Longitudinal Patterns of Antimicrobial Resistance in *Escherichia coli* Isolated from Children <5 Years of Age Following Hospital Discharge in Kenya and the Impact of a 5-Day Course of Azithromycin

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ABSTRACT

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Introduction: Antimicrobial resistance (AMR) is a growing concern worldwide, especially among gram-negative bacteria. One gram-negative species, *E. coli,* is responsible for most deaths attributed to AMR, especially in sub-Saharan Africa. Hospitalization is a time of increased exposure to pathogens and antibiotics. However, there is limited data on the burden of AMR post-hospital discharge and factors associated with the occurrence of extended-spectrum β-lactamase (ESBL) - producing *E. coli* within communities.

Methods: A sequential cross-sectional data analysis from *E. coli* isolated from fecal samples collected from children <60 months old in Homabay and Kisii counties of Western Kenya. Fecal samples were collected at hospital discharge, and at three-month and six-month follow-ups.

Fecal samples from each unique child were cultured in triplicate for *E. coli*. If *E. coli* was isolated from at least one of the samples tested in triplicate, the child was considered to have a positive *E. coli* culture. Any unique child with *E. coli* isolated at enrollment with antimicrobial susceptibility testing (AST) results was included in our analysis.

We evaluated changes in the proportion of AMR to twelve selected antibiotics over six months following hospital discharge and determined AMR decline by comparing the proportion of AMR between discharge and three months and between three- and six-months post-discharge.

We also determined the occurrence of ESBL-producing *E. coli* at six months post-hospital discharge. We conducted a univariate analysis to determine the burden of AMR post-hospital discharge and a Multivariate Poisson regression to evaluate the association between the occurrence of ESBL-producing *E. coli* at six months post-hospital discharge and selected risk factors. We then conducted a sensitivity analysis to examine differences in phenotypic AMR among isolates detected at discharge, three months, and six months post-hospital discharge.

Results: 406 unique children were enrolled in the study, and all had *E. coli* isolated at the discharge time point. Most of the children (323, or 80%) had *E. coli* isolated at each time point (discharge, month three, and month six). *E. coli* isolates were predominantly from males (59.5%) and the median age of the included children was 19 months (IQR 23 months) at enrollment. Most children were hospitalized for at least three days, were of low socio-economic status (65.2%), and were HIV unexposed (84%).

There was a statistically significant decline in the proportional non-susceptibility to all antibiotics from hospital discharge to three months. The proportion of non-susceptibility isolates between three months and six months was not statistically significant for most antibiotics, except for ceftazidime 0.58 (0.36 - 0.95, p0.031), gentamicin 0.44 (0.30 - 0.63, p<0.001), and ESBL-producing *E. coli* 0.55 (0.32 - 0.94, p0.029). Non-susceptibility to ampicillin (AMP) and trimethoprim-sulfamethoxazole (TMP/SMX) remained highest at the end of follow-up at month six (72% and 84%, respectively). Carriage of ESBL-producing *E. coli* dropped from 44% at hospital discharge to 11% at six months post-hospital discharge. There were no statistically significant risk factors associated with the occurrence of ESBL-producing *E. coli* at six months post-hospital discharge.

Conclusions: At hospital discharge, non-susceptibility to *E. coli* remained high, suggesting that exposure to antibiotics in the hospital is a driver of AMR in these children. *N*on-susceptibility to all antibiotics significantly declined up to three months post-discharge suggesting that when antibiotic pressure associated with illness and hospitalization is removed, AMR emergence also stabilizes. ESBL-producing *E. coli* remained stable up to six months post-hospital discharge. There were no significant correlates of ESBL-producing *E. coli* at six months post-hospital discharge among the risk factors examined. Overall, these findings highlight the urgent need for facility-based interventions, including increased surveillance, antibiotic stewardship, and other control measures, to reduce the spread of antimicrobial-resistant bacteria in sub-Saharan Africa.

INTRODUCTION

Children in sub-Saharan Africa (SSA) are disproportionately impacted by bacterial communicable diseases (1) which often result in hospitalization and/or mortality. Antibiotics are commonly used to treat bacterial infections in the hospital and community (1,2). While these interventions can be life-saving, frequent exposure to antibiotics can lead to antimicrobial resistance (AMR) (1). Multidrug-resistant (MDR) and Extensively Drug Resistant (XDR) organisms in particular may lead to post-hospital discharge morbidity, rehospitalization, and mortality and are disproportionately prevalent in developing countries (1,3). AMR contributes to about five million deaths, about one million of which are attributable to bacterial AMR (1). Globally, enteric *E. coli* is the most common pathogen responsible for deaths attributed to AMR (1). Non-pathogenic commensal *E. coli* has been reported to serve as reservoirs of mobile genetic elements that lead to AMR acquisition by other bacteria (2,4).

Wide-spread clinical and community use of antibiotics such as ampicillin and trimethoprim-sulfamethoxazole in SSA and azithromycin, and ciprofloxacin in Asia, have been associated with community-wide non-susceptibility to these antibiotics (2,5). In addition, mass drug administration (MDA) with azithromycin has been shown to reduce mortality in some settings in SSA and is currently being scaled up in some settings (4,5). The widespread use of antibiotics in these settings is associated with significant increases in non-susceptibility in these communities although declines in non-susceptibility have also been observed following removal of antibiotic pressure (4,6–8). For example, in studies where there was a six month follow-up after MDA, participants who received a single treatment of azithromycin had a much lower weighted average of non-susceptibility compared to those who continued to receive periodic treatment (7).

High-level exposure to antibiotics has also been associated with the occurrence of non-susceptible *E. coli* within the community, even among individuals not reporting recent antibiotic use (9). Factors considered important in the carriage of AMR include overcrowding, socioecological behaviors, socioeconomic status, geographic location, food safety, contaminated waste, previous hospitalization, previous antibiotic use, and livestock ownership (9,10). In addition, health care contact is an important driver of non-susceptibility among bacteria. A diagnosis of meningitis, being hospitalized in the prior year, in-hospital exposure to penicillin, ceftriaxone and gentamicin, longer duration of hospitalization have all been associated with the occurrence of extended-spectrum β-lactamase (ESBL) - producing *E. coli* (10). ESBL-producing *E. coli* are relevant since their occurrence in the community limits therapeutic options, encourages spread of AMR and are also responsible for numerous outbreaks (11). The persistence of ESBL-producing *E. coli* has important clinical consequences for children recovering from hospitalization, as ESBL carriage has been associated with increased morbidity (12). However, the burden of ESBL-producing *E. coli* following hospital discharge has not been well described and it is not known whether high levels of antibiotic non-susceptibility persist following hospitalization, for how long, and what factors may be associated with occurrence of ESBL-producing *E. coli* in children who return to the community.

Understanding such dynamics may shed light into potential sources and potential intervention points to reduce the spread of AMR in children and in their communities following hospital discharge.

We conducted a repeated cross-sectional study nested within a previously completed randomized placebo-controlled trial of azithromycin (the Toto Bora clinical trial - registration number NCT02414399) to investigate changes in non-susceptibility among *E. coli isolates* over a six-month period post-hospital discharge among children < 5 years old in Western Kenya. Additionally, we determined correlates associated with the occurrence of ESBL-producing *E. coli* at six months post-hospital discharge (10,13).

METHODS

Parent study: The parent study was a randomized double-blind placebo clinical trial (Toto Bora trial - Trial registration number NCT02414399) testing the efficacy of a 5-day course azithromycin delivered at hospital discharge on rates of rehospitalization and death six-months post-hospital discharge (14).

Study Setting: Homabay District hospital and Kisii Teaching and Referral Hospital.

Study participants: Children aged 1- 59 months, weighing at least 2 kilograms, were hospitalized, and subsequently discharged and were residents in the study area for at least six months post-hospital discharge were included in the parent trial. Children with trauma, poisoning, and congenital disorders and those who did not provide informed consent were excluded from the study. Out of the children (aged 1 to 59 months) recruited at hospital discharge following informed consent from the caregivers in the parent study, approximately 400 children with *E. coli* isolated were randomly selected to participate in an antimicrobial susceptibility testing (AST) sub study.

Data collection: Standardized questionnaires were used to collect sociodemographic information and medical history at enrollment (hospital discharge) as well as three and six months thereafter.

Sample collection: Stool or rectal swabs (if a child could not produce whole stool) were collected from enrolled children at hospital discharge (prior to randomization), three months, and six months after. Fecal samples were immediately placed in Cary Blair media to maintain sample quality with bacterial cultures performed within 24 hours.

Laboratory procedures: Briefly, rectal swabs and stool samples were streaked onto selective differential media i.e., MacConkey agar (MAC) and eosin methylene blue agars (EMB) with incubation at 37°C for 18 – 24 hours. Suspected *E. coli* isolates were confirmed following a negative oxidase test and identified using the API 20E system (bioMérieux, Inc). Once *E. coli* was confirmed, they were suspended in tryptic soy broth with 15% glycerol and frozen at -80˚C. Frozen isolates were thawed and revived on MacConkey agar with incubation at 37°C for 18 – 24 hours in triplicate for each unique child. AST was performed on any *E. coli* positive isolate using disc diffusion using Clinical and Laboratory Standards Institute (CLSI) guidelines (15).

The *E. coli* cultures on MacConkey agar were suspended in 5ml of sterile normal saline and adjusted to a density of 0.5 MacFarland standard prior to homogenous spread on Mueller-Hinton agar. A disc dispenser was used to place ten selected antibiotics onto the Mueller-Hinton agar. These antibiotics included ampicillin (AMP), ceftriaxone (CRO), cefotaxime (CTX), ceftazidime (CAZ), cefoxitin (FOX), imipenem (IMP), ciprofloxacin (CIP), gentamicin (GEN), amoxicillin/clavulanic acid (AMC), trimethoprim-sulfamethoxazole (TMP/SMX), azithromycin (AZM), and chloramphenicol (CHL).

CLSI-2020 M-100 was used for interpreting zone diameters for Enterobacterales for each antibiotic and isolates were classified as susceptibility or non-susceptible (included intermediate or resistant). However, Salmonella enterica serovar Typhi standard was used to reference the zone diameter for azithromycin (AZM) since there are no specified cut offs.

ESBL-producing *E. coli* were determined using double disc diffusion i.e., using CTX and CAZ with and without clavulanic acid. For any of the isolates, if the differences in zone diameters between CTX and CTX/clavulanic acid or CAZ and CAZ/clavulanic was <5mm, then the isolate was considered non-ESBL-producing *E. coli*. However, if this difference was ≥5mm, then the isolate was considered an ESBL-producing *E. coli*. Quality control for non-ESBL-producing *E. coli* was evaluated using ATCC 25922 strains of *E. coli* while ESBL-producing *E. coli* were evaluated using NCTC 13351 *E. coli*.

Statistical analysis:

Socio-demographic and clinical features of the children were summarized and compared between the different counties. We also compared ESBL-producing *E. coli* to non-ESBL-producing *E. coli*. Continuous variables were summarized using medians and interquartile range (IQR) and compared using nonparametric Wilcoxon-Mann-Whitney test. Categorical variables were summarized using counts and proportions and compared using Pearson's chi-square tests or Fisher's exact tests as appropriate.

For each variable, where values are missing, the denominator is stated in the table or number of missing observations in a footnote to the corresponding summary table.

We conducted exploratory analyses to identify any outliers or missing variables.

Additionally, the proportion of *E. coli* isolates non-susceptible to each antibiotic i.e., ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefoxitin, imipenem, ciprofloxacin, gentamicin, amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole, azithromycin, and chloramphenicol and presence of ESBL-producing *E. coli* at each timepoint (hospital discharge, three months, and six months post-hospital discharge) were determined. In analysis, if any of the three isolates per unique individual was *E. coli* positive, then that was considered as one isolate per unique child. Additionally, ESBL-producing *E. coli* was considered if any of the child's isolate had a zone diameter ≥5mm using either CTX and CTX/clavulanic acid or CAZ and CAZ/clavulanic or both.

The change in proportion of non-susceptibility over time were modelled using generalized estimating equations (GEE) with a Poisson link and exchangeable correlation structure to account for the repeated sample collection per child over time. We tested for time trends (non-susceptible proportion post-hospital discharge vs. three months and three months vs. six months).

The proportion of ESBL-producing *E. coli* isolates six months post-hospital discharge were modelled with potential correlates (independent variables identified at discharge) using Poisson regression. Each correlate was modelled to determine univariate associations with occurrence of ESBL-producing *E. coli*. Factors considered included gender, age (in months), randomization arm, length of hospital stay, hospitalized in the previous year, adhered to treatment, crowding, breast feeding status, household income, water source, caregiver level of education, treatment of drinking water, toilet type, shared toilet, livestock ownership and HIV status.

RESULTS

Of the 1398 children enrolled, 87 % had *E. coli* isolated from fecal samples and of those, 406 children were included in the AMR sub study. Of the 406 children with *E. coli* at hospital discharge that had AST results, 358 had *E. coli* isolated at month three and 342 had *E. coli* isolates at month six and were all subjected to AST (Figure 1). Among those samples isolated from unique individuals at hospital discharge, 323 (80%) were isolated at all three follow-up visits.

The majority of the 406 included children were male (59%), had a median age of 19 months (IQR = 23 months), had a median of 3 days in hospital (54%), were not hospitalized in the prior year to the study (79%), had improved water sources (84%), used a pit latrine (88%), kept livestock (71%) and were HIV unexposed (84%) (Table1). Of the 406 children, 54% were randomized to azithromycin and adherence to azithromycin or placebo was 94% among included participants.

On examining non-susceptibility to the twelve antibiotics across the six months, there was high non-susceptibility to ampicillin (AMP) and trimethoprim-sulfamethoxazole (TMP/SMX) at enrollment, month three and month six i.e., 91%, 74% and 72% for AMP and 93%, 85% and 84%, for TMP/SMX and respectively. Conversely, non-susceptibility to imipenem (IMP) and cefoxitin (FOX) remained low across the months i.e., 3%,1%,0% for IMP and 10%, 3%, 2% for FOX respectively (Figure 1).

Most of the antibiotics had <50% non-susceptibility at all time points. At the end of follow-up at month six, non-susceptibility to azithromycin (AZM) (19%) and ESBL-producing *E. coli* (11%) remained stable.

On examining the proportion of non-susceptible isolates over time, non-susceptibility to all tested antibiotics was significantly lower when comparing non-susceptibility at month three to those at hospital discharge (Figure 2 and Table 2). Compared to the proportion of children with ESBL-producing *E. coli* at discharge (44%), the proportion with ESBL-producing *E. coli* at month 3 was over 80% lower with a prevalence ratio (PR) of 0.18 (0.13 - 0.24, p<0.001). Similar associations between proportion non-susceptibility at month three compared to hospital discharge were found for all the cephalosporins i.e., ceftriaxone 0.28 (0.21 - 0.37, p<0.001), cefotaxime 0.35 (0.26 - 0.46, p<0.001), ceftazidime 0.28 (0.20 - 0.39, p<0.001) and cefoxitin 0.35 (0.19 - 0.65, p<0.001).

The proportion of children with non-susceptibility in *E. coli* isolates between months three and six was not significantly different except for ceftazidime 0.58 (95%CI 0.36 - 0.95, p0.031), gentamicin 0.44 (95%CI 0.30 - 0.63, p<0.001**)** and ESBL-producing *E. coli* 0.55 (95%CI 0.32 - 0.94, p0.029).

ESBL-producing *E. coli* were slightly more likely to be isolated from females compared to males (1.32, 95%CI 0.67 – 2.59), those who were in the treatment arm of the parent study to receive azithromycin compared to those who received a placebo (1.51, 95%CI 0.75 – 3.07), those who did not complete their required five doses of azithromycin compared to those who completed their doses (1.83, 95%CI 0.34 – 9.76), those who shared a toilet compared to those who practice open defecation (2.63, 95%CI 0.18 – 38.4), those who came from families who had livestock compared to those without (1.32, 95%CI 0.55 – 3.17) and children who were HIV infected compared to those who are HIV exposed, but uninfected (3.21, 95%CI 0.48 – 21.4).

Only 11% of children had ESBL-producing *E. coli* six months after randomization and we found no independent predictors of ESBL-producing *E. coli* at this timepoint (Table 3).

DISCUSSION

In this study, children discharged from hospital in Kenya had a high proportion of *E. coli* isolated with non-susceptibility to a number of commonly used antibiotics, in many cases higher than that shown in other studies in sub-Saharan Africa (5). Non-susceptibility to ampicillin and trimethoprim-sulfamethoxazole remained high among these children, even at six months post-hospital discharge when children are living in their communities.

Previous studies have suggested that non-susceptibility to these antibiotics is linked to misuse and over prescription in SSA (2,5). Additionally, it has been reported that daily prophylaxis of trimethoprim-sulfamethoxazole in management of opportunistic infections among HIV-infected persons in sub-Saharan Africa leads to significantly increases non-susceptibility among *E. coli* isolates (16).

Previous studies have monitored resistance over time up to six months after MDA (17). Our study was different since we assessed in-person carriage over time post-hospital discharge. However, non-susceptible *E. coli* to ampicillin and trimethoprim-sulfamethoxazole was similar (17). In contrast, non-susceptibility to imipenem and cefoxitin was relatively low among *E. coli* isolates in this population throughout follow-up. It is likely that non-susceptibility to these antibiotics was less common because these antibiotics are not ubiquitous potentially due to their high cost.

In our study non-susceptibility among *E. coli* isolates significantly reduced from hospital discharge up to 3 months post-hospital discharge.

It has previously been suggested that reducing treatment duration using antibiotics reduces non-susceptibility carriage among isolates by reducing the drug pressure (18). We also examined non-susceptibility among cephalosporins which is often associated with the occurrence of ESBL-producing *E. coli*. In this population, ESBL-producing *E. coli* was 11% at six months post-hospital discharge suggesting that empirical treatment options need to be evaluated for improved outcomes. Non-susceptibility to most antibiotics declined significantly at three months post-hospital discharge and then stabilized by six months post-hospital discharge, which is similar to one study in Tanzania who followed up children for up to six months, but compared MDA to non-MDA (5).

None of the correlates considered were associated with the occurrence of ESBL-producing *E. coli* six months post-hospital discharge. From this study, some selected correlates including completion of antibiotic doses, the presence of livestock in the homestead and HIV status needs to be evaluated in larger studies. There is paucity of data related to risk factors associated with ESBL-producing *E. coli* post-hospital discharge.

This study had several strengths including tracking individuals post hospital discharge unlike previous studies which focused on MDA. Additionally, we had less loss to follow-up as children were easily tracked for additional data and sample collection. However, there were some limitations including collecting data and samples at only three time points for up to six months, and therefore not designed to examine longitudinal trends.

Additionally, we used a serial cross-sectional approach for this study and therefore cannot establish temporal sequence. On analyzing ESBL-producing *E. coli* at six months post-hospital discharge, the study was not powered to detect the differences in correlates between these and non-ESBL-producing *E. coli*.

CONCLUSION

In this study we report high non-susceptibility to selected antibiotics at hospital discharge, further highlighting the impact of exposure to antibiotics drives high levels of non-susceptibility among hospitalized children. The proportion of *E. coli* non-susceptibility to all tested antibiotics declined during follow-up, with a statistically significant decline when comparing *E. coli* isolated at enrollment to those isolated three months post-hospital discharge. This further demonstrates that when intensive antibiotic pressure is removed, AMR also reverts towards levels observed in the community. However, after the third month post-hospital discharge, AMR did not continue to decline for most antibiotics. Non-susceptibility stabilized suggesting that there is still relatively high resistance in these children that likely mirrors the burden of circulating non-susceptible *E. coli*.

Persistent AMR in children returning to the community following hospitalization may limit treatment options, resulting in increased morbidity and longer hospital stays that burden the healthcare system. In addition, these children may continue to serve as reservoirs for the transfer of AMR genes to others in the community.

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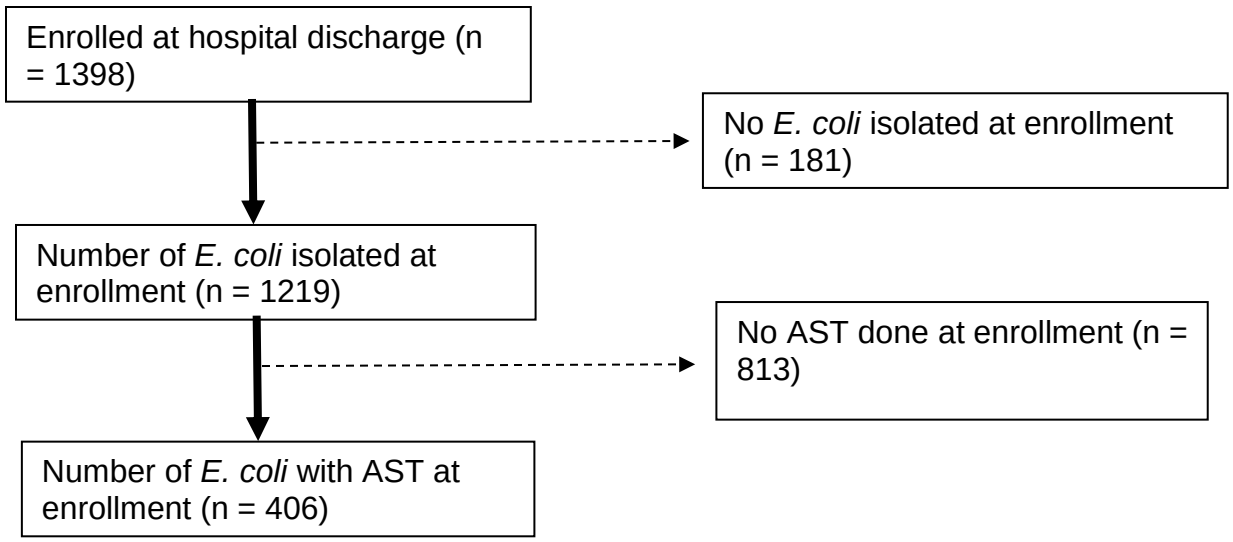

Fig 1: Participants' flow diagram

Table 1: Descriptive statistics of differences in *E. coli* at enrollment

	Homabay	Kisii	Overall
	(n=164)	(n=242)	(n=406)
Gender			
Female	66 (40%)	99 (41%)	165 (41%)
Male	98 (60%)	143 (59%)	241 (59%)
Age in months			
Median (IQR)	20.0 (22)	17.0 (23)	19.0 (23)
Randomization arm			
Placebo arm	75 (46%)	112 (46%)	187 (46%)
Treatment arm	89 (54%)	130 (54%)	219 (54%)
Hospital stay (in days)			
Median (IQR)	4.0 (3.3)	3.0 (3.0)	3.0 (3.0)
Hospitalized (previous year)			
No	130 (80%)	190 (79%)	320 (79%)
Yes	33 (20%)	51 (21%)	84 (21%)
Adhere to treatment			
< 5 doses	13 (7.9%)	1 (0.4%)	14 (3.4%)
All 5 doses	150 (91%)	233 (96%)	383 (94%)
Not reported	1 (0.6%)	8 (3.3%)	9 (2.2%)
Crowding			
Yes	45 (27%)	36 (15%)	81 (20%)
No	119 (73%)	206 (85%)	325 (80%)
Breastfeeding			
Exclusively breastfed	111 (68%)	73 (30%)	184 (45%)
Never breastfed	3 (1.8%)	2 (0.8%)	5 (1.2%)
Partially breastfed	49 (30%)	147 (61%)	196 (48%)
Unknown	1 (0.6%)	20 (8.3%)	21 (5.2%)
Household Income at enrollment			
< 5000 KES	26 (16%)	38 (16%)	64 (16%)
>= 5000 KES	126 (78%)	193 (82%)	319 (80%)
Unknown (refused to answer)	9 (5.6%)	5 (2.1%)	14 (3.5%)
Water source at enrollment			
Improved	124 (76%)	217 (90%)	341 (84%)
Unimproved	40 (24%)	25 (10%)	65 (16%)
Caregiver highest education level			
<= Primary	101 (62%)	100 (41%)	201 (50%)
>= secondary	62 (38%)	141 (59%)	203 (50%)
unknown	1 (0%)	1 (0%)	2 (0%)
Treated drinking water			
No	44 (27%)	157 (66%)	201 (50%)
Yes	118 (73%)	82 (34%)	200 (50%)

Missing	2 (0%)	3 (0%)	5 (0%)
Toilet type			
Flush toilet	6 (3.7%)	23 (9.5%)	29 (7.1%)
Other (bush)	20 (12%)	0 (0%)	20 (4.9%)
Pit latrine	138 (84%)	219 (90%)	357 (88%)
Shared toilet			
Open defecation	20 (12%)	0 (0%)	20 (5%)
Private toilet	55 (34%)	127 (53%)	182 (45%)
Shared toilet	86 (53%)	114 (47%)	200 (50%)
Unknown	3 (0%)	1 (0%)	4 (0%)
Livestock ownership			
No	39 (24%)	80 (33%)	119 (29%)
Yes	125 (76%)	161 (67%)	286 (71%)
Unknown	0 (0%)	1 (0%)	1 (0%)
Child HIV Status			
HIV-exposed, uninfected	34 (21%)	13 (5%)	47 (12%)
HIV infected	5 (3.0%)	3 (1.2%)	8 (2%)
HIV unexposed	122 (74%)	218 (90%)	340 (84%)
Uninfected, exposure status unknown	3 (1.8%)	8 (3.3%)	11 (2.7%)

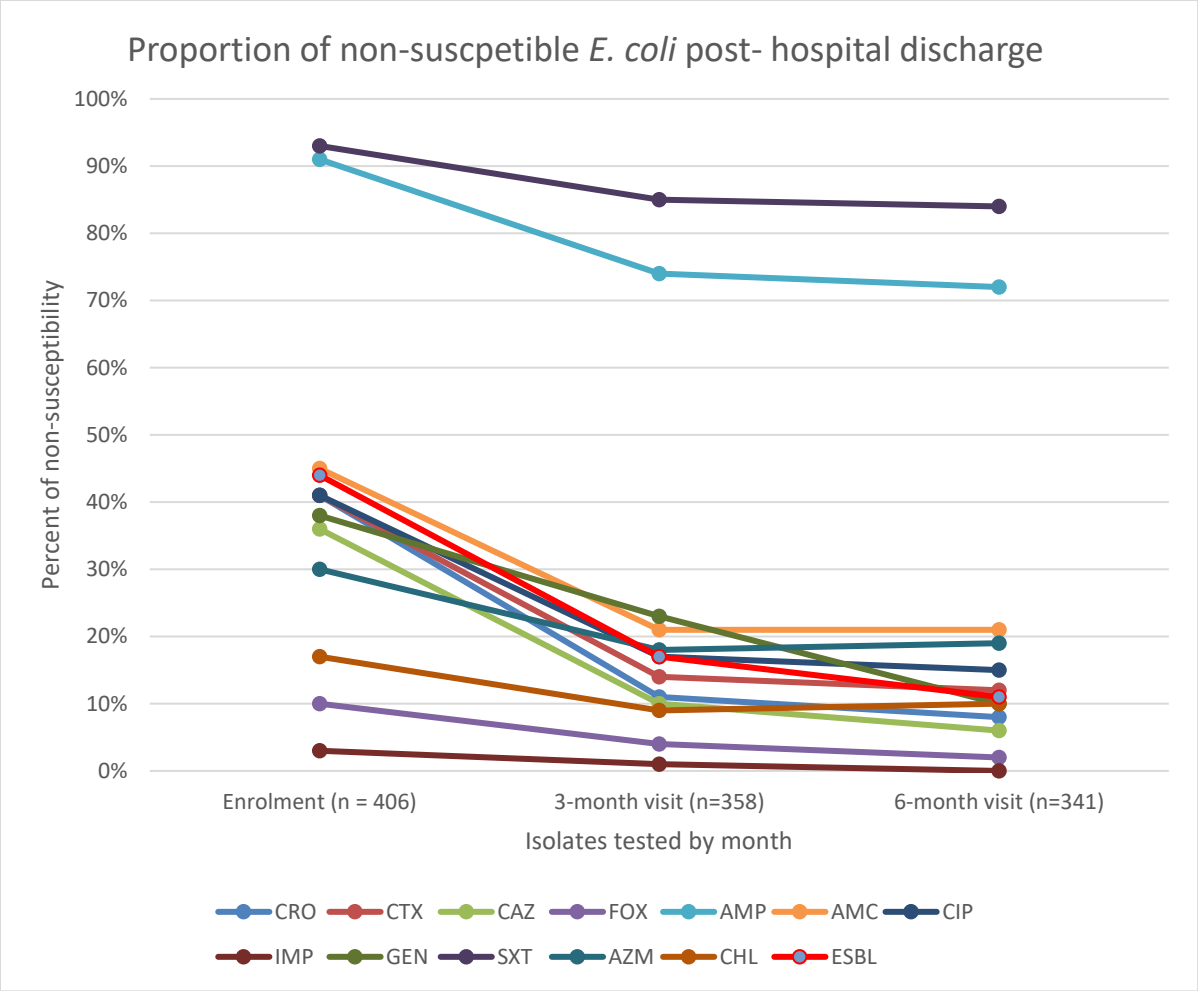

Fig 2: Proportion of non-susceptible *E. coli* post hospital discharge

Table 2: Description of changes in non-susceptibility to different antibiotics over time

Antibiotic	PR* 0 - 3 months (95%CI), p-value	PR* 3 - 6 months (95%CI), p-value
Ceftriaxone (CRO)	**0.28 (0.21 - 0.37), p<0.001**	0.67 (0.43 - 1.03), p0.070
Cefotaxime (CTX)	**0.35 (0.26 - 0.46), p<0.001**	0.84 (0.59 - 1.20), p0.348
Ceftazidime (CAZ)	**0.28 (0.20 - 0.39), p<0.001**	**0.58 (0.36 - 0.95), p0.031**
Cefoxitin (FOX)	**0.35 (0.19 - 0.65), p<0.001**	0.65 (0.28 - 1.49), p0.305
Ampicillin (AMP)	**0.81 (0.76 - 0.87), p<0.001**	0.99 (0.90 - 1.08), p0.755
Amoxillin/clavulanic acid (AMC)	**0.46 (0.37 - 0.58), p<0.001**	1.04 (0.78 - 1.38), p0.808
Ciprofloxacin (CIP)	**0.41 (0.32 - 0.53), p<0.001**	0.91 (0.65 - 1.27), p0.579
Imipenem (IMP)	**0.19 (0.04 - 0.85), p0.029**	0.52 (0.05 - 5.76), p0.597
Gentamicin (GEN)	**0.60 (0.48 - 0.75), p<0.001**	**0.44 (0.30 - 0.63), p<0.001**
Trimethoprim/Sulfamethoxazole (TMP/SMX)	**0.91 (0.87 - 0.96), p<0.001**	1.00 (0.94 - 1.06), p0.945
Azithromycin (AZM)	**0.60 (0.45 - 0.79), p<0.001**	1.07 (0.79 - 1.44), p0.676
Chloramphenicol (CHL)	**0.55 (0.38 - 0.80), p0.002**	1.11 (0.71 - 1.74), p0.636
ESBL	**0.18 (0.13 - 0.24), p<0.001**	**0.55 (0.32 - 0.94), p0.029**

*PR = Prevalence ratio

Table 3: Factors associated with ESBL-producing E. coli six months post-hospital discharge.

Variable	ESBL, n = 39	Non-ESBL, n = 303	Crude PR	Adjusted PR
Gender				
Male	20 (9.8%)	184 (90%)	REF	REF
Female	19 (14%)	119 (86%)	1.40 (0.74 – 2.64)	1.32 (0.67 – 2.59)
Age (in months)	16 (7, 25)	19 (10, 33)	0.99 (0.96 - 1.01)	0.98 (0.96 – 1.01)
Location				
Kisii	21 (10%)	185 (90%)	REF	REF
Homabay	18 (13%)	118 (87%)	1.30 (0.68 – 2.44)	1.10 (0.47 – 2.54)
Randomization arm				
Placebo	14 (9.1%)	140 (91%)	REF	REF
Treatment	25 (13%)	163 (87%)	1.46 (0.77 - 2.89)	1.51 (0.75 – 3.07)
Hospital stay (in days)	4.0 (2.0, 7.0)	3.0 (2.0, 5.0)	1.05 (0.99 - 1.10)	1.06 (0.99 – 1.14)
Hospitalized (previous year)				
Yes	8 (11%)	63 (89%)	REF	REF
No	31 (12%)	238 (88%)	1.02 (0.49 – 2.39)	0.75 (0.32 – 1.75)
Adhere to treatment				
All 5 doses	37 (11%)	294 (89%)	REF	REF
< 5 doses	2 (18%)	9 (82%)	1.63 (0.26 – 5.31)	1.83 (0.34 – 9.76)
Crowding				
Crowding	4 (5.8%)	65 (94%)	REF	REF
No crowding	35 (13%)	238 (87%)	2.21 (0.88 - 7.40)	2.36 (0.73 – 7.61)
Breastfeeding				
Exclusively breastfed	22 (14%)	132 (86%)	REF	REF
Never breastfed	0 (0%)	5 (100%)	0.00 (0.00 - Inf)	0.00 (0.00 - Inf)
Partially breastfed	16 (9.6%)	151 (90%)	0.67 (0.35 - 1.28)	0.60 (0.30 – 1.23)
Unknown	1 (6.3%)	15 (94%)	0.44 (0.06 - 3.25)	0.50 (0.06 – 4.03)
Household Income at enrollment				
< 5000 KES	5 (8.9%)	51 (91%)	REF	REF
>= 5000 KES	30 (11%)	238 (89%)	1.25 (0.53 - 3.68)	1.30 (0.45 – 3.77)
Unknown	3 (25%)	9 (75%)	2.80 (0.57 - 11.4)	2.25 (0.46 – 10.9)
Water source at enrollment				
Improved	32 (11%)	256 (89%)	REF	REF
Unimproved	7 (13%)	47 (87%)	1.17 (0.47 - 2.49)	1.02 (0.40 – 2.59)
Caregiver highest education level				
<= Primary	18 (10%)	154 (90%)	REF	REF
>= Secondary	21 (13%)	147 (88%)	1.19 (0.64 - 2.27)	1.40 (0.69 – 2.83)
Treated drinking water				
No	17 (10%)	149 (90%)	REF	REF
Yes	22 (13%)	149 (87%)	1.26 (0.67 - 2.40)	1.08 (0.52 – 2.24)

Toilet type				
Flush Toilet	2 (8.0%)	23 (92%)	REF	REF
Other (Bush)	1 (5.6%)	17 (94%)	0.69 (0.03 - 7.25)	0.38 (0.03 – 5.57)
Pit Latrine	36 (12%)	263 (88%)	1.51 (0.46 - 9.26)	1.14 (0.24 – 5.55)
Shared toilet				
Open defecation	1 (5.6%)	17 (94%)	REF	REF
Private Toilet	17 (11%)	141 (89%)	1.94 (0.40 - 34.9)	1.96 (0.15 – 26.3)
Shared Toilet	21 (13%)	141 (87%)	2.33 (0.49 - 41.9)	2.63 (0.18 – 38.4)
Livestock ownership				
No	8 (8.3%)	88 (92%)	REF	REF
Yes	31 (13%)	214 (87%)	1.52 (0.73 - 3.55)	1.32 (0.55 – 3.17)
HIV status				
HIV exposed, uninfected	5 (12%)	35 (88%)	REF	REF
HIV infected	2 (29%)	5 (71%)	2.29 (0.44 – 11.8)	3.21 (0.48 – 21.4)
HIV unexposed	32 (11%)	256 (89%)	0.89 (0.35 – 2.28)	1.06 (0.38 – 2.96)
Uninfected, exposure status unknown	0 (0%)	7 (100%)	0.00 (0 - Inf)	0.00 (0 - Inf)