14.3% in *E. coli* and 46.7% in *Klebsiella spp.* in Eastern Africa[7]. Learning more about asymptomatic colonisation, a key step prior to infection in vulnerable patient groups, is therefore crucial to prevent transmission, and consequently, reduce drug-resistant infections.

Prior to 2016, no studies described risk factors for colonisation among healthy individuals in the community in sSA [5]. Antibiotic use, age, hospital admission and income have since been identified in community-based studies [8-11]. However, most of them focused on a specific subset of the community, therefore we cannot be confident these risk factors are generalisable

across the population. Moreover, although it is believed that inadequacies in water, sanitation and hygiene (WASH) infrastructure and associated behaviours play an important role in transmission of AMR-bacteria [12, 13], risk factors related to WASH in this context are not well described.

To be able to reduce transmission of, and colonisation by, AMR-enteric bacteria in sSA, we need to explore the dynamics of colonisation in order to tailor appropriate interventions; for example the role of seasonality. A first step in understanding the temporal dynamics of within-household transmission is to determine how long a household is at risk of colonisation once one member has been colonised. This will help inform the design of public health policies that interrupt transmission of AMR-bacteria.

Here, we describe an analysis of an 18-month longitudinal cohort study using microbiological, household and WASH surveys, where WASH refers to water, sanitation (containment of human and animal faeces), hygiene and food hygiene. We fit a serially-correlated generalised linear mixed model, exploring household, individual and WASH risk factors for ESBL-E colonisation in settings with different degrees of urbanisation in Malawi.

METHODS

The Drivers of Resistance in Uganda and Malawi (DRUM) consortium was an interdisciplinary consortium studying AMR transmission in a One Health setting, working across urban, peri-urban and rural communities in Uganda and Malawi (www.drumconsortium.org). DRUM was a repeated-measures study in which individuals, clustered into households, were sampled at four timepoints over 6 months. Longitudinal human stool, animal and environmental sampling were used to isolate ESBL *E. coli* (ESBL-Ec) and ESBL *K. pneumoniae* (ESBL-K). Samples were cultured in enrichment broth (BPW) and placed in an aerobic incubator at $37 \pm 1°C$ for 18–24 hours. The samples were then plated onto ESBL chromogenic agar (CHROMagar™, France), cultured aerobically at $37 \pm 1°C$ for 18–24 hrs and read for growth of ESBL bacteria. Pink and white colonies were categorised as ESBL-Ec while blue colonies underwent speciation for *K. pneumoniae*, using PCR, to identify ESBL-K isolates. Throughout this paper, colonisation refers to asymptomatic carriage. The detailed protocol is available at [14].

This analysis focuses on the Malawian areas: Ndirande (urban), Chileka (peri-urban), and Chikwawa (rural). We model the presence or absence of colonisation with ESBL-Ec and ESBL-K in individuals and aim to detect associations with individual-level demographic and health characteristics, household-level WASH indicators, and the social context represented by the study area. In order to capture seasonality and household structure, we used a hierarchical multivariable harmonic regression, with temporal correlation at the household level.

Covariate selection

In order to investigate WASH practices, reported variables (i.e. *presence* of a toilet) were collected by asking participants at baseline, whilst observed variables (i.e. *type* of toilet) were answered by field teams observing the household infrastructure. These variables were screened for importance by environmental health specialists (Morse, Chidziwisano) with expertise on the risks and control points for faecal-oral transmission in Malawi [15-17].

Individual- and household-level covariates were also selected. Antibiotic use was defined as the reported use of any antibiotics in the last six months (at baseline) and subsequently between each follow-up visit. The survey question was supported by the use of the "drug bag" method and also cross referenced in the participants health passports [18]. Cotrimoxazole prophylactic therapy was excluded to capture only the immediate change expected to occur following short courses of therapy.

Univariable logistic models were used to investigate the effect of WASH infrastructure and associated behaviours on colonisation. We started by looking at the effect of the study area on colonisation to separate its potential effect from the effects of other risk factors and ran the univariable models accordingly. This allowed for a refinement of the variables to be included in the modelling framework, by retaining them only if p-value <0.2. We pragmatically set this threshold to avoid missing the identification of important covariates. A spline model was used to detect non-linear effects of age, but none was found.

Multilevel harmonic hierarchical regression

The modelling framework is detailed in Supplementary Methods. Briefly, we used a hierarchical multivariable harmonic logistic regression, incorporating covariates selected by the univariable analysis. We included study region, annual and bi-annual harmonic terms to capture seasonality, and a serially-correlated household-level random effect to reflect temporal correlation in household-level prevalence between timepoints and heterogeneity in prevalence between households over and above that explained by the covariates. The analysis was carried out for both binary colonisation response variables. The Bayesian model was fitted using priors [19] and implemented in R v4.1.1 with RStan [20, 21].

Initial model exploration considered adding the random effect at the individual-level, but found no evidence of temporal correlation. We therefore applied the serial correlation to the household-level, enabling us to quantify both a household-effect and to account for possible "household contamination" with an ESBL as a result of a longer-term transmission process operating within the home. To ensure maximum identifiability of the random effects, we performed our analysis only on households with sample results for all time points.

Ethical approval was obtained from Liverpool School of Tropical Medicine Research and Ethics Committee, UK (REC, #18-090) and College of Medicine Research and Ethics Committee, Malawi (#P.11/18/2541).

RESULTS

Exploratory analysis

After covariate selection and cleaning, the dataset contained 2493 samples from 259 households (Supplementary Dataset, Supplementary Results). The list of covariates can be found in Supplementary Table 1. The distribution of samples, individuals, and households is presented in Table 1.

The 129 households for which individuals had four available samples were used for the hierarchical model. The distribution of samples over time can be found in Fig 1.

Participant data

Our age distribution data reflect Malawi's population structure. People were considered adults at ≥16 years (54.0%, 483/894) and school age was ≥5 to 15 years (27.1%, 242/894). 57.1% (510/894) of participants were female. At baseline, 15.2% (129/851) of participants reported having taken at least one course of antibiotics, while between subsequent visits, 6% (37/616), 9.4% (54/570) and 8.3% (38/456) reported it. This varied by region, with 15.4% (137/891) in Chikwawa, 9.3% (66/709) in Ndirande and 6.2% (55/893) in Chileka.

Overall, the prevalence of ESBL-Ec was 37.0% (922/2493) and the prevalence of ESBL-K was 11.9% (296/2493). Both varied over time, following a similar pattern (Fig 2). Some evidence of seasonality can be discerned for both, especially for *E. coli*, with a decrease in prevalence during the dry season (May-October) followed by an increase during the wet season (November-April).

Household data

Over 75% (202/259) of households had between 3 and 6 members, with a median of 4 individuals. 9% (24/259) had two individuals whilst 13% (33/259) had over 6 individuals. More than 50% (156/259) had a monthly income between 15000 and 50000 Malawi Kwacha (mwk). Thirty-eight households had less than 15000 mwk, whilst sixty-five households had over 50000 mwk. The median income was 30000 mwk.

WASH data

Correlations between household-level variables are depicted in Fig 3. There was a positive correlation between animal-exposure related factors, e.g. bird owners appeared more likely to keep animals inside, who were more likely to come into contact with food preparation areas.

With increasing income, there was increasing likelihood the household's water drinking source came from a pipe, rather than a tube well or borehole. Moreover, increasing income correlated with presence of hand washing facilities and soap in the household, and of cleaning materials near the toilet. Eating from shared plates appeared to be negatively correlated with these sanitation factors - the higher the household income is, the less chance individuals used shared plates.

Impact of WASH infrastructure and associated behaviours on ESBL colonisation

In order to start exploring the effect of WASH variables on ESBL colonisation, and to look at variability between regions, we first ran a generalised linear model including only the study area. There was a higher risk of being colonised in Ndirande (OR 1.39 CI[1.13-1.70];p=0.002) compared to Chileka. No significant effect was detected for ESBL-K. Consequently, univariable analysis was run with the study area included as a covariate for ESBL-Ec, but not for ESBL-K.

Human gut mucosal colonisation with ESBL-producing *E. Coli*

Results from the univariable models showed that having a drinking source coming from a tube well or a borehole, having a drop hole cover on the toilet and allowing animal contact with food areas appeared to be highly significant. Whilst a positive association with colonisation was detected for the source coming from a tube well or a borehole, the opposite can be said for piped water. Animal contact with food areas, keeping animals inside and having a toilet floor surfaced with soil were significant and positively associated with colonisation. Having a drop hole cover and having clean paper in the toilet were also significant but appeared to have a protective effect. Older age, visible open defecation in the area, owning cattle, sheep or goats, contact with river water were significant (<0.05) and positively associated with colonisation. Male sex, higher income, having a disposal mechanism for animal waste, having a piped water drinking source, storing water in a container with lid and tap were negatively associated with ESBL-Ec colonisation (Supplementary Table 2).

The hierarchical model showed that men are less at risk of becoming colonised with ESBL-Ec (OR 0.786 CrI[0.678-0.910]) and that having a tube well or a borehole as a drinking source increases your risk (OR 1.550 CrI[1.003-2.394]). Coming into contact with standing water appeared to be negatively associated with colonisation (OR 0.749 CrI[0.574-0.978]). Finally, an apparent signal of annual seasonality was noticeable from the presence of part of the harmonic term (Table 2).

Using the covariance structure (Supplementary Methods), the range of temporal correlation was estimated at 77.85 days (CrI[30.85-140.60]), thus samples from the same household obtained more than 77 days apart are effectively uncorrelated, meaning that an individual's ESBL status at 78 days is not influenced by their status at baseline (Supplementary Results).

Human gut mucosal colonisation with ESBL-producing *K. Pneumoniae*

Univariable models showed that household size was highly significant (p<0.01), revealing a greater risk of being colonised with increasing household size. Eating street food and eating from shared plates appeared to have a significant protective effect on colonisation. Contrarily, owning birds and coming into contact with drains were significant and positively associated with colonisation (Supplementary Table 3).

The hierarchical model for ESBL-K found that antibiotic use increased the risk of being colonised (OR 1.281 CrI[1.049-1.565]). Eating from shared plates was negatively associated with colonisation (OR 0.672 CrI[0.460-0.980]). Finally, a signal of annual seasonality was also detected (Table 3). The range of temporal correlation for ESBL-K colonisation was estimated at 54.29 days (CrI[12.91-130.43]).

DISCUSSION

This study identified varying prevalence of ESBL colonisation over time; with higher prevalence during the wet season. Potential explanations include the accumulation of mud and floodwater, which might lead to more contact with contaminated soil or water. Additionally, the increased time spent indoors during periods of heavy rain might lead to higher within-household transmission. The prevalence is consistent with previous studies on ESBL colonisation in sSA[5, 6, 22]. However, while most of these studies were from a specific subset of the population, our study focused on the general population in community settings. Comparing to community studies exclusively, we found a prevalence of 37% which is much higher than the previous estimate of 18% [95% CI 11–28%] for community members in sSA[5]. This prevalence is close to some of the highest reported in the world[6].

The correlation heatmap (Fig 3) suggested that the socioeconomic status of the household influences the household WASH situation, and that higher income allows for a better access to cleaner water and sanitation and hygiene products. Being female was identified as a risk factor for ESBL-Ec, perhaps because women are more likely to perform domestic duties i.e. laundry, housework, and childcare, which would place them at higher risk of being in contact with the faecally-contaminated environment. However, no direct association was found between income and colonisation here, perhaps because the vast majority of households in the study were below the threshold of absolute poverty and that income alone is a poor indicator of wealth.

Study area, keeping animals inside, and animal contact with food preparation areas also conferred an increased risk for colonisation with ESBL-Ec in the univariable models. These animal husbandry practices are common in sSA [23], however they put household members at higher risk of exposure to faecal pathogens and enteric infections [24, 25]. Having access to cleaning materials and a drop hole cover were negatively associated with colonisation. The latter

being used to prevent flies from accessing faecal matter, this association is consistent with studies showing the role of flies in transporting and transmitting *E. coli* [26, 27].

Eating from shared plates and the presence of standing water around the household appeared to have a protective effect. This is a surprising result, since plate-sharing, which is common in LMICs [28], has been associated with transmission of enteric pathogens in other settings [29]. Similarly, since wastewater is known to play a role in the transmission of AMR [12], both would seem to promote transmission. As with all statistical inference, we caution that these results might represent at Type I error (in a Bayesian context), and that further research into WASH behavioural patterns and interactions with other explanatory variables would be necessary to confirm our findings.

Antibiotic use was identified as a risk factor for ESBL-K, consistent with previous studies in sSA [5, 8-10]. In Malawi, antibiotics are widely available by formal and informal routes [30]. Lack of healthcare workers at many facilities results in day-long waiting times for patients, driving them to search for alternative ways to access antimicrobials, usually without consultation or prescription. Additionally, to alleviate waiting times and accommodate the high volume of patients, workers may dispense antimicrobials directly to patients without a prescription [31]. Therefore, the identification of antibiotic use as a risk factor may also reflect recent healthcare exposure. The reason for the variation between study areas is uncertain. In Chikwawa, the rural area of the study [14], participants are likely to have greater exposure to livestock, and may have had increased access to antibiotics through non-governmental organisations [32]. This emphasizes the importance of antimicrobial exposure in driving ESBL colonisation, thus highlighting the need for optimising community antibiotic usage.

For ESBL-K, at the univariable level, household size was the only highly significant risk factor, highlighting the importance of the household in driving ESBL transmission. Other significant variables included owning birds, which are known to be responsible for faecal contamination of the household environment in LMICs [33], and contact with drains, highlighting again the importance of interactions between animals, humans and environment.

Temporal models detected a temporal correlation range of 8-11 weeks. In other words, two samples taken within that time frame are more likely to both be colonised than if spread apart any further in time. Though our method was designed only to detect association between ESBL prevalence in subsequent follow-ups, it does suggest that within-household transmission occurs within this time frame. Subsequent causal inference studies would be required to confirm this. An understanding of how long colonisation lasts after acquisition is needed to tailor appropriate interventions.

This study had some limitations. Variables had to be pre-selected based on perceived importance. We could not find temporal correlation at individual-level, which suggested that an individual's samples could be seen as independent from each other. Potential explanations

include the use of stool samples over rectal swabs, which may have been better for screening. Additionally, the laboratory testing protocol was qualitative, discriminating only between presence or absence of ESBLs. Further work is needed to consider the impact of microbiological methods on informing these models. Whole genome sequencing will allow a thorough investigation of the linkage between sequence types. The COVID-19 pandemic caused the sampling and microbiological testing to be suspended, causing some delay in our data collection (April-July 2020).

Our study suggests that WASH factors and environmental hygiene are key drivers of AMR-transmission in Malawi, consistent with findings in other African settings [34]. Our results also point towards acquisition of ESBL-producing *E. coli* through contaminated water and/or inappropriate WASH infrastructure. Additionally, seasonality and gender also suggest the importance of environmental hygiene and practices in driving ESBL-producing *E. coli* transmission. This underlines the need for improved access to clean water and suggests that associating WASH behavioural practice with better WASH conditions would be instrumental in decreasing transmission. However, for ESBL-producing *K. pneumoniae*, antibiotic use was identified as a risk factor, therefore emphasizing the importance of antimicrobial exposure in driving transmission and the need for improved infection prevention and control measures and antibiotic usage and stewardship training. A better understanding of how the WASH factors of the different communities impact ESBL colonisation and transmission will inform public health responses to the challenge presented by AMR and enable design of effective intervention strategies in sub-Saharan Africa.

NOTES

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Table 1. Distribution of the number of households, individuals and samples

	Ndirande	Chikwawa	Chileka	Total
Households	96	64	99	259
Individuals	285	259	350	894
Samples	709	891	893	2493

Table 2. Temporal model results for ESBL-producing *E. coli* colonisation status

	Log-odds	Odds ratio (95% CrI)
(Intercept)	-0.716	0.489 (0.360-0.663)
Reactive to HIV testing (vs non-reactive)	0.027	1.027 (0.863-1.223)
Unknown HIV status (vs non-reactive)	-0.031	0.969 (0.808-1.163)
Recent use of antibiotics	0.093	1.097 (0.946-1.274)
Age	0.132	1.141 (0.970-1.343)
Being male (vs female)*	**-0.241**	**0.786 (0.678-0.910)**
Average household monthly income	0.226	1.254 (0.916-1.715)
Open defecation	0.054	1.055 (0.826-1.349)
Presence of a disposal mechanism for animal waste	0.104	1.110 (0.857-1.437)
Eating from shared plates	-0.245	0.783 (0.598-1.024)
Having a pipe as drinking water source	0.132	1.141 (0.818-1.592)
Having a tube well/ borehole as drinking water source	**0.438**	**1.550 (1.003-2.394)**
Use of alternative water for cleaning utensils	0.014	1.014 (0.802-1.283)
Owning cattle, goats or sheep	0.139	1.149 (0.892-1.480)
Keeping animals inside	0.075	1.078 (0.852-1.364)
Contact with river water	0.048	1.049 (0.791-1.391)
Toilet floor material: none (vs concrete/wood)	0.123	1.131 (0.799-1.600)

Toilet floor material: soil (vs concrete/wood)	0.131	1.140 (0.820-1.585)
Presence of drop hole cover on the toilet	-0.202	0.817 (0.626-1.067)
Presence of newspaper/paper in the toilet	-0.155	0.856 (0.670-1.094)
Frequency of soap presence in handwashing facilities	-0.000	1.000 (0.640-1.563)
Storing water covered	-0.179	0.836 (0.537-1.302)
Storing water in a container with lid/tap	-0.034	0.967 (0.754-1.240)
Contact between animal and food areas	0.218	1.244 (0.983-1.573)
Presence of standing water around the household	**-0.289**	**0.749 (0.574-0.978)**
Number of days since the first sample	0.167	1.182 (0.869-1.608)
Harmonic term (sinday)	**-0.466**	**0.628 (0.453-0.869)**
Harmonic term (cosday)	0.371	1.449 (0.958-2.191)
Harmonic term (sinday2)	-0.084	0.919 (0.644-1.314)
Harmonic term (cosday2)	0.018	1.018 (0.711-1.457)
Living in Chikwawa (vs Chileka)	-0.276	0.759 (0.527-1.093)
Living in Ndirande (vs Chileka)	0.386	1.471 (0.980-2.207)

*Significant variables highlighted in bold

Table 3. Temporal model results for ESBL-producing *K. pneumoniae* colonisation status

	Log-odds	Odds ratio (95% CrI)
(Intercept)	-3.432	0.032 (0.017-0.060)
Recent use of antibiotics*	**0.248**	**1.281 (1.049-1.565)**
Number of people living in the household	0.298	1.347 (0.932-1.947)
Presence of a toilet in the household	-0.136	0.873 (0.468-1.628)
Eating street food	0.091	1.095 (0.797-1.505)
Eating from shared plates	**-0.398**	**0.672 (0.460-0.980)**
Having a pipe as drinking water source	-0.253	0.776 (0.506-1.190)

Having a communal tap as drinking water source	-0.423	0.655 (0.408-1.051)
Use of alternative water for cleaning utensils	-0.241	0.786 (0.563-1.097)
Owning birds	0.015	1.015 (0.706-1.459)
Owning dogs or cats	0.024	1.024 (0.735-1.427)
Owning pigs	0.157	1.170 (0.853-1.604)
Contact with drains	0.219	1.245 (0.906-1.710)
Toilet type: other (vs no toilet)	0.203	1.225 (0.752-1.996)
Toilet type: pit latrine (vs no toilet)	0.204	1.226 (0.550-2.734)
Toilet type: shared toilet (vs no toilet)	-0.395	0.674 (0.395-1.148)
Visible human faeces around the household	0.224	1.251 (0.881-1.777)
Storing water uncovered	-0.326	0.722 (0.478-1.089)
Number of days since the first sample	-0.066	0.936 (0.587-1.493)
Harmonic term (sinday)	**-0.753**	**0.471 (0.289-0.767)**
Harmonic term (cosday)	0.448	1.565 (0.883-2.774)
Harmonic term (sinday2)	-0.304	0.738 (0.434-1.255)
Harmonic term (cosday2)	0.483	1.621 (0.961-2.736)
Living in Chikwawa (vs Chileka)	-0.038	0.963 (0.606-1.529)
Living in Ndirande (vs Chileka)	0.274	1.315 (0.812-2.130)

*Significant variables highlighted in bold

FIGURE LEGENDS

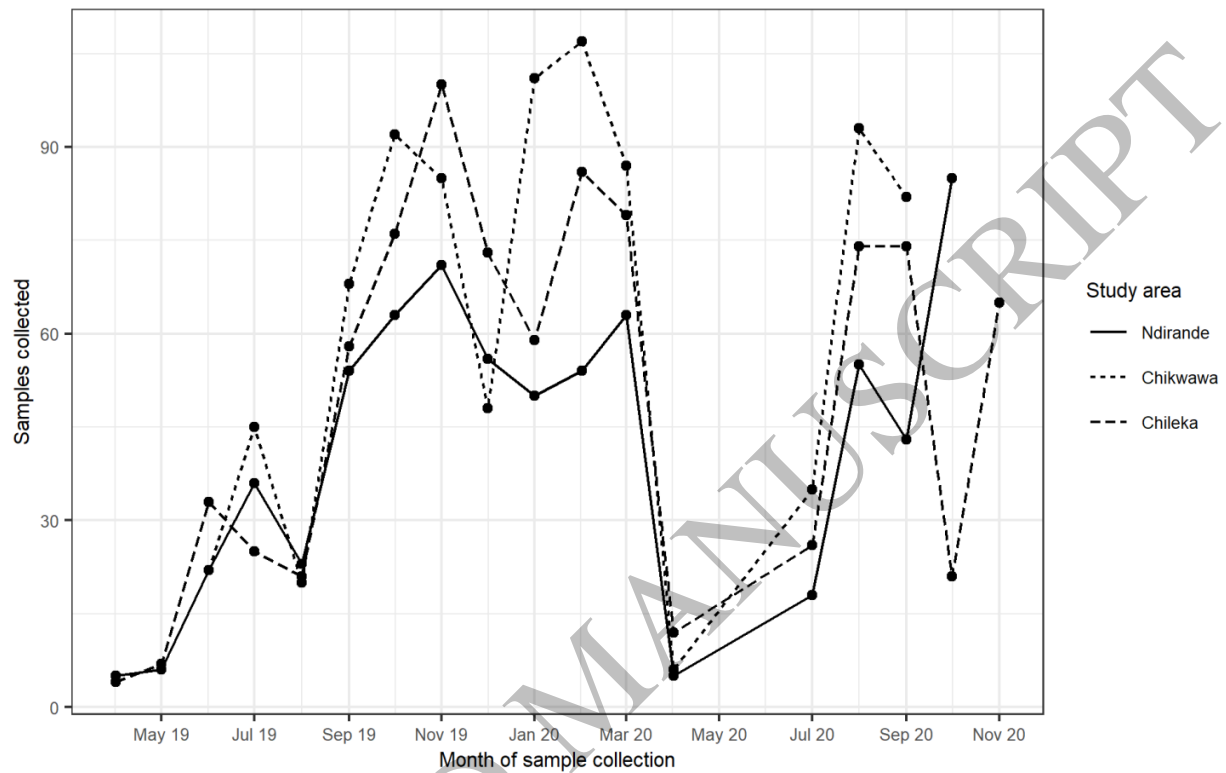**Fig 1.** Distribution of samples collected, over time and by study area

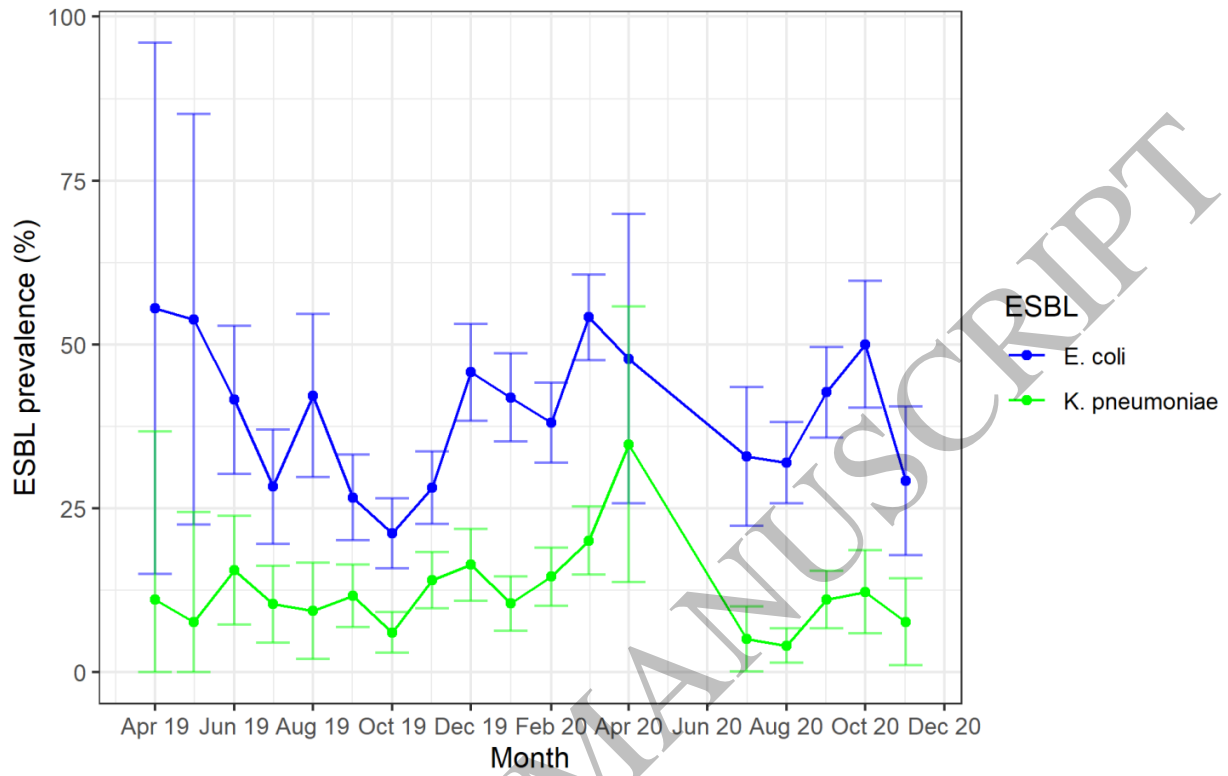**Fig 2.** Prevalence of ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* per month.

Fig 3. Correlation heatmap of household-level covariates. Values range from -0.5 (blue) to 1 (red) and represent the correlation coefficient between every pair of household-level covariates. Covariates are in the same order on rows and columns.