Antimicrobial Resistance in Children at Hospital Discharge in Western Kenya

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A dissertation submitted

in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2022

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Program Authorized to Offer Degree:

Public Health - Epidemiology

University of Washington

ABSTRACT

Antimicrobial Resistance in Children at Hospital Discharge in Western Kenya

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Introduction: Antimicrobial resistance **(AMR)** is associated with millions of deaths per year worldwide. The global burden of AMR is disproportionately high in Sub-Saharan Africa, primarily as a result of the high incidence of infectious diseases. Children who have been hospitalized and subsequently discharged in this region are at high risk for morbidity and mortality. It is possible that AMR plays a role in these poor outcomes through treatment failure due to antibiotic resistant pathogens or through the acquisition of new resistant infections either during hospitalization or the post-discharge period. In addition, children carrying resistance determinants may serve as an important reservoir of AMR that can be transferred to other household and community members. Conditions favorable to the spread of bacteria, and therefore AMR, such as crowded living conditions, insufficient sanitation and hygiene facilities and contaminated water sources, may exacerbate community transmission from these children to others. **Methods:** This dissertation used bacterial isolates from fecal samples from a recently completed clinical trial examining whether azithromycin reduced death and rehospitalization during the discharge period in children aged 6 – 59 months in western Kenya. Fecal samples were collected at the time of enrollment, prior to the administration of the first dose of the study drug or placebo. Study staff performed medical record extractions, conducted interviews with caregivers, and study clinicians performed a medical examination. Fecal samples were also collected at follow-up visits 3-months and 6-months post-enrollment. Bacterial isolates, including *Escherichia coli* **(*E. coli*)**, *Salmonella*, and *Shigella* were isolated and underwent antimicrobial susceptibility testing **(AST)**. Additionally, a random subset of

caregivers were enrolled and provided fecal samples for the isolation of *E. coli* and subsequent AST. The proportion of *E. coli* isolated from children at enrollment resistant to a panel of antibiotics and extended-spectrum beta-lactamase **(ESBL)** production and risk factors for carriage of ESBL-producing *E. coli* was determined (Chapter 1). Patterns of AMR in *E. coli* isolated from children and their caregivers at the time of hospital discharge were examined and correlates of concordance assessed (Chapter 2). Agreement of susceptibility to antibiotics in *Salmonella* or *Shigella* with *E. coli* isolated from the same fecal sample were also examined (Chapter 3). **Results:** The proportion of *E. coli* isolated from children resistant to ampicillin (95%), gentamicin (44%), ceftriaxone (46%), and the presence of ESBL (44%) was high. Use of antibiotics during the hospitalization (adjusted prevalence ration [aPR] = 2.23; 95% CI: 1.29 – 3.83) and being hospitalized within the prior year (aPR = 1.32 [1.07 – 1.69]) were associated with the presence of ESBL producing *E. coli*. Additionally, being female (aPR = 1.42; 95% CI: 1.15 – 1.76), practicing open defecation (aPR = 2.02; 95% CI: 1.39 – 2.94), and having a toilet shared with other households (aPR = 1.49; 95% CI: 1.17 – 1.89) were also associated with carriage of ESBL *E. coli*. Across all antibiotics tested, caregivers had less AMR than children. Concordance of AMR was more common when resistance to a particular antibiotic was particularly high (cefoxitin, chloramphenicol, and cotrimoxazole) or low (imipenem). ESBL concordance was less common in child-caregiver pairs where there was more than one child in the household compared to those without additional children (55.0% vs. 79.9% [p = 0.02]) and with caregivers who reported being employed or a student than those who were not employed or a student (45.0% vs. 55.0%) [p = 0.05]). *E. coli* had high concordance for susceptibility and was a reliable predictor of the outcome to cephalosporins and gentamicin but was a poor predictor of susceptibility to azithromycin and ciprofloxacin. **Conclusion:** Children who have been discharged from hospital have a high burden of AMR, which may contribute to poor outcomes in the post-discharge period. Given the frequency of AMR in this high risk population, new guidelines for the treatment of common infectious syndromes in children with a recent hospitalization should be considered. Additionally, because these children are likely to carry AMR from the hospital back into the community, they may pass AMR to family and other community members. Interventions which reduce the transmission of infectious agents may also reduce the transmission of AMR.

INTRODUCTION

Antimicrobial resistance **(AMR)** was associated with approximately 1.3 million deaths in 2019, making AMR one of the leading causes of deaths worldwide.[1] The burden of AMR is greatest in Sub-Saharan Africa **(SSA),** where infectious diseases are more common than in other regions. While country-level estimates of AMR exist, information on specific vulnerable populations, such as young children who have been hospitalized and discharged, are scarce.[2] More than 5 million children under the age of 5 years die in SSA per year and 40% of those illnesses are due to infectious diseases.[3,4] While AMR and infectious diseases are known to result in illness and death in children in SSA, limited laboratory capacities and systemic surveillance also limit knowledge of the problem.[1,5-7]

Children in SSA who have been discharged from hospital remain at high risk of illness and death for at least 6-months into the post-discharge period.[8,9] The full mechanisms of why these children fare poorly are not fully understood. However, AMR may play an important role. Children may leave the hospital with incompletely treated or recurring infections due to AMR or may become infected with new resistant infections during this delicate period. Guidelines from the World Health Organization **(WHO)** prioritize the use of beta-lactam antibiotics for many infectious syndromes, such as penicillins and 3rd generation cephalosporins.[10] Resistance to these drugs may be due to antibiotic specific mutations and may also be a result of broader resistance patterns, such as extended-spectrum beta-lactamase **(ESBL)** production.[11-13] Limited availability and high costs of alternative antibiotics in these settings may also be associated with increased mortality and morbidity in children post-hospital discharge.[14,15] No additional guidelines exist for children who return to hospital, therefore they may receive the same antibiotic prescribed during their initial hospitalization, regardless of whether the drug was effective in clearing a prior illness and independent of the underlying risk of AMR.

Resistance in bacterial isolates from children who have been discharged from hospital in SSA has not been well described.[2] Ascertaining correlates of AMR in this population could distinguish children at higher risk of treatment failure using current international prescribing guidelines. While AMR is known to be a major underlying cause of illness and death, individual testing for AMR is often not conducted due to constraints on laboratory infrastructure in SSA. Data highlighting risk factors for carriage of AMR may

provide important targets for intervention to reduce morbidity and mortality in this highly vulnerable population.

As a result of both direct and indirect exposures to antibiotics, children who have been discharged from hospital may be at significant risk of carrying AMR determinants at hospital discharge. As they return to their communities, these children may transmit pathogens with AMR and/or resistance genes to caretakers, siblings, and the community at large. Such transmission may increase the burden of AMR, especially in communities with reduced access to clean drinking water, sanitation facilities, and crowded living conditions.[7,16,17] Additionally, primary caretakers often spend significant time caring for their children when their child is hospitalized. Because AMR is frequently transmitted from the environment and between people, the exposure to the hospital environment and patients may place them at higher risk of acquiring AMR bacteria. This in turn could lead to transmission back to their child during the post-discharge period and resistant infections. As with children who have been hospitalized and return to their communities, if primary caregivers do have high carriage of AMR, they too can be a source of transmission to other family members and their communities.

Children who have been hospitalized and discharged in low-resource countries are frequently exposed to antibiotics.[9,18-20] Given the prevalence of infectious diseases and AMR in SSA, these children are highly likely to carry AMR bacteria, including harboring bacteria broad resistance, such as that conferred by ESBL producing organisms. However, few studies have examined AMR in children who have been discharged from hospitals in SSA. We examined resistance to antibiotics in *Escherichia coli* **(***E. coli)* in children who participated in the Toto Bora trial and their primary caregivers. *E. coli* may be an ideal bacteria for surveillance of AMR, as they are ubiquitous in the colon, easy to grow in the laboratory, and share resistance elements with other bacteria.[21-23] The gastrointestinal tract may be a reservoir of genetic AMR elements that can be exchanged between *E. coli* and pathogenic bacteria belonging to the same family, *Enterobacteriaceae*, such as *Salmonella* or *Shigella* species **(spp.)**.[22,23] Chapter 1 ascertained the prevalence of AMR in children at hospital discharge and correlates of harboring ESBL-producing *E. coli*. Chapter 2 assessed AMR in primary caregivers of children enrolled in the Tota Bora study and concordance between patterns of AMR in the children their caregivers. Lastly, Chapter 3

assessed the similarities of AMR between *Salmonella* or *Shigella*, two pathogenic gut bacteria, and *E. coli* isolated from the same fecal sample to determine the utility of using *E. coli* as a proxy for resistance among common enteric pathogens. This study provides much needed information regarding AMR in a population of children at high risk for death and illness in SSA.

ACKNOWLEDGEMENTS

This dissertation would not be possible without the children and caregivers that participated in the Toto Bora trial and I offer my sincerest gratitude. I would also like to give thanks to everyone involved with the study, both in Seattle and in Kenya for their knowledge and kindness over the years.

I would like to thank my committee members for their time, expertise, and guidance as I navigated this research project and the doctoral program.

Specifically, I would like to thank my advisers and mentors. Thank you so much for giving me the opportunity to be associated with such an amazing working group and projects that taught me so much. Thank you for all the meetings, feedback, and answering my [many] questions. Thank you to the Gut Health and Child Survival group in Global WACh for the support, laughs, and collaboration. I would also like to thank everyone associated with the Department of Epidemiology that I have interacted with during my time here.

I want to especially thank my family for their love and support. Thank you for believing in me and always being at my side.

Chapter 1: Antimicrobial Resistance including Extended Spectrum Beta Lactamases (ESBL) among *E. coli* isolated from Kenyan Children at Hospital Discharge

INTRODUCTION

In most low- and middle-income countries, infectious diseases account for approximately 40% of child deaths [3]. Children living in these settings are regularly exposed to bacterial pathogens from poor sanitation, crowding, and immune-deficiencies, leading to frequent bacterial infections that require antibiotic therapy [24,25]. Children in Sub-Saharan Africa **(SSA)** are at highest risk of death from infection, where limited antibiotic options and a lack of systematic surveillance of antimicrobial resistance **(AMR)** further complicate management decisions [15,24,26-28].

Children living in SSA who have been discharged from an inpatient hospital stay are at particularly high risk of mortality in SSA [8,29-31]. For example, post-discharge mortality rates in the year following a hospital stay are often similar to inpatient mortality and 8 – 9 times higher than mortality rates in age-matched community children [8,18,32]. The mechanisms driving this increased mortality are not clearly understood, though AMR may be a contributing factor [9]. AMR may lead to treatment failure in the post-discharge period and transmissible AMR genes may pose future health risks both to these children and to their communities.

AMR in commensal *Escherichia coli* ***(E. coli)*** may act as a surrogate marker for resistance in other pathogenic bacteria in the gut [23,33,34]. Carriage of AMR in commensal *E. coli* has also been shown to be a risk factor for the acquisition of resistant *E. coli* infections [23,35-37]. Few studies have quantified AMR in commensal *E. coli* in children in SSA, and fewer have characterized AMR in *E. coli* at hospital discharge [2]. We conducted a cross-sectional analysis to determine the prevalence of resistance to commonly used antibiotics in commensal *E. coli* isolated from Kenyan children discharged from hospital. In addition, we report risk factors for extended-spectrum beta-lactamase (**ESBL**) producing bacterial isolates, an indicator of broad resistance to commonly used beta-lactam antibiotics [28,38-40].

METHODS

Study Design

This cross-sectional nested study utilized the antimicrobial susceptibility patterns of *E. coli* isolated from children enrolled at hospital discharge as well as clinical, sociodemographic, and child health history information collected during interviews with the primary caregiver and physical exams collected during a recently completed clinical trial. The protocol of the parent trial has been published [41].

Parent Trial

Population

Children aged 1 – 59 months were enrolled at the time of hospital discharge at Kisii and Homa Bay hospitals in western Kenya to assess whether a 5-day course of azithromycin reduces rehospitalization and/or death in the subsequent 6-month period [41]. Eligible children were those who weighed at least 2kg, had been hospitalized and subsequently discharged, planned to remain in the area for at least 6 months, did not have a contraindication to azithromycin, and were not prescribed other macrolide antibiotics [41]. Children were excluded from the study if their hospital admission was solely due to trauma, poisoning, or obvious congenital disorder, if a twin of the same sex was enrolled in the study the same day, or if a legal guardian did not provide consent.

Data Collection

After providing informed consent, caregivers were interviewed by study staff to gather sociodemographic information and relevant medical history using a standardized questionnaire. Diagnoses, procedures, and other relevant medical information were extracted from medical records. Enrolled children underwent a physical examination by a study clinician, which included anthropometric measurements (height (or length if < 24 months), weight and middle upper arm circumference (MUAC)). Weight-for-length/height z-score **(WLZ/WHZ)**, weight-for-age z-score **(WAZ)**, length/height-for-age z-score **(LAZ/HAZ)** were determined using the WHO's anthropometric macro code for Stata [42].

Collection and transportation of fecal samples and E. coli culture

During enrolment in the parent trial, whole stool (or rectal swabs if whole stool was unavailable) was collected from all children. Samples were placed in Cary-Blair media to maintain bacterial integrity during transport for microbiological culture within 24 hours. Briefly, swabs or a sample of stool were streaked onto Mueller-Hinton (**MH**), MacConkey (**MAC**), and eosin methylene blue agars (**EMB**) and incubated in ambient air at 37°C for 18 – 24 hours. Results from differential media (MAC and EMB) were recorded and the isolation of *E. coli* was confirmed using the API 20E system (bioMérieux, Inc) and oxidase reactions as described previously [43]. Confirmed *E. coli* isolates were suspended in tryptic soy broth with 15% glycerol and frozen at -80°C. A random subset of enrolled children were chosen to participate in an AMR sub-study. A random number generator from Microsoft Excel was assigned to each child's patient identification (PID) number from the two main recruiting sites (Kisii and Homa Bay) and PIDs corresponding to random numbers of 0 and 5 were selected for this sub-study. In April 2019, the random selection changed to the selection of child PIDs with caregivers enrolled in an AMR study, whose selection was also based on a random number generator in excel. This switch in random-selection methods occurred due to a secondary aim added to the parent trial to evaluate resistance patterns in both children and their caregivers. *E. coli* isolates of selected children were subjected to Antimicrobial Susceptibility Testing (**AST**) using disc diffusion following methods described by the Clinical and Laboratory Standards Institute (**CLSI**) [44]. Isolates were thawed, and quadrant-streaked for isolation onto MAC and incubated at 37°C in ambient air. If more than one colony morphology was seen following restoration on MAC agar, isolates from each distinct morphology (up to 3) were separately subjected to AST. Having any *E. coli* isolates with non-susceptibility or ESBL-production was considered as indicative of AMR in the child.

Antimicrobial susceptibility testing

The overnight cultures of *E. coli* isolates were placed into 5ml of sterile normal saline and adjusted to be uniform with a 0.5 MacFarland standard. Bacterial diluents were used to homogenously cover the surfaces of MH agar using disposable sterile swabs. Prior to incubation, antibiotic discs were added on

top of the inoculated MHA using a disc dispenser, ensuring appropriate spacing between discs. The plates were placed in an incubator at 37°C in ambient air for 18 – 24 hours. Antibiotics tested included ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefoxitin, and imipenem; ciprofloxacin; gentamicin; amoxicillin/clavulanic acid; trimethoprim-sulfamethoxazole; azithromycin; and chloramphenicol. Zone diameters, measured in millimeters, established by CLSI-2020 M-100 (Table 2A, Zone Diameter and MIC Breakpoints for Enterobacteriaceae) were used to determine susceptibility, resistance, or an intermediate designation [44]. Both intermediate and resistant isolates were classified as resistant. As there are no established zone diameters for azithromycin in *E. coli,* we used standards for *Salmonella* enterica serovar Typhi.[4,44]

Determination of ESBL-producing E. coli

ESBL-production was determined using the double-disc diffusion test, which tests for ceftazidime and cefotaxime with and without clavulanic acid as previously described [12,45]. Quality control was assured by appropriate differences in zone sizes between the two selected antibiotics with and without clavulanic acid for ESBL-producing (NCTC 13351) and ESBL-negative (ATCC 25922) strains of *E. coli*. Specifically, suitable performance of the test the ESBL-negative QC strain of *E. coli* was confirmed if the difference in the zone size between ceftazidime and the zone size of ceftazidime with clavulanic acid was <5mm and if the difference in the zone size between cefotaxime and the zone size of cefotaxime with clavulanic acid was <5mm. Suitable performance of the test for the ESBL-producing (ESBL-positive) QC strain of *E. coli* was confirmed if the difference in the zone size between ceftazidime and the zone size of ceftazidime with clavulanic acid was ≥5mm and if the difference in the zone size between cefotaxime and the zone size of cefotaxime with clavulanic acid was ≥5mm as described in the CLSI guidelines.[44]

Ethical Considerations

The study was approved by the Institutional Review Boards of the Kenya Medical Research Institute (SERU 3086), the Kenyan Pharmacy and Poisons Board (Ref. No. PPB/ECCT/15/10/04), and the University of Washington (IRB# 49120). The parent trial was registered at ClinicalTrials.gov (Identifier: NCT02414399). Caregivers provided informed written consent in their preferred language (English,

Kiswahili, Kisii, or Luo). If a caregiver was not literate, information was read in the language of their choice and consent was obtained using a witnessed thumbprint.

Nested Study

This nested study included children who participated in the AMR sub-study of the parent trial and from whom *E. coli* were isolated at the enrollment visit.

Statistical Analysis

The prevalence of non-susceptibility to each antibiotic of interest was determined and confidence intervals (95%) calculated assuming a binomial distribution. Univariate Poisson regression using robust standard errors was used to determine risk factors for ESBL producing *E. coli*. Correlates of interest chosen a priori included child age (in months), HIV infection or exposure, length of hospitalization and antibiotic use in hospital, WAZ, LAZ/HAZ, WLZ/WHZ, enrollment hospital, household toilet type, water source and treatment, household crowding, and ownership of livestock (defined as cows, goats, sheep, pigs and/or chickens). Being referred from another hospital facility and having a hospitalization in the year prior to the one immediately preceding discharge were also examined as risk factors. Having an improved water source was determined by the reporting of water piped to the home or community, a borehole or tube well, spring water through a pipeline, a lined well with a pump or bucket or bottled water as a primary source of water. Household crowding was defined as >2 individuals to a room. Variables were chosen based on their association with AMR in previously published literature or due to clinical and biological plausibility.[7,25,26,46-51]

Multivariate Poisson regression models with robust standard errors were used to identify independent risk factors for the presence of ESBL producing *E. coli* at the time of discharge from hospital. Age, sex, and enrollment hospital were added to multivariable models as were variables that were found to be associated, at the alpha of 0.05 level, with the resistance outcome in univariate analyses. A separate analysis was conducted to determine whether resistance was associated with the type of sample provided, either whole stool or rectal swabs, to determine whether there may be differences depending

on the sample type (Appendix I). Multivariate Poisson regression models adjusting only for variables chosen a priori (age, sex, and hospital) were performed separately (Appendix II). Associations were considered statistically significant at an alpha of 0.05. Analyses were performed in Stata 14 (StataCorp, College Station, Texas).

RESULTS

Study Population

Children were enrolled in the parent trial between June 2016 and November 2019. Among the 448 children randomly selected to have AST performed, *E. coli* were identified in the fecal sample from 406 (90.6%). (Fig 1). The median age of the 406 included children was 19 months (interquartile range [IQR] 10 – 33 months), about 59.4% were male, 2.1% were HIV-infected, and 11% were HIV exposed, uninfected (HEU) (Table 1). During their hospitalization, most children received antibiotics (87.2%); in the subset of children who received antibiotics, the most common were penicillins (82.6%), ceftriaxone (71.6%), and gentamicin (62%). The most frequent diagnoses at admission and discharge were pneumonia, diarrhea, anemia, and malaria. The median length of hospital stay was 3 days (IQR 2 – 5 days). Children typically came from homes with improved water (84%) and pit latrines (87.9%), although more than half shared toilet facilities with another household. Almost half of the children lived in homes with more than 2 people to a room and most caregivers reported owning livestock (70.6%). The random selection of participants resulted in few significant differences in characteristics between children randomized to this study (Appendix III) or between children with and without *E. coli* isolated (Appendix IV).

Participants were included from Kisii Teaching & Referral Hospital (59.7%) and Homa Bay County Referral Hospital (40.3%). Children enrolled in Kisii were more likely to receive antibiotics during hospitalization (94.6%) compared to children enrolled in Homa Bay hospital (76.1%). Children enrolled at Kisii Teaching & Referral Hospital were also less likely to be HIV-exposed or infected or live in a household with a shared toilet, that was crowded, and that owned livestock (Table 1).

Burden of AMR *E. coli*

Of the 406 children included in this study, 94 had two morphologically distinct *E. coli* identified, and another 19 had three distinct morphologies tested, totaling 538 unique isolates. All 406 children harbored *E. coli* with reduced susceptibility to at least one of the tested antibiotics isolated from a fecal sample. Almost all (92.6%) children had *E. coli* isolated that lacked phenotypic susceptibility to ampicillin (95% Confidence Interval [CI]: 89.6% – 95.0%), while 43.8% (95% CI: 39% - 48.8%) of children harbored isolates resistant to gentamicin (Fig 2). Lack of susceptibility to ceftriaxone was common (46.1% [95% CI: 41.1% - 51.0%]) as was presence of ESBL (44.3% [95% CI: 39.4% - 49.3%]). Non-susceptibility to cephalosporins other than ceftriaxone was also commonly identified, with 46.1% (95% CI: 41.1% - 51.0%) of *E. coli* isolated from children had non-susceptibility to cefotaxime, and 41.4% (95% CI: 36.5% - 46.3%) to ceftazidime. There was relatively low resistance to cefoxitin identified (13.3% [95% CI: 10.2% - 17%]). About half of the children (50.6% (95% CI: 45.6% - 55.6%) had an *E. coli* isolate with reduced susceptibility to amoxicillin/clavulanic acid. Children also harbored *E. coli* with phenotypic non-susceptibility to less commonly used antibiotics including ciprofloxacin (46.8% [95% CI: 41.9% - 51.8%], azithromycin (37.7% [95% CI: 33% - 42.6%]), and chloramphenicol (22.7% [95% CI: 18.7% – 27.0%]). Only 3% (95% CI: 1.5% - 5.1%) of children were colonized with *E. coli* resistant to imipenem.

Risk Factors for ESBL Producing *E. coli* in Kenyan Children at Hospital Discharge. Receiving any antibiotic while hospitalized was positively associated with the presence of ESBL producing *E. coli* (adjusted prevalence ratio [aPR] = 2.23; 95% CI: 1.29 – 3.83, Table 2). Specifically, receiving ceftriaxone while hospitalized was positively associated with the presence of ESBL producing *E. coli* (aPR = 3.01; 95% CI: 1.78 – 5.09) compared to not receiving any antibiotics. Prior hospitalization in the previous year was also positively associated with ESBL producing *E. coli* (aPR 1.32 [1.07 – 1.69]) as were hospital stays of at least 4 days (aPR = 1.34; 95% CI 1.07 – 1.69). A diagnosis of gastroenteritis (aPR = 1.42; 95% CI 1.08 – 1.89) was associated with ESBL-producing *E. coli* being isolated from fecal samples. Being diagnosed with meningitis was also positively associated with ESBL-producing bacteria (crude prevalence ratio [cPR] = 1.26; 95% CI 1.01 – 1.56), as was a diagnosis of malaria (cPR = 1.26; 1.01 –

1.56). However, these associations were not seen in adjusted models (aPR 0.90; 0.68 – 1.19 and 1.20; 95% CI 0.94 – 1.53, respectively). Other hospital diagnoses, enrolling hospital, nor whole stool versus rectal swab collection (Appendix I) were associated with higher presence of ESBL producing *E. coli*. The practice of open defecation was associated with ESBL-producing *E. coli* (aPR = 2.02; 95% CI: 1.39 – 2.94), as was the sharing of sanitation facilities with other households (aPR = 1.49; 95% CI: 1.17 – 1.89). Living in crowded housing and availability of improved water source were also not associated with ESBL producing *E. coli*. Female sex was the only host characteristic with increased likelihood of isolating ESBL-producing *E. coli* (aPR = 1.42; 95% CI: 1.15 – 1.76).

When adjusting for variables chosen *a priori* (sex, age, and facility) female sex was no longer associated with carriage of ESBL *E. coli* (aPR = 1.20; 0.97 – 1.49) nor was sharing a toilet with at least 1 other household (aPR 1.16; 0.92 – 1.47) (Appendix II). In this secondary analysis, meningitis was associated with carriage of ESBL-producing *E. coli* in the adjusted model (aPR = 1.43; 1.09 – 1.87) while other diagnoses did not demonstrate any association. As in the primary multivariate analyses, hospitalization in the prior year (aPR = 1.39; 1.11 – 1.76), length of hospital stay of 4 days or greater (aPR 1.39; 1.11 – 1.74), receiving an antibiotic (aPR = 1.93; 1.19 – 3.12), receiving ceftriaxone (aPR = 2.66; 1.66 – 4.26), and open defecation (aPR = 1.66; 1.14 – 2.41) were associated with higher carriage of ESBL-producing *E. coli*.

DISCUSSION

In this study of children recently discharged from two hospitals in Western Kenya, a high burden of AMR was identified. AMR is associated with mortality, longer hospital stays, and high treatment costs [52]. Factors related to hospitalization, including recent antibiotic use, were strongly associated with ESBL-producing *E. coli*. Antibiotic use is common in this population of severely ill hospitalized children and complicated by a lack of available diagnostic tests to rule out non-bacterial causes. The high rates of AMR carriage may be particularly problematic during the post-discharge period, when children are at high risk of subsequent illness.

Most children in this study received antibiotics recommended in national and international guidelines for the management of common childhood infections, including beta-lactam and/or aminoglycoside antibiotics [10,15,53]. Recent receipt of an antibiotic more than doubled the likelihood of having ESBL-producing *E. coli* isolated, suggesting that the frequent use of these drugs in hospitals leads to significant drug pressure driving AMR, as has been described previously [10]. Lack of susceptibility to ceftriaxone, a commonly used second-line antibiotic for many infections, was present in over 40% of children with *E. coli* isolates. The presence of ESBL was similarly common. These resistance rates are similar to those reported among hospitalized children in other East African settings [36]. The high rate of ESBL documented here is concerning given how critical beta-lactam antibiotics are to treatment of common illnesses. In addition, the presence of these beta-lactamases are also associated with resistance to aminoglycosides, another antimicrobial class commonly used in many settings [13]. Being hospitalized within the prior year was also associated with carrying ESBL-producing *E. coli*. Children with prior hospitalization may have been more likely to have received antibiotics or to have been exposed to a community in which antibiotic use drove selection pressure.

We found 44.3% of children to harbor ESBL-producing *E. coli,* as determined by examining 3rd generation cephalosporins with and without the beta-lactamase inhibitor clavulanic acid. By contrast, resistance to individual 3rd generation cephalosporins tested ranged from 41.4% (ceftazidime) to 46.1% (ceftriaxone and cefotaxime). Although we report phenotypic resistance patterns for individual antibiotics, clinicians may avoid treating with any 3rd generation cephalosporin if there is resistance reported to another drug in the same class as the presence of broader resistance by ESBL-producing bacteria may result in the avoidance of most beta-lactams.

Nearly all (94%) of *E. coli* isolates were resistant to trimethoprim-sulfamethoxazole, despite only 2% of children having received trimethoprim-sulfamethoxazole during their hospital stay. Other studies in Sub-Saharan Africa have reported similarly high rates of trimethoprim-sulfamethoxazole resistance among children [35]. The high rate of resistance observed, despite the relatively low in-hospital use, likely reflects the widespread use of daily trimethoprim-sulfamethoxazole for persons living with HIV and for HIV-exposed children in many communities in the region [54]. Individual exposure may be less important

in driving trimethoprim-sulfamethoxazole resistance than exposure to widespread resistance in the community, as has been observed elsewhere [55].

We found female sex to be associated with ESBL-producing *E. coli* as has been previously reported [56]. However, this relationship has not been consistently observed in other studies in SSA [36]. In low resource settings, female children may be more likely to experience delays in care seeking [57], which may lead to more severe disease by the time of presentation. Additionally, female children may be more likely to participate in duties for the household, potentially resulting in greater exposure to contaminated environments. This may result in the need for extended antimicrobial therapies and longer exposure to the hospital environment.

Child age, living in a crowded household environment, use of an improved water source, access to treated water, and livestock ownership were not associated with AMR in this analysis, although these findings differ from those reported in other studies in SSA [17,25,38]. Sanitation and hygiene in the forms of open defecation or sharing a toilet with other households were related to carriage of ESBL-producing bacteria, suggesting that not all ESBL was acquired in hospital [25]. The fact that children recently discharged from hospital harbor high levels of bacteria resistant to multiple classes of antibiotics used for the treatment of common infectious syndromes is a major public health concern due to potential implications for the broader population given sharing of AMR organisms or mobile genetic elements to others in the household and community. Such sharing of AMR has been documented [58], and is likely most pronounced in crowded household environments with limited sanitation and hygiene facilities common in some rural SSA settings [2].

Although hospital-based antibiotic stewardship programs are a cornerstone of AMR prevention in many high-resource settings, in low-resource settings such programs may face additional challenges to implementation due to a reliance on syndromic diagnosis in the absence of diagnostic confirmatory testing, the limited availability of resistance testing and monitoring, difficulties in access to care and limited alternative antibiotic treatment options [15,59]. In SSA, antibiotics are available outside of hospital and clinical settings, which may reduce the effectiveness of hospital-based antibiotic stewardship programs in the region [26]. Some success has been seen with antibiotic stewardship programs in South Africa

without increasing mortality caused by infectious diseases, which may be an adverse consequence of limiting antibiotic use in these settings [60,61].

There were several strengths to this analysis. Few studies have examined carriage of AMR bacteria in children at hospital discharge. In addition, most studies focus on pathogenic bacteria although AMR transmission from commensal flora may be important to community spread [2]. Additionally, AMR may be contributing to the high rate of rehospitalization and death in the post-discharge period. The study also was conducted in a rural setting which adds to available data from major African cities where other studies have been conducted previously [34,36,38]. However, this study does have several limitations. By limiting our study population to children who survived hospitalization, we may have preferentially selected children less likely to be harboring resistance. Due to this study's cross-section design we are unable to differentiate AMR that was acquired prior to a child's hospitalization from that acquired in-hospital nor were we able to discern risk-factors that may be associated with carriage of ESBL-producing *E. coli* prior to discharge. However, hospitalizations in children under the age of 5 years are common in western Kenya, suggesting our study population may be relatively generalizable to a sizeable proportion of children in the region [20,62-64]. Future studies should include assessments of AMR at admission, discharge, and into the post-discharge period to further discern when resistant *E. coli* is acquired. We evaluated a relatively small number of isolates, which means we may have missed AMR present in non-sampled isolates from these children.

CONCLUSIONS

The prevalence of AMR in *E. coli* from children who have been hospitalized and subsequently discharged from hospital in Western Kenya is high. AMR is increasing globally and the public health relevance of these findings is likely significant, both for individual children and for communities. Limited laboratory capacities inhibit the ability to perform bacterial culture and AST, resulting in empiric antibiotic treatment from international and national guidelines. Health care exposure appears to be a major driver of AMR and interventions to prevent and reduce AMR transmission and acquisition in the health care

setting are urgently needed. Interventions are urgently needed that include the prevention of illnesses that lead to children being hospitalized and attention to alternative antimicrobial therapies for children who experience AMR-related clinical failure in LMIC settings.

Figure 1. Participant flow chart

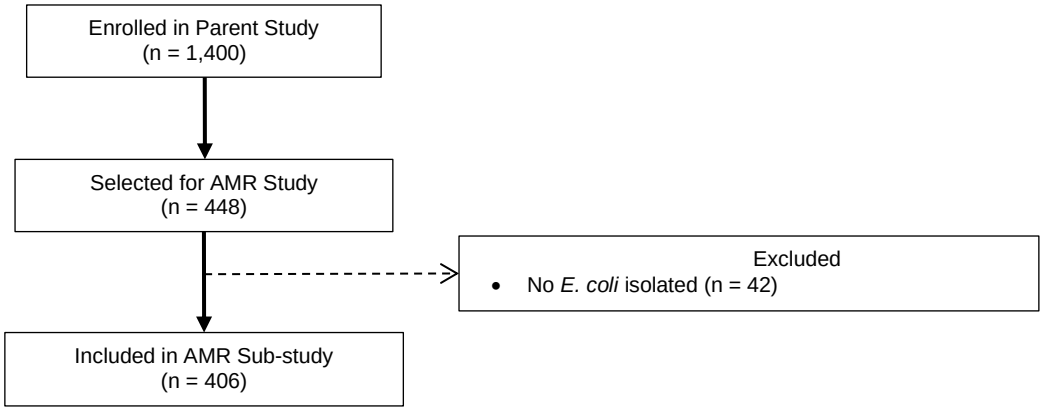


Table 1. Descriptive Characteristics.

	Total (n = 406) n (%)	Kisii (n = 242) n (%)	Homa Bay (n = 164) n (%)
Participant Characteristics			
Sex			
Male	241 (59.4%)	143 (59.1%)	97 (59.5%)
Female	165 (40.6%)	99 (40.9%)	66 (40.5%)
Age (months)			
24 and over	160 (39.5%)	93 (38.4%)	67 (41.1%)
12 – 23	121 (29.9%)	70 (28.9%)	51 (31.3%)
6 – 11	80 (19.8%)	50 (20.7%)	30 (18.4%)
1 – 5	44 (10.9%)	29 (12.0%)	15 (9.2%)
Breastfeeding[i]			
Exclusively Breastfed	184 (47.9%)	74 (33.3%)	110 (67.9%)
Partially Breastfed	195 (50.8%)	146 (65.8%)	49 (30.2%)
Never Breastfed	5 (1.3%)	2 (0.9%)	3 (1.9%)
HIV Status[ii]			
HIV Uninfected	339 (86.9%)	218 (93.2%)	121 (77.6%)
HIV Uninfected, Exposed	43 (11.0%)	13 (5.6%)	30 (19.2%)
HIV Infected	8 (2.1%)	3 (1.3%)	5 (3.2%)
Nutritional Characteristics[iii]			
Neither Stunted nor Wasted	271 (66.9%)	166 (68.6%)	105 (64.4%)
Wasted, not Stunted	34 (8.4%)	21 (8.7%)	13 (8.0%)
Stunted, not Wasted	89 (22.0%)	51 (21.1%)	38 (23.3%)
Stunted and Wasted	11 (2.7%)	4 (1.7%)	7 (4.3%)
Hospitalization Information			
Referred from another Heath Facility	106 (26.1%)	72 (29.8%)	34 (20.7%)
Hospitalized ≤1 Year Prior to this Hospitalization[iv]	84 (20.8%)	51 (21.2%)	33 (20.3%)
Length of Hospital Stay (in days)[vvi]	3 (2 – 5)	3 (2 – 5)	4 (2 – 6)
Received Antibiotic during Hospitalization	354 (87.2%)	229 (94.6%)	125 (76.2%)
Antibiotic Received during Hospitalization[vii]			
Penicillins	247 (82.6%)	182 (79.5%)	65 (52.4%)
Ceftriaxone	131 (71.6%)	70 (84.3%)	61 (61.0%)
Gentamicin	219 (62.0%)	168 (73.4%)	51 (41.1%)
Other	52 (14.7%)	29 (12.7%)	23 (18.6%)
Admitting Diagnosis[viii]			
Anemia	82 (20.3%)	29 (12.0%)	53 (32.5%)
Gastroenteritis/Diarrhea	82 (20.3%)	49 (20.3%)	33 (20.3%)
Malaria	191 (47.2%)	101 (47.7%)	90 (55.2%)
Meningitis	41 (10.1%)	29 (12.0%)	12 (7.4%)
Pneumonia/LRTI	146 (36.1%)	105 (43.4%)	41 (25.2%)
Sickle Cell	43 (10.6%)	10 (4.1%)	33 (20.3%)
Sepsis	13 (3.2%)	2 (0.8%)	11 (6.8%)
Tuberculosis	11 (2.7%)	5 (2.1%)	6 (3.7%)
URTI	28 (6.9%)	21 (8.7%)	7 (4.3%)
Other	15 (3.7%)	11 (4.6%)	4 (2.5%)
Prescribed Antibiotic at Discharge	242 (59.8%)	163 (67.4%)	79 (31.7%)
Household Information			
Crowding (>2 people/room)	192 (47.4%)	91 (37.6%)	101 (62.0%)
Livestock Ownership	286 (70.6%)	161 (66.5%)	125 (76.7%)
Improved Water Source (31)	340 (84.0%)	217 (89.7%)	123 (75.5%)
Treated Drinking Water[ix]	200 (49.9%)	82 (34.3%)	118 (72.8%)
Shared Toilet[x]	200 (49.4%)	114 (47.1%)	86 (52.8%)
Toilet Type			
Flushing	29 (7.2%)	23 (9.5%)	6 (3.7%)
Pit Latrine	356 (87.9%)	219 (90.5%)	137 (84.1%)
Open Defecation	20 (4.9%)	0 (0%)	20 (12.3%)

[i] Of those with data available (n = 384) Current breastfeeding for children ≤6 months; breastfeeding practiced when children were under 6 months; n = 21 unknown

[ii] Uninfected, Exposure Status unknown (n = 11), Exposed, infection status unknown (n = 4); Column percentages of children with exposure and infection status known (n = 390)

[iii] Wasted is defined as WHZ < -2 or MUAC <11.5cm while Stunted is determined by HAZ <-2; MUAC is only taken into consideration in children 6 months or older

[iv] Of the 404 with previous hospitalizations, if any, known

[v] Median and interquartile range provided

[vi] Of those with admission and discharge dates both available (n = 401)

[vii] Not mutually exclusive. Total n = 354 (87.2%) received antibiotics, column percentages are of these children. Other antibiotics given: azithromycin (n = 4), cefuroxime (n = 5), trimethoprim-cotrimoxazole (n = 10), chloramphenicol (n = 16), ciprofloxacin (n = 1), clarithromycin (n = 5), erythromycin (n = 1), tetracycline (n = 1), metronidazole (n = 18)

[viii] Not mutually exclusive. Other diagnoses at admission include: HIV (n = 2), UTI (n = 2), tuberculosis (n = 5), sepsis (n = 9), Poisoning/herbal toxicity (n = 3), asthma (n = 8), upper respiratory tract infection (n = 31), unknown (n = 15)

[ix] Among those who did not report use of bottled water and responded using filters, boiling, or chlorinating drinking water (n = 401)

[x] Shared Toilets are those used by more than 1 household and did not include open defecation (n = 20 all in Homa Bay) and excluding those who did not answer (n = 3)

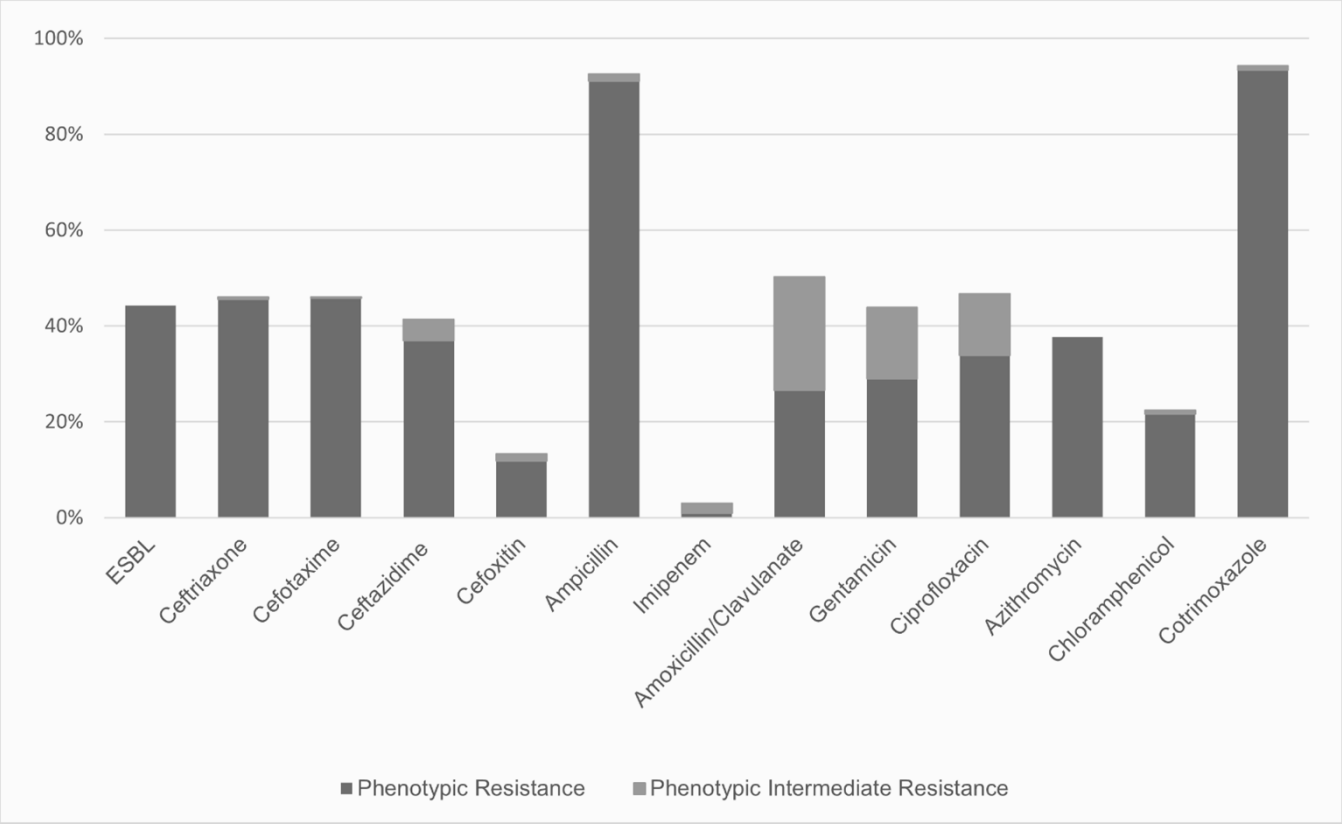


Fig 2. Prevalence of children with *E. coli* isolates at hospital discharge in Kenya resistant to selected antibiotics (N = 406). Resistant isolates are determined by the sizes of clearings around antibiotics measured using disc diffusion antimicrobial susceptibility testing and classified as “resistant” or “intermediate” by CLSI guidelines.

Table 2. Risk Factors for ESBL Producing *E. coli* from Fecal Samples

	ESBL + (N = 177) N (%)[ii]	ESBL - (N = 229) N (%)[ii]	Prevalence Ratios (95% CI)	p-value	Adjusted Prevalence Ratios (95% CI)[i]	p-value
Facility						
Kisii	100 (56.1%)	142 (62.4%)	REF.		REF.	
Homa Bay	77 (43.9%)	87 (37.6%)	1.13 (0.91 – 1.42)	0.26	0.87 (0.69 – 1.11)	0.26
Child Characteristics						
Sex						
Male	98 (55.4%)	143 (62.5%)	REF.		REF.	
Female	79 (44.6%)	86 (37.6%)	1.18 (0.94 – 1.47)	0.15	1.42 (1.15 – 1.76)	0.00***
Age (months)						
24 and over	76 (42.9%)	84 (36.7%)	REF.		REF.	
12 – 23	49 (27.7%)	73 (31.9%)	0.85 (0.64 – 1.11)	0.23	1.01 (0.77 – 1.33)	0.92
6 – 11	30 (17.0%)	50 (21.8%)	0.79 (0.57 – 1.09)	0.16	1.24 (0.92 – 1.68)	0.16
1 – 5	22 (12.4%)	22 (9.6%)	1.05 (0.75 – 1.48)	0.77	1.23 (0.75 – 1.81)	0.49
Breastfeeding[iii]						
Exclusively Breastfed	87 (52.1%)	98 (45.0%)	REF.		REF.	
Partially Breastfed	79 (47.3%)	116 (53.2%)	0.45 (0.07 – 2.74)	0.39	1.21 (0.74 – 1.98)	0.44
Never Breastfed	1 (0.6%)	4 (1.8%)	0.91 (0.58 – 1.44)	0.69	0.64 (0.13 – 3.04)	0.58
HIV[iv]						
HIV Uninfected	150 (88.2%)	190 (86.0%)	REF.			
HIV Uninfected, Exposed	15 (8.8%)	28 (12.7%)	0.79 (0.52 – 1.21)	0.28	0.70 (0.41 – 1.21)	0.20
HIV Infected	5 (2.9%)	3 (1.4%)	1.42 (0.82 – 2.45)	0.22	1.45 (0.91 – 2.31)	0.12
Nutritional Characteristics						
Neither stunted nor wasted	119 (67.2%)	153 (66.8%)	REF.			
Wasted, not Stunted	12 (6.8%)	22 (9.6%)	0.81 (0.50 – 1.30)	0.38	0.75 (0.46 – 1.21)	0.24
Stunted, not Wasted	40 (22.6%)	49 (21.4%)	1.03 (0.79 – 1.34)	0.84	1.03 (0.76 – 1.40)	0.84
Stunted and Wasted	6 (3.4%)	5 (2.2%)	1.25 (0.71 – 2.18)	0.44	0.81 (0.31 – 2.11)	0.67
Hospitalization Information						
Referred from Another Health Facility						
No	125 (70.6%)	175 (76.4%)	REF.		REF.	
Yes	52 (29.4%)	54 (23.6%)	1.18 (0.93 – 1.49)	0.18	0.77 (0.53 – 1.12)	0.17
Hospitalizations in the Prior Year[v]						
No	132 (72.9%)	189 (84.8%)	REF.		REF.	
Yes	49 (27.1%)	34 (15.3%)	1.41 (1.13 – 1.77)	0.00***	1.32 (1.07 – 1.64)	0.01*
Length of Hospital Stay (in days)[vi]						
< 4	78 (44.8%)	135 (59.2%)	REF		REF.	
≥ 4	96 (55.2%)	93 (40.8%)	1.40 (1.12 – 1.75)	0.03***	1.34 (1.07 – 1.69)	0.01*
Received Antibiotic During Hospitalization						
No	12 (6.8%)	40 (17.5%)	REF.		REF.	
Yes	165 (93.2%)	189 (82.5%)	2.02 (1.21 – 3.36)	0.01***	2.23 (1.29 – 3.83)	0.00***
Antibiotic Received During Hospitalization[vii]						
Penicillins	92 (88.5%)	155 (79.5%)	1.61 (0.96 – 2.72)	0.07	1.73 (0.96 – 3.12)	0.07
Ceftriaxone	95 (88.8%)	36 (47.4%)	3.14 (1.90 – 5.23)	0.00***	3.01 (1.78 – 5.09)	0.00***
Gentamicin	81 (87.1%)	138 (77.5%)	1.69 (0.95 – 2.71)	0.08	1.68 (0.93 – 3.06)	0.09
Admitting Diagnosis[viii]						
Anemia	39 (22.0%)	44 (19.2%)	1.07 (0.82 – 1.39)	0.62	1.05 (0.81 – 1.34)	0.72
Gastroenteritis/Diarrhea	31 (17.5%)	52 (22.7%)	0.84 (0.62 – 1.12)	0.24	1.42 (1.08 – 1.89)	0.01*
Malaria	94 (53.1%)	98 (42.8%)	1.26 (1.01 – 1.56)	0.04*	1.20 (0.94 – 1.53)	0.15
Meningitis	25 (12.1%)	16 (7.0%)	1.43 (1.09 – 1.87)	0.01*	0.90 (0.68 – 1.19)	0.45
Pneumonia/LRTI	55 (31.1%)	91 (39.7%)	0.82 (0.64 – 1.04)	0.10	0.91 (0.70 – 1.18)	0.46
Household Information						
Crowding						
No	97 (54.8%)	117 (51.1%)	REF.		REF.	
Yes	80 (45.2%)	112 (48.9%)	0.92 (0.74 – 1.15)	0.46	1.02 (0.83 – 1.25)	0.88
Livestock Ownership						
No	59 (33.3%)	61 (26.6%)	REF.		REF.	
Yes	118 (66.7%)	168 (73.4%)	0.84 (0.67 – 1.05)	0.13	0.93 (0.52 – 1.16)	0.52
Improved Water Source						
No	28 (15.8%)	37 (16.2%)	REF.		REF.	
Yes	149 (84.2%)	192 (83.8%)	1.01 (0.75 – 1.38)	0.93	1.07 (0.82 – 1.39)	0.62
Treated Drinking Water[ix]						
No	89 (49.4%)	112 (50.7%)	REF.		REF.	
Yes	91 (50.6%)	109 (49.3%)	1.02 (0.83 – 1.28)	0.81	0.91 (0.72 – 1.16)	0.45

Toilet[x]								
Private, for Household Only	73	(40.6%)	109	(49.1%)	REF.		REF.	
Shared with ≥1 Other Household	93	(51.7%)	107	(48.2%)	1.16 (0.92 – 1.46)	0.21	1.49 (1.17 – 1.89)	0.00***
Open Defecation	14	(7.8%)	6	(2.7%)	1.75 (1.24 – 2.35)	0.00***	2.02 (1.39 – 2.94)	0.00***

[i] Adjusted for a priori determined potential confounders (facility, age, gender) in addition to variables deemed associated in univariate models at the p ≤.05 level (length of hospital stay, in-hospital use of cephalosporins, whether any hospitalizations occurred within the year preceding enrollment, toilet, and diagnosis of meningitis or malaria at admission). The multivariable model did not include whether or not an antibiotic was received in the hospital due to potential collinearity with cephalosporin use.

[ii] Column Percentages shown

[iii] Current breastfeeding for children ≤6 months and breastfeeding practiced when children were under 6 months; n = 21 unknown

[iv] Uninfected, Exposure Status unknown (n = 11), Exposed, infection status unknown (n = 4); Column percentages of children with exposure and infection status known (n = 390)

[v] Of the 404 with previous hospitalizations, if any, known

[vi] Among those with both admission and discharge dates available (n = 401)

[vii] Reference group are those who did not receive an antibiotic (n = 52), which make up the denominator of the column percentage shown (12 of those who did not receive an antibiotic had ESBL-producing *E. coli* isolated, while 38 did not have ESBL-producing isolates). Antibiotics given in hospital were not mutually exclusive. Other antibiotics given: azithromycin (n = 4), cefuroxime (n = 5), trimethoprim-cotrimoxazole (n = 10), chloramphenicol (n = 16), ciprofloxacin (n = 1), clarithromycin (n = 5), erythromycin (n = 1), tetracycline (n = 1), metronidazole (n = 18). Of the antibiotics tested: 247 were given a penicillin class antibiotic(s). Of these, a total of 155 did not have ESBL-producing *E. coli* isolated, while 92 given a penicillin drug had ESBL-producing *E. coli* isolated. 131 were given ceftriaxone; 36 did not have ESBL-producing bacteria isolated, while 95 did. 219 children were given gentamicin, 63% of whom did not have ESBL-producing bacteria. 53 children received antibiotics not belonging to the prior three classes, 25 had ESBL-producing E. coli isolated.

[viii] Not mutually exclusive. The reference group for each diagnosis from the time of admission is not having the corresponding diagnosis. Other diagnoses at admission include: asthma (n = 7), HIV (n = 2), poisoning/herbal toxicity (n = 4), UTI (n = 2)

[ix] Treated water included those who did not report use of bottled water and reported filters, boiling, or chlorinating to treat drinking water. The comparison group was those who did not treat drinking water and did not use bottled water (n = 401)

[x] Of those who answered (n = 402)

***Significant at an alpha of 0.01 *Significant at an alpha of 0.05

Appendix I. Association of swabs or whole stool and the identification of ESBL-Producing *E. coli*.

	ESBL Producing		ESBL Negative		Prevalence Ratio (95% CI)	p-value
	N	(%)	N	(%)		
ESBL						
Whole Stool	21	(11.9%)	48	(21.0%)	REF.	
Rectal Swab	156	(88.1%)	181	(79.0%)	1.27 (0.87 – 1.86)	0.22

Appendix II. Risk Factors for ESBL Producing *E. coli* from Fecal Samples with Multivariate Models adjusting solely for *a priori* defined variables

	ESBL + (N = 177) N (%)[ii]	ESBL - (N = 229) N (%)[i]	Prevalence Ratio (95% CI)	p-value	Adjusted Prevalence Ratio (95% CI)[i]	p-value
Facility						
Kisii	100 (56.1%)	142 (62.4%)	REF.		REF.	
Homa Bay	77 (43.9%)	87 (37.6%)	1.13 (0.91 – 1.42)	0.26	1.14 (0.91 – 1.41)	0.25
Child Characteristics						
Sex						
Male	98 (55.4%)	143 (62.5%)	REF.		REF.	
Female	79 (44.6%)	86 (37.6%)	1.18 (0.94 – 1.47)	0.15	1.20 (0.97 – 1.49)	0.09
Age (months)						
24 and over	76 (42.9%)	84 (36.7%)	REF.		REF.	
12 – 23	49 (27.7%)	73 (31.9%)	0.85 (0.64 – 1.11)	0.23	1.03 (0.67 – 1.14)	0.32
6 – 11	30 (17.0%)	50 (21.8%)	0.79 (0.57 – 1.09)	0.16	1.08 (0.62 – 1.88)	0.39
1 – 5	22 (12.4%)	22 (9.6%)	1.05 (0.75 – 1.48)	0.77	1.50 (0.80 – 2.81)	0.52
Breastfeeding[iii]						
Exclusively Breastfed	87 (52.1%)	98 (45.0%)	REF.		REF.	
Partially Breastfed	79 (47.3%)	116 (53.2%)	0.45 (0.07 – 2.74)	0.39	0.90 (0.57 – 1.41)	0.64
Never Breastfed	1 (0.6%)	4 (1.8%)	0.91 (0.58 – 1.44)	0.69	0.43 (0.07 – 2.61)	0.36
HIV[iv]						
HIV Uninfected	150 (88.2%)	190 (86.0%)	REF.			
HIV Uninfected, Exposed	15 (8.8%)	28 (12.7%)	0.79 (0.52 – 1.21)	0.28	0.74 (0.48 – 1.13)	0.16
HIV Infected	5 (2.9%)	3 (1.4%)	1.42 (0.82 – 2.45)	0.22	1.23 (0.71 – 2.13)	0.47
Nutritional Characteristics						
Neither stunted nor wasted	119 (67.2%)	153 (66.8%)	REF.			
Wasted, not Stunted	12 (6.8%)	22 (9.6%)	0.81 (0.50 – 1.30)	0.38	0.78 (0.48 – 1.26)	0.32
Stunted, not Wasted	40 (22.6%)	49 (21.4%)	1.03 (0.79 – 1.34)	0.84	1.02 (0.78 – 1.33)	0.88
Stunted and Wasted	6 (3.4%)	5 (2.2%)	1.25 (0.71 – 2.18)	0.44	1.22 (0.72 – 2.04)	0.46
Hospitalization Information						
Referred from Another Health Facility						
No	125 (70.6%)	175 (76.4%)	REF.		REF.	
Yes	52 (29.4%)	54 (23.6%)	1.18 (0.93 – 1.49)	0.18	1.30 (0.95 – 1.79)	0.10
Hospitalization within the Prior Year[v]						
No	132 (72.9%)	189 (84.8%)	REF.		REF.	
Yes	49 (27.1%)	34 (15.3%)	1.41 (1.13 – 1.77)	0.00***	1.39 (1.11 – 1.76)	0.01***
Length of Hospital Stay (in days)						
< 4	78 (44.8%)	135 (59.2%)	REF		REF.	
≥ 4	96 (55.2%)	93 (40.8%)	1.40 (1.12 – 1.75)	0.03***	1.39 (1.11 – 1.74)	0.00***
Received Antibiotic During Hospitalization						
No	12 (6.8%)	40 (17.5%)	REF.		REF.	
Yes	165 (93.2%)	189 (82.5%)	2.02 (1.21 – 3.36)	0.01***	1.93 (1.19 – 3.12)	0.01***
Antibiotic Received During Hospitalization[vi]						
Penicillins	92 (88.5%)	155 (79.5%)	1.61 (0.96 – 2.72)	0.07	1.48 (0.87 – 2.52)	0.15
Ceftriaxone	95 (88.8%)	36 (47.4%)	3.14 (1.90 – 5.23)	0.00***	2.66 (1.66 – 4.26)	0.00***
Gentamicin	81 (87.1%)	138 (77.5%)	1.69 (0.95 – 2.71)	0.08	1.40 (0.81 – 2.44)	0.23
Admitting Diagnosis[vii]						
Anemia	39 (22.0%)	44 (19.2%)	1.07 (0.82 – 1.39)	0.62	1.04 (0.79 – 1.36)	0.78
Gastroenteritis/Diarrhea	31 (17.5%)	52 (22.7%)	0.84 (0.62 – 1.12)	0.24	0.86 (0.63 – 1.16)	0.31
Malaria	94 (53.1%)	98 (42.8%)	1.01 (1.01 – 1.56)	0.04*	1.23 (0.99 – 1.54)	0.07
Meningitis	25 (12.1%)	16 (7.0%)	1.43 (1.09 – 1.87)	0.01*	1.43 (1.09 – 1.87)	0.01***
Pneumonia/LRTI	55 (31.1%)	91 (39.7%)	0.82 (0.64 – 1.04)	0.10	0.85 (0.66 – 1.09)	0.20
Household Information						
Crowding						
No	97 (54.8%)	117 (51.1%)	REF.		REF.	
Yes	80 (45.2%)	112 (48.9%)	0.92 (0.74 – 1.15)	0.46	0.89 (0.71 – 1.11)	0.30
Livestock Ownership						
No	59 (33.3%)	61 (26.6%)	REF.		REF.	
Yes	118 (66.7%)	168 (73.4%)	0.84 (0.67 – 1.05)	0.13	0.83 (0.66 – 1.05)	0.11
Improved Water Source						
No	28 (15.8%)	37 (16.2%)	REF.		REF.	
Yes	149 (84.2%)	192 (83.8%)	1.01 (0.75 – 1.38)	0.93	0.96 (0.72 – 1.28)	0.78
Treated Drinking Water						
No	89 (49.4%)	112 (50.7%)	REF.		REF.	

Yes	91	(50.6%)	109	(49.3%)	1.02 (0.83 – 1.28)	0.81	0.97 (0.77 – 1.23)	0.82
Toilet[viii]								
Private, for Household Only	71	(43.8%)	111	(50.5%)	REF.		REF.	
Shared with ≥1 Other Household	91	(56.2%)	109	(49.6%)	1.16 (0.92 – 1.47)	0.21	1.16 (0.92 – 1.47)	0.21
Open Defecation					1.66 (1.14 - .2.41)	0.01***	1.66 (1.14 – 2.41)	0.01***

[i] Adjusted for a priori determined potential confounders (facility, age, sex).
[ii] Column Percentages shown
[iii] Current breastfeeding for children ≤6 months and breastfeeding practiced when children were under 6 months; n = 21 unknown
[iv] Uninfected, Exposure Status unknown (n = 11), Exposed, infection status unknown (n = 4); Column percentages of children with exposure and infection status known (n = 390)
[v] Of those with hospitalizations in the prior year known (n = 404)
[vi] Reference group are those who did not receive an antibiotic (n = 52), which make up the denominator of the column percentage shown (12 of those who did not receive an antibiotic had ESBL-producing *E. coli* isolated, while 38 did not have ESBL-producing isolates). Antibiotics given in hospital were not mutually exclusive. Other antibiotics given: azithromycin (n = 4), cefuroxime (n = 5), trimethoprim-cotrimoxazole (n = 10), chloramphenicol (n = 16), ciprofloxacin (n = 1), clarithromycin (n = 5), erythromycin (n = 1), tetracycline (n = 1), metronidazole (n = 18). Of the antibiotics tested: 247 were given a penicillin class antibiotic(s). Of these, a total of 155 did not have ESBL-producing *E. coli* isolated, while 92 given a penicillin drug had ESBL-producing *E. coli* isolated. 131 were given ceftriaxone; 36 did not have ESBL-producing bacteria isolated, while 95 did. 219 children were given gentamicin, 63% of whom did not have ESBL-producing bacteria. 53 children received antibiotics not belonging to the prior three classes, 25 had ESBL-producing E. coli isolated.
[vii] Not mutually exclusive. The reference group for each diagnosis is not having the corresponding diagnosis. Other diagnoses at admission include: asthma (n = 7), HIV (n = 2), poisoning/herbal toxicity (n = 4), UTI (n = 2)
[viii] Of those who do not practice open defecation (n = 381)
***Significant at an alpha of 0.01 *Significant at an alpha of 0.05

Appendix III. Comparison of characteristics of children selected for the AMR study compared to the other children in the parent study eligible for sampling

	Selected for AST (n = 455) n (%)	Not Selected for AST (n = 890) n (%)	p-value
Participant Characteristics			
Sex			
Male	271 (59.6%)	526 (59.1%)	0.87
Female	184 (40.4%)	364 (40.9%)	
Age (months)			
24 and over	170 (37.4%)	332 (37.3%)	
12 – 23	140 (30.8%)	253 (28.4%)	0.61
6 – 11	90 (19.8%)	176 (19.8%)	
1 – 5	55 (12.1%)	129 (14.5%)	
Breastfeeding[i]			
Exclusively Breastfed	217 (50.2%)	443 (50.2%)	
Partially Breastfed	210 (48.6%)	421 (47.7%)	0.42
Never Breastfed	5 (1.2%)	19 (2.2%)	
HIV Status[ii]			
HIV Uninfected	381 (87.0%)	763 (88.8%)	
HIV Uninfected, Exposed	47 (10.7%)	88 (10.2%)	0.14
HIV Infected	10 (2.3%)	8 (0.9%)	
Nutritional Characteristics[iii]			
Neither Stunted nor Wasted	304 (66.8%)	668 (75.1%)	
Wasted, not Stunted	39 (8.6%)	62 (8.6%)	0.01*
Stunted, not Wasted	101 (22.2%)	141 (22.2%)	
Stunted and Wasted	11 (2.4%)	19 (2.4%)	
Hospitalization Information			
Length of Hospital Stay (in days)[iv,v]	3 (2 – 5)	3 (2 – 5)	0.95
Received Antibiotic during Hospitalization	401 (88.3%)	801 (90.0%)	0.29
Antibiotic Received during Hospitalization[vi]			
Penicillins	278 (69.3%)	551 (68.8%)	0.85
Ceftriaxone	150 (37.4%)	362 (45.2%)	0.01*
Gentamicin	250 (62.3%)	482 (60.2%)	0.47
Other	66 (16.5%)	119 (14.9%)	0.47
Admitting Diagnosis[vii]			
Anemia	93 (20.4%)	145 (16.3%)	0.06
Gastroenteritis/Diarrhea	94 (20.7%)	163 (18.3%)	0.30
Malaria	239 (52.5%)	452 (50.8%)	0.55
Meningitis	49 (10.8%)	115 (12.9%)	0.25
Pneumonia/LRTI	164 (36.0%)	356 (40.0%)	0.16
Sickle Cell	49 (10.8%)	71 (8.0%)	0.09
Suspected Sepsis	17 (3.7%)	55 (6.2%)	0.06
Tuberculosis	11 (2.4%)	28 (3.2%)	0.45
Other	15 (3.3%)	55 (6.2%)	0.02*
Prescribed Antibiotic at Discharge	275 (60.4%)	558 (62.7%)	0.42
Household Information			
Crowding (>2 people/room)	211 (46.4%)	407 (45.7%)	0.82
Livestock Ownership	316 (69.5%)	587 (66.0%)	0.28
Improved Water Source[viii] (31)	379 (83.3%)	728 (81.8%)	0.50
Shared Toilet[ix]	225 (52.5%)	389 (45.5%)	0.02*
Toilet Type			
Flushing	37 (8.1%)	83 (9.3%)	
Pit Latrine	398 (87.5%)	774 (87.1%)	0.33
Open Defecation	20 (4.4%)	32 (3.6%)	

[i] Of those with data available (n = 1,268) Current breastfeeding for children ≤6 months or breastfeeding practiced when children were under 6 months; n = 77 unknown

[ii] Uninfected, Exposure Status unknown (n = 41), Exposed, infection status unknown (n = 7); Column percentages of children with exposure and infection status known (n = 1,297)

[iii] Wasted is defined as WHZ < -2 or MUAC <11.5cm while Stunted is determined by HAZ <-2; MUAC is only taken into consideration in children 6 months or older

[iv] Median and interquartile range provided

[v] Of those with admission and discharge dates both available (n = 1,336)

[vi] Not mutually exclusive. Total n = 1,202 (89.4%) received antibiotics, column percentages are of these children. Other antibiotics given: azithromycin (n = 23), co-amoxiclav (n = 1), cefuroxime (n = 16), trimethoprim-sulfamethoxazole (n = 22), chloramphenicol (n = 41), ciprofloxacin (n = 8), clarithromycin (n = 22), erythromycin (n = 7), tetracycline (n = 6), metronidazole (n = 60)

[vii] Not mutually exclusive. Other diagnoses at admission include: HIV (n = 5), urinary tract infection (n = 16), poisoning/herbal toxicity (n = 14), asthma (n = 34), upper respiratory tract infection (n = 91), fever of unknown origin (n = 2), and unknown (n = 70)

[viii] Those had drinking water included participants who did not report use of bottled water and responded using filters, boiling, or chlorinating drinking water

[ix] Shared Toilets are those used by more than 1 household and did not include open defecation (n = 52) and excluding those who did not answer (n = 9)

Appendix IV. Comparison of characteristics of children selected for the AMR study with *E. coli* compared to children without *E. coli* isolated from fecal samples

	AST n	(n = 406) (%)	No AST n	(n = 42) (%)	p-value
Participant Characteristics					
Sex					
Male	241	(59.4%)	25	(59.5%)	0.98
Female	165	(40.6%)	17	(40.5%)	
Age (months)					
24 and over	160	(39.4%)	8	(19.1%)	
12 – 23	122	(30.1%)	15	(35.7%)	0.02*
6 – 11	80	(19.7%)	9	(21.4%)	
1 – 5	44	(10.8%)	10	(23.8%)	
Breastfeeding[i]					
Exclusively Breastfed	185	(48.1%)	28	(68.3%)	
Partially Breastfed	195	(50.7%)	13	(31.7%)	0.04
Never Breastfed	5	(1.3%)	0	(0%)	
HIV Status[ii]					
HIV Uninfected	340	(87.0%)	35	(87.5%)	
HIV Uninfected, Exposed	43	(11.0%)	4	(10.0%)	0.97
HIV Infected	8	(2.1%)	1	(2.5%)	
Nutritional Characteristics[iii]					
Neither Stunted nor Wasted	273	(67.2%)	30	(67.0%)	
Wasted, not Stunted	34	(8.4%)	3	(7.1%)	0.03*
Stunted, not Wasted	88	(21.7%)	9	(21.4%)	
Stunted and Wasted	11	(2.7%)	0	(2.0%)	
Hospitalization Information					
Length of Hospital Stay (in days)[ivv]	3	(2 – 5)	4	(2 – 5)	0.26
Received Antibiotic during Hospitalization	354	(87.2%)	41	(97.6%)	0.046*
Antibiotic Received during Hospitalization[vi]					
Penicillins	247	(69.8%)	25	(96.2%)	0.07
Ceftriaxone	133	(77.6%)	19	(95.0%)	0.03*
Gentamicin	219	(61.9%)	25	(96.2%)	0.05
Other	53	(15.0%)	9	(90.0%)	0.75
Admitting Diagnosis[vii]					
Anemia	83	(20.4%)	8	(19.1%)	0.83
Gastroenteritis/Diarrhea	83	(20.4%)	9	(21.4%)	0.88
Malaria	192	(47.3%)	21	(50.0%)	0.74
Meningitis	41	(10.1%)	8	(19.1%)	0.08
Pneumonia/LRTI	146	(36.0%)	14	(33.3%)	0.74
Sickle Cell	43	(10.6%)	6	(14.3%)	0.47
Suspected Sepsis	13	(3.2%)	4	(9.5%)	0.04*
Tuberculosis	11	(2.7%)	0	(0%)	0.79
URTI	28	(6.9%)	3	(7.1%)	0.95
Other	15	(3.7%)	8	(19.1%)	0.83
Prescribed Antibiotic at Discharge	242	(59.8%)	29	(69.1%)	0.23
Household Information					
Crowding (>2 people/room)	192	(47.3%)	17	(40.5%)	0.40
Livestock Ownership	286	(70.4%)	26	(61.9%)	0.25
Improved Water Source[65]	341	(84.0%)	32	(76.2%)	0.20
Shared Toilet[viii]	200	(52.4%)	414	(45.9%)	0.03*
Toilet Type					
Flushing	29	(7.1%)	7	(9.7%)	
Pit Latrine	357	(87.9%)	33	(86.8%)	0.03
Open Defecation	20	(4.9%)	0	(0%)	

[i] Of those with data available (n = 426) Current breastfeeding for children ≤6 months; breastfeeding practiced when children were under 6 months; n = 2 unknown

[ii] Uninfected, Exposure Status unknown (n = 13), Exposed, infection status unknown (n = 4); Column percentages of children with exposure and infection status known (n = 431)

[iii] Wasted is defined as WHZ < -2 or MUAC <11.5cm while Stunted is determined by HAZ <-2; MUAC is only taken into consideration in children 6 months or older

[iv] Median and interquartile range provided

[v] Of those with admission and discharge dates both available (n = 443)

[vi] Not mutually exclusive. Total n = 395 (88.2%) received antibiotics, column percentages are of these children. Other antibiotics given: azithromycin (n = 4), co-amoxiclav (n = 1), cefuroxime (n = 5), trimethoprim-sulfamethoxazole (n = 10), chloramphenicol (n = 17), ciprofloxacin (n = 2), clarithromycin (n = 22), erythromycin (n = 2), tetracycline (n = 2), metronidazole (n = 23)

[vii] Not mutually exclusive. Other diagnoses at admission include: HIV (n = 2), urinary tract infection (n = 2), poisoning/herbal toxicity (n = 4), asthma (n = 7), upper respiratory tract infection (n = 31),

[viii] Shared Toilets are those used by more than 1 household and did not include open defecation (n = 20) and excluding those who did not answer (n = 6)

Chapter 2: Sharing of Antimicrobial Resistance among Hospitalized Children and their Caregivers in Kenya

INTRODUCTION

Antimicrobial resistance **(AMR)** has emerged as a major public health threat, responsible for millions of global deaths in 2019.[1] The burden of AMR is highest in sub-Saharan Africa **(SSA),** due primarily to the high incidence of infectious diseases in these settings.[1,26,34,66] Children who have been hospitalized are at a particularly high risk of developing or becoming infected with resistance-harboring bacteria, as a direct result of antibiotic pressure leading to the selection of genetic mutations that confer resistance, exchanges of resistant genetic material between bacterial communities and the transmission of AMR carrying bacteria or genes from the community or within the hospital environment.[14,67-69] Caregivers of hospitalized children may also be at high risk of AMR as a result of spending time within the hospital setting, where surfaces, and other parts of the hospital environment, can spread AMR bacteria. [70,71] The close proximity to their hospitalized child, as well as other children potentially carrying AMR bacteria, may also contribute to caregiver risk of AMR.[72] Hospitalized children and their caregivers may serve as important reservoirs of community spread of AMR upon their return to the community. Such spread may be particularly common in settings of crowded living conditions and a lack of access to suitable sanitation and hygiene facilities.

While AMR is often measured in pathogens isolated in clinical settings to guide patient management, AMR can also be detected in commensal bacterial communities.[68,73] Some evidence suggests that commensal carriage of AMR may be a proxy for AMR detection among bacterial pathogens in the host.[14,33,68] *Escherichia coli* **(*E. coli*)** are commensal bacteria ubiquitous to the gastrointestinal systems of humans that have both pathogenic potential and may be a reservoir of AMR-conferring genes for other pathogenic bacteria. We isolated *E. coli* from fecal samples of hospitalized children and their primary caregivers and evaluated the presence of resistance to commonly used antibiotics. We sought to determine whether hospitalized children and their primary caregivers had similar patterns of AMR. We were particularly interested in the presence of extended-spectrum beta-lactamase **(ESBL)** producing *E.*

coli, as ESBL are associated with resistance to most beta-lactams and often co-occur with resistance to other classes of antibiotics including fluoroquinolones and aminoglycosides.[12,74] In addition, we identified characteristics and environmental factors associated with concordant carriage of ESBL-producing *E. coli* in child-caregiver pairs and evaluated risk factors for carriage of ESBL producing *E. coli* among caregivers.

METHODS

Study Design

This cross-sectional study was nested within a clinical trial evaluating the impact of azithromycin to reduce hospitalization and death among young children discharged from hospital in Kenya.[4,41] We evaluated the antimicrobial susceptibility patterns of *E. coli* isolated from hospitalized children and their primary caregivers at the time of discharge and prior to the administration of the study drug. Interviews were conducted with caregivers to collect clinical, sociodemographic, and child health history information in addition to physical exams of the children. The protocol and primary analysis of the parent trial have been published.[4,41]

Parent Trial

Population

At the time of hospital discharge, children aged 1 – 59 months were enrolled from Kisii and Homa Bay County Teaching and Referral hospitals in western Kenya. The parent study examined whether a 5-day course of azithromycin reduces rehospitalization and/or death in the subsequent 6-month period.[41,4] Children were considered eligible if they weighed at least 2kg, had been hospitalized and subsequently discharged, planned to stay in the area for a minimum of 6 months, and were not prescribed macrolide antibiotics at discharge.[4,41] Reasons for exclusion included hospitalization solely due to trauma, poisoning, or a congenital syndrome. Having a twin of the same sex enrolled on the same day, failure to obtain consent from a legal guardian, contraindications to azithromycin, discharge with a prescription to

a macrolide drug, or taking a protease inhibitor were also reasons for exclusion.[4,41] A random subset of caregivers, either a parent or legal guardian, were enrolled at the same time as the index children.[41]

Data Collection

Standardized questionnaires were used to collect sociodemographic information and relevant medical history of both the child and caregiver after informed consent was provided. Relevant medical information and procedures from the child's hospitalization were extracted from medical records and a study clinician performed a physical examination of enrolled children. Body mass index **(BMI)** was calculated using caregiver weight and height. Anthropometric measures appropriate for the child's age: either length, if less than 24 months, or height, weight, and middle upper arm circumference **(MUAC)** were measured. Weight-for-length/height z-score **(WLZ/WHZ)**, weight-for-age z-score **(WAZ)**, length/height-for-age z-score **(LAZ/HAZ)** were determined using the macro code for STATA from the WHO to determine anthropometry.[42]

Collection and transport of fecal samples

Whole stool or rectal swabs (FLOQSwabs, Copan Diagnostics), when whole stool was not available, were collected from all enrolled children and the subset of enrolled caregivers at the time of the child's discharge from hospital. Samples were immediately placed in Cary-Blair media, maintained between 2 - 8°C, and shipped to the Center for Microbiology at the Kenya Medical Research Institute **(KEMRI CMR)** within 24 hours of collection for microbial culture. The fecal samples were first plated on Mueller-Hinton **(MH)** agar followed by MacConkey **(MAC)** and eosin methylene blue **(EMB)** agars. All agar plates were incubated at 37°C in ambient air for 18 hours. Agar plates were prepared in the laboratory according to manufacturer's instructions. Quality control was done by checking the sterility of the agar plates by incubating a clean plate for 24 hours at 37°C to ensure no contaminants grew. The ability to support the growth of bacteria was also tested by plating *E. coli* and *Salmonella* on MH agar plates. These were incubated for 18-24 hours at 37°C to verify the media would support the growth of *Enterobacteriaceae*. Culture results from the selective and differential media (MAC and EMB) were recorded and isolated *E. coli* colonies were confirmed using the API 20E system (bioMérieux, Inc) and

oxidase reactions as described previously.[43] Isolates from primary cultures were placed in tryptic soy broth with 15% glycerol and frozen at -80°C. *E. coli* isolates of randomly selected children and their caregivers were subjected to antimicrobial susceptibility testing (**AST**) using disc diffusion following methods described by the Clinical and Laboratory Standards Institute (**CLSI**).[44] Isolates were thawed, and quadrant-streaked for isolation onto MAC and incubated at 37°C in ambient air. While most *E. coli* are indole-positive, lactose fermenting, and motile there other morphologies exist and some atypical morphologies have demonstrated AMR.[75,76] Therefore, if more than one colony morphology was seen following restoration on MAC agar, isolates from each morphology (up to 3) were separately subjected to AST.

Antimicrobial Susceptibility Testing

Overnight cultures of *E. coli* isolates were placed into 5ml of sterile normal saline and adjusted for uniformity with a 0.5 MacFarland standard. Sterile swabs were placed into the bacterial diluent then used to homogenously cover the surfaces of MH agar plates. Antibiotic discs were added using a disc dispenser on top of the inoculated MH agar followed by placement into in an ambient air incubator maintained at 37°C for 18 – 24 hours. The following antibiotics were tested: ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefoxitin, imipenem, ciprofloxacin, gentamicin, azithromycin, chloramphenicol, combined amoxicillin and clavulanic acid **(co-amoxiclav)**, and combined trimethoprim/sulfamethoxazole **(cotrimoxazole)**. Zone diameters were measured in millimeters and susceptibility, resistance, or an intermediate designation were interpreted using guidelines in the CLSI-2021 M-100 (Table 2A, Zone Diameter and MIC Breakpoints for Enterobacteriaceae).[44] Intermediate and resistant isolates were collapsed and classified as non-susceptible. To date, there are no zone diameters established to determine phenotypic resistance to azithromycin for *E. coli.* Therefore, we used the 2021 guidelines for *Salmonella enterica* serovar Typhi as has been done in other studies.[4,44] When more than one isolate was tested from the same fecal sample, the presence of any isolate with phenotypic non-susceptibility was determined to be resistant.

Determination of ESBL-producing E. coli

Double-disc diffusion was used to determine phenotypic ESBL-producing *E. coli*. This test compares the zone sizes of ceftazidime and cefotaxime with and without clavulanic acid, a beta-lactamase inhibitor, as previously described.[12,45] Quality control was assured by appropriate differences in zone sizes between the two selected antibiotics with and without clavulanic acid for ESBL-producing (NCTC 13351) and ESBL-negative (ATCC 25922) strains of *E. coli*.[77,78] If the difference in zone size between cefotaxime and cefotaxime with clavulanic acid and ceftazidime and ceftazidime with clavulanic acid was <5mm then the ESBL-negative QC strain of *E. coli* functioned as expected. Conversely, if the difference in zone sizes between cefotaxime and cefotaxime with clavulanic acid or the zone sizes of ceftazidime and ceftazidime with clavulanic acid was ≥5mm then the ESBL positive QC strain verified the performance of the test.[44]

Ethical Considerations

The study was approved by the Institutional Review Boards of the Kenya Medical Research Institute (SERU 3086), the Kenyan Pharmacy and Poisons Board (Ref. No. PPB/ECCT/15/10/04), and the University of Washington (IRB# 49120). The parent trial was registered at ClinicalTrials.gov (Identifier: NCT02414399).

Statistical Analysis

The proportion of isolates not susceptible to each antibiotic tested was determined for children and caregivers. Confidence intervals were calculated assuming a binomial distribution. A heatmap was constructed to demonstrate the level of concordance in terms of resistance versus susceptibility for each antibiotic in the child-caregiver pairs. Each pair was determined to be concordant for resistance to an antibiotic if both the child and caregiver had *E. coli* that demonstrated phenotypic resistance to that given antibiotic. Similarly, concordance in harboring ESBL-producing *E. coli* was determined for pairs where both harbored isolates with this phenotypic result. Concordance for susceptibility to an antibiotic (or negativity for ESBL-production) was determined for pairs if neither had *E. coli* isolated that was resistant to the given antibiotic. Pairs discordant for AMR were further delineated to indicate which participant

(child or caregiver) had AMR to a given antibiotic. Percent agreement for each antibiotic was calculated with both susceptible and non-susceptible pairs in the numerator and all pairs in the denominator.

We determined the positive likelihood ratio **(LR+)** for each antibiotic by taking the probability a caretaker was positive for AMR if their child had AMR and dividing that by the probability a caretaker would have AMR if their child had susceptible *E. coli* isolated. Conversely, the negative likelihood ratio **(LR-)** was calculated by dividing the probability *E. coli* isolated from a caretaker was susceptible to a given antibiotic if *E. coli* from their child was resistant by the probability a caretaker will susceptible with a child who has susceptible bacteria isolated.

We evaluated whether concordant ESBL-producing *E. coli* in caregiver and child pairs were associated with specific caregiver and child characteristics. Chi^2 tests were used to identify factors associated with concordance for ESBL-producing *E. coli*. To compare ESBL-producing concordant pairs solely to discordant pairs, a sub-analysis removed concordant susceptible pairs from the comparator group. Fisher's Exact Test were used for instances where bins were present with 5 or less observations. These risk factors were chosen a priori and included caregiver sex, age, HIV status, BMI, marital status, other children in their care, employment, highest education completed, household income, household crowding, ownership of livestock, use of an improved water source, the type of toilet used by the household and whether the toilet was shared with other households. Characteristics associated with the child included, age, breastfeeding during the first 6 months of life, HIV status, nutritional characteristics (stunting and/or wasting), whether the child was referred to the hospital from another health facility, the length of hospitalization prior to discharge, and whether antibiotics were received during the hospital stay. Associations were considered significant at a 2-sided alpha of 0.05 or less.

Potential risk factors for carriage of ESBL-producing *E. coli* among the caregivers in this study were chosen a priori. These included those chosen for the analysis of associations with concordance of ESBL-producing *E. coli* in child-caregiver pairs, with the removal of child-specific characteristics. Univariate Poisson regressions with robust standard errors were used and associations were considered significant at a two-sided alpha of ≤0.05. Multivariate Poisson regressions adjusted for the site of enrollment and any variables considered significant in univariate analyses at an alpha less than or equal

to 0.05. Statistical analyses were performed in StataMP 17 (StataCorp, College Station, Texas). Risk factors of ESBL-producing *E. coli* in children from this cohort are presented in Chapter 1.

RESULTS

Study Population

Two hundred and twenty-nine children and their caregivers were included in this analysis (Figure 1). The majority of children were unexposed to HIV (n = 191; 86.8%), while 25 (11.4%) were HIV exposed uninfected and 4 (1.8%) were HIV-infected. The median hospitalization length preceding discharge was 3 days and 196 (85.6%) of children received antibiotics during the hospitalization. Most primary caregivers were female (n = 221; 96.5%). Thirty-one (14.1%) were HIV infected, and 36 (15.8%) were older than 35 years of age (Table 1). Most caregivers (n = 178; 77.7%) reported caring for more than one child and almost half (n = 106; 46.3%) reported crowded living conditions (Table 1).

AMR in Children Discharged from Hospital and their Caregivers

Prevalence of AMR

Non-susceptibility to all antibiotics tested was higher in *E. coli* isolated from children compared to caregivers (Figure 2). ESBL producing *E. coli* were present in 16.6% (95% confidence interval [CI] 12.0 – 22.1%) of *E. coli* isolates from caregivers and 44.5% (95% CI 38.0% - 51.2%) of children. Isolates from nearly all children (91.7% [95% CI 87.3% – 94.9%) demonstrated resistance to ampicillin while 65.1% (95% CI 58.5% – 71.2%) of isolates from caregivers were designated resistant. *E. coli* with AMR to ciprofloxacin was present in 48.5% (95% CI 41.8% – 55.1%) of children and 29.7% (23.9% – 36.1%) of caregivers. Resistance to cotrimoxazole was high in both children (94.3% [95% CI 90.5% – 96.9%]) and caregivers (83.0% [95% CI 77.5 – 87.6%]). Imipenem resistance was low in both children (3.5% [95% CI 1.5% - 6.8%]) and caregivers (2.2% [95% CI 0.7% - 5.0%]) (Figure 2).

Percent agreement of resistance and susceptibility to antibiotics in children and their caregivers

AMR concordance to imipenem was 96.9% between children and caregivers (Table 2, Figure 3). Cefoxitin, chloramphenicol, and cotrimoxazole also had high agreement; 81.7%, 74.7%, and 79.9%, respectively. Agreement was slightly less for other antibiotics, including co-amoxiclav (53.7%) and ceftriaxone (64.6%). Discordance was most common when child isolates were non-susceptible and caregivers susceptible (Figure 3).

Likelihood of AMR in caregiver given child's carriage of AMR E. coli

LR+ values for most antibiotics were close to 1, suggesting no greater likelihood of AMR in caregivers that had children with AMR (Table 2). Similarly, LR- for most antibiotics were near 1, indicating greater likelihood of antibiotic susceptibility in isolates of caregivers with children who also had susceptible isolates. The exception was imipenem, where the LR+ was 87.6 and LR- 0.6, driven primarily by the very low rate of observed non-susceptibility to imipenem in both the children and the caregivers.

Predictors of Concordant ESBL-Producing E. coli from Child-Caregiver Pairs

Child-caregiver pairs were less likely to have concordant ESBL-producing *E. coli* if the caregiver had other children in their care (other children concordant [55.0%] versus not concordant for ESBL-producing *E. coli* [79.9% p=0.05]) and among those who were employed or in school (concordant for ESBL-producing *E. coli* [55.0%] compared to other pairs [78.6% p = 0.02]) (Table 3). Other caregiver characteristics such as sex, age, and BMI were not significantly associated with concordance of ESBL-producing *E. coli*. Similarly, socioeconomic indicators such as income, access to improved water sources, the practice of open defecation, and the sharing of toilet facilities with other households were not associated with concordance in the child-caregiver pairs. None of the child characteristics or hospitalization information analyzed were significantly associated with both the child and caregiver harboring ESBL-producing *E. coli*. When compared solely to discordant pairs in secondary analyses, child-caregiver ESBL concordance was also associated with employment or student status and children in caregivers' care other than the child enrolled in this study (Appendix I). Additionally, crowded conditions were also associated with ESBL-producing concordance (Appendix I).

Risk factors for carriage of ESBL-Producing E. coli in Caregivers of Children who have been Hospitalized and Subsequently Discharged in Kenya

There were 38 (10.5%) caregivers who had ESBL-producing *E. coli* isolated from their fecal samples (Table 4). Individual characteristics, including age, HIV infection status, and BMI did not demonstrate a statistically significant relationship with harboring ESBL-producing bacteria. There was no statistically significant association with having ESBL-producing *E. coli* isolated from caregivers with factors related to children's hospitalizations or household risk-factors (Table 4).

DISCUSSION

In this study, AMR to clinically relevant antibiotics was frequently detected in *E.coli* isolated from primary caregivers of these children. However, despite their proximity to hospitalized children and the hospital environment, caregivers were less likely than their children to have AMR, suggesting that environmental spread of AMR may be less important than the contribution of direct antibiotic selection pressure in the hospital setting. Resistance to cotrimoxazole and ampicillin, antibiotics commonly used in SSA, were detected in greatest frequency, suggesting that community spread of resistance to these antibiotics has occurred.

In general, the patterns of AMR detected in isolates from hospitalized children differed from patterns identified in isolates from their primary caregivers. Similarities were present only when the prevalence of AMR was low (imipenem, chloramphenicol, and cefoxitin) or very high (cotrimoxazole). Given the higher prevalence of AMR in hospitalized children compared to their primary caregivers, the direct pressure of antibiotic use while ill is likely the greatest source of AMR in this setting. The changing epidemiology of predominant genetic determinants of ESBL suggests that resistance can pass between communities and hospitals.[2,66] Therefore, it is possible that AMR in the community plays a role in the AMR present in hospital settings.[2,73,79]

Previously hospitalized children who return to an environment with crowding and a lack of adequate sanitation and hygiene facilities may share AMR bacteria and genetic elements with others in the household and/or community.[14] In fact, community exposure to antibiotics may be a more important

determinant of resistance in individuals than individual exposure to antibiotics.[55] Differences in caregiver responsibilities may partly explain a lack of concordant AMR between child-caregiver pairs. Having other children to care for, being employed or being a student, were all negatively associated with concordant ESBL. These responsibilities may reduce the time a caregiver spends at the hospital with their child and therefore may reduce the risk of acquiring AMR from the hospital setting.

Transmission of ESBL producing bacteria in settings that lack adequate sanitation has been previously noted.[17] However, like this study, some have also not found a significant association between access to hygiene and sanitation facilities.[16,38,80] Access to toilet facilities at the hospital may confound what interpretations we can make regarding toilets at home and the isolation of ESBL-producing bacteria.

This study had several notable strengths. Data and samples were systematically collected as part of large rigorous randomized trial and able to be linked to well-characterized clinical and social datasets to evaluate risk factors and associations. Fecal samples were available at the same time from caregivers and their children to demonstrate AMR at the time of the child's hospital discharge. Most studies which examine intra-familial sharing of AMR have smaller sample sizes and most occur in high income countries. The study also has several limitations. The cross-sectional nature of this study does not allow us to determine when the children and caregivers were colonized with AMR bacteria. Longitudinal assessments of AMR carriage are needed to confirm the temporal patterns of transmission between children and their caregivers. We also did not examine molecular determinants of resistance or clonality and it is possible that the mechanisms driving resistance differed even when AMR patterns were concordant. We were also unable to make conclusions about hospital transmission due to the lack of community caregiver child controls.

CONCLUSION

Resistance to a wide range of frequently used antibiotics is common in isolates of *E. coli* identified from hospitalized children in rural western Kenya. Primary caregivers of these children are exposed to the same hospital environment but are unlikely to be receiving antibiotics. We found that caregivers often

carried antibiotic resistant *E. coli*, albeit less frequently than their children. Hospitalized children who are discharged may contribute to the burden of AMR infections in SSA through transmission of AMR to family and community members and lack of hygiene and sanitation access may be important risk factors for this transmission. In addition to antibiotic stewardship interventions and hospital infection control measures, targeted approaches to reducing transmission of AMR following discharge, including improvements in hygiene and sanitation, may reduce the burden of AMR in communities in low-resource settings.

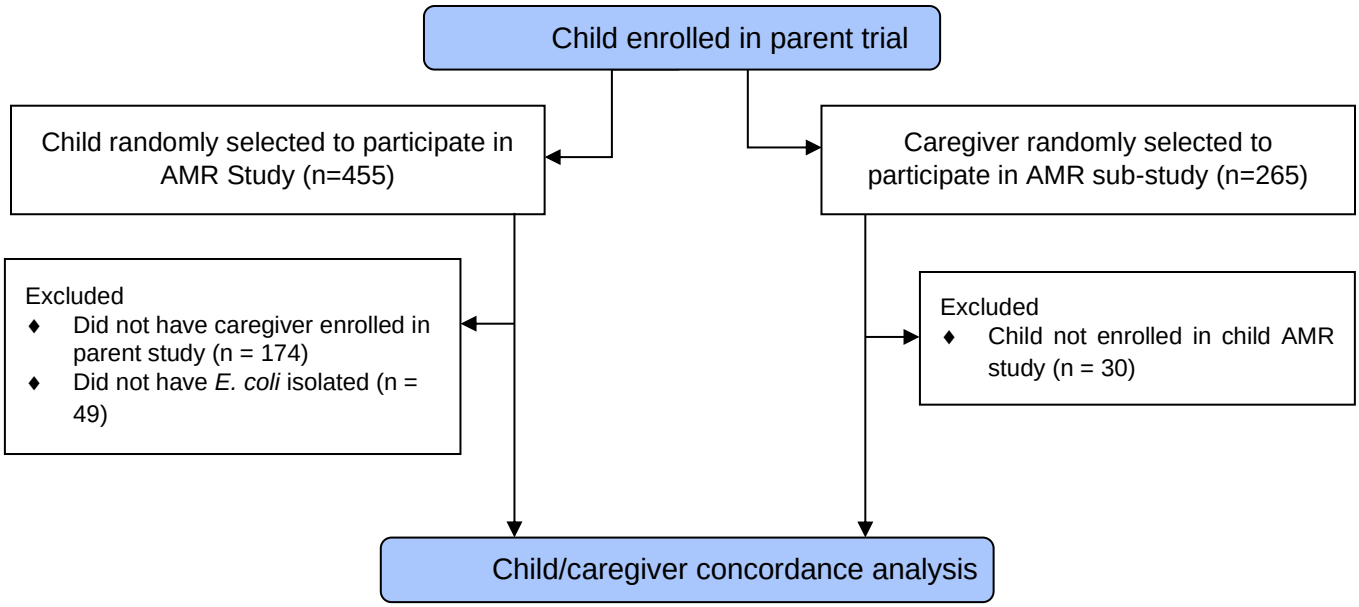


Figure 1. Participant flow chart

Table 1. Descriptive Characteristics

	Total (n = 229) n (%)	Kisii (n = 138) n (%)	Homa Bay (n = 91) n (%)
Caregiver Characteristics			
Sex			
Male	8 (3.5%)	6 (4.3%)	2 (2.2%)
Female	221 (96.5%)	132 (95.7%)	89 (97.8%)
Age (years)			
≤ 24	97 (42.5%)	55 (40.1%)	42 (46.2%)
25 - 35	95 (41.7%)	61 (48.9%)	34 (37.4%)
36 and over	36 (15.8%)	21 (15.3%)	15 (16.5%)
HIV[i]			
HIV Uninfected	189 (85.9%)	122 (92.4%)	67 (76.1%)
HIV Infected	31 (14.1%)	10 (7.6%)	21 (23.9%)
BMI			
≤18.5	13 (5.7%)	5 (3.6%)	8 (8.8%)
18.6 – 24.9	138 (60.3%)	79 (57.3%)	59 (64.8%)
≥25.0	78 (34.1%)	54 (39.1%)	24 (26.4%)
Marital Status			
Married	159 (69.4%)	109 (79.0%)	50 (55.0%)
Married – Polygamous	25 (10.9%)	7 (5.1%)	18 (19.8%)
Not Married/Widowed/Separated	45 (19.6%)	22 (15.9%)	23 (25.3%)
Total Children Under Care			
One	51 (22.3%)	33 (23.9%)	18 (19.8%)
Two	65 (28.4%)	42 (30.4%)	23 (28.4%)
Three or More	113 (49.3%)	63 (45.7%)	50 (49.3%)
Employment[ii]			
Student or Employed	169 (76.5%)	100 (75.2%)	69 (78.4%)
Not Employed or a Student	52 (22.5%)	33 (23.9%)	19 (20.9%)
Education Completed[iii]			
Primary or Less	115 (48.0%)	71 (52.6%)	35 (40.7%)
Secondary or Higher	106 (52.0%)	64 (47.4%)	51 (59.3%)
Income (Kenyan Shillings) Household[iv]			
≥ 5,000	175 (87.5%)	107 (86.3%)	68 (89.5%)
< 5,000	25 (12.5%)	17 (13.7%)	8 (10.5%)
Crowding (>2 people/room)	106 (46.3%)	54 (39.1%)	52 (57.1%)
Livestock Ownership	162 (70.7%)	95 (68.8%)	67 (73.6%)
Improved Water Source	192 (83.4%)	121 (87.7%)	71 (78.0%)
Shared Toilet[v]	113 (51.8%)	76 (44.9%)	51 (63.8%)
Toilet Type			
Flushing	14 (6.1%)	11 (8.0%)	3 (3.3%)
Pit Latrine	205 (89.5%)	127 (92.0%)	78 (85.7%)
Open Defecation	10 (4.3%)	0 (0%)	10 (11.0%)
Child Characteristics			
Sex			
Male	139 (60.7%)	85 (61.6%)	54 (59.3%)
Female	90 (39.3%)	53 (38.4%)	37 (40.7%)
Age (months)			
24 and over	93 (40.6%)	56 (40.6%)	37 (40.7%)
12 – 23	63 (27.5%)	36 (26.1%)	27 (29.7%)
6 – 11	49 (21.4%)	28 (20.3%)	21 (23.1%)
1 – 5	24 (10.5%)	18 (13.0%)	6 (6.6%)
HIV Status[vi]			
HIV Uninfected	191 (86.8%)	122 (92.4%)	69 (78.4%)
HIV Uninfected, Exposed	25 (11.4%)	8 (6.1%)	17 (19.3%)
HIV Infected	4 (1.8%)	2 (1.5%)	2 (2.3%)
Nutritional Characteristics[vii]			
Neither Stunted nor Wasted	149 (65.1%)	93 (67.4%)	56 (61.5%)
Wasted, not Stunted	24 (10.5%)	15 (10.9%)	9 (9.9%)
Stunted, not Wasted	50 (21.8%)	27 (19.6%)	23 (25.3%)
Stunted and Wasted	6 (2.6%)	3 (2.2%)	3 (3.3%)
Child's Hospitalization Information			
Referred from other health facility	68 (29.7%)	46 (33.3%)	22 (24.2%)
Length of Hospital Stay (in days)[viiiix]	3 (2 – 5)	3 (2 – 5)	4 (3 – 6)
Received ≥ 1 Antibiotic During Hospitalization	196 (85.6%)	129 (93.5%)	67 (73.6%)

Antibiotic Received During Hospitalization			
Penicillin(s)	132 (78.4%)	99 (91.7%)	26 (57.9%)
Ceftriaxone	80 (70.8%)	43 (60.7%)	37 (60.7%)
Gentamicin	120 (78.4%)	94 (91.3%)	26 (52.0%)

[i] Of those with HIV infection status information data available (n = 220)
[ii] Of those with employment or student status known (n =221)
[iii] Of those with education attainment information provided (n =221)
[iv] Of those with reporting household information data (n = 220)
[v] Shared Toilets are those used by more than 1 household and did not include open defecation (n = 10 all in Homa Bay) and excluding those who did not answer (n = 1)
[vi] Uninfected, exposure Status unknown (n = 9); column percentages of children with exposure and infection status known (n = 220)
[vii] Wasted is defined as WHZ < -2 or MUAC <11.5cm while stunted is determined by HAZ <-2; MUAC is only taken into consideration in children 6 months or older
[viii] Median and interquartile range provided
[ix] Of those with admission and discharge dates both available (n = 401)

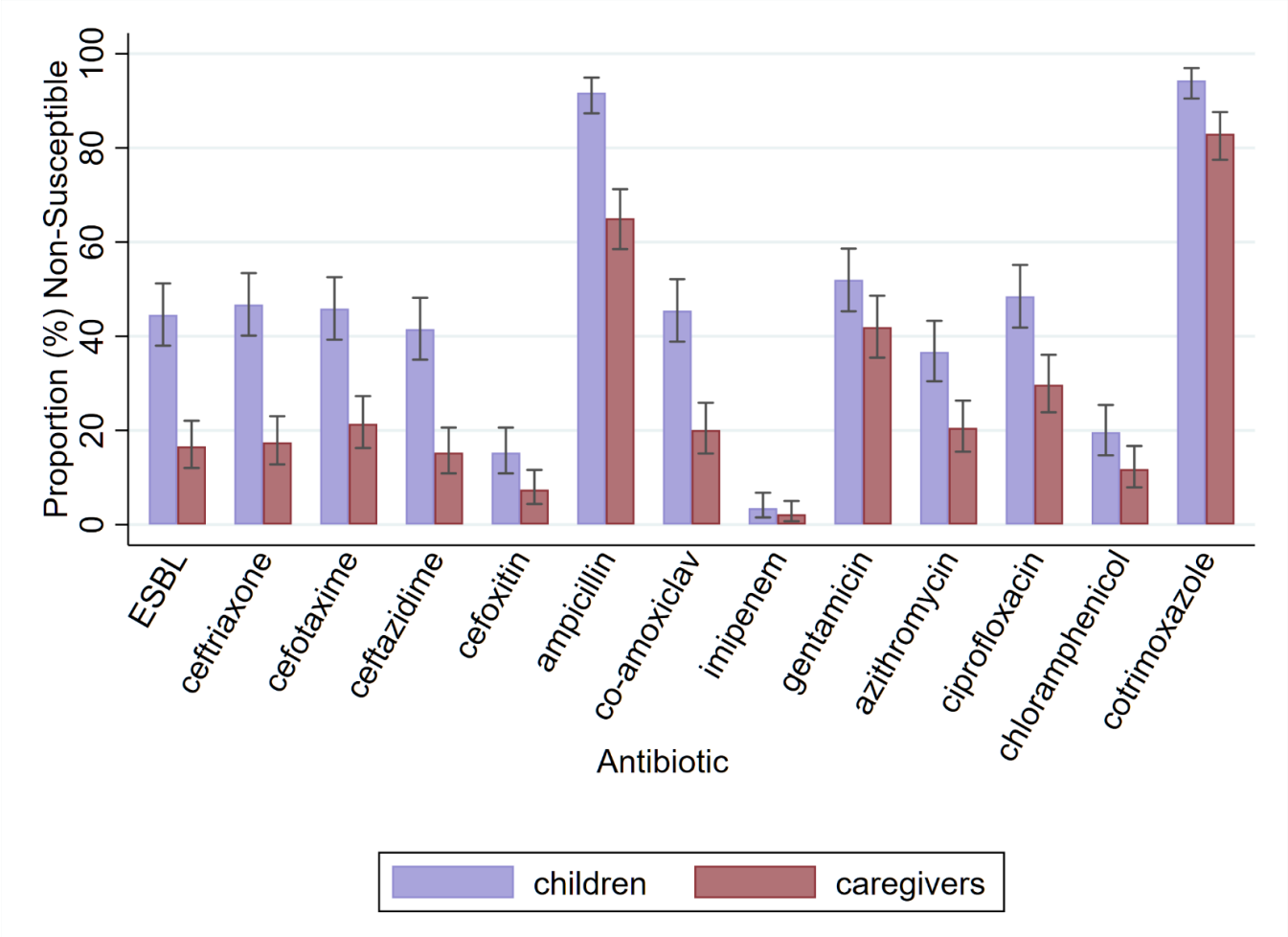
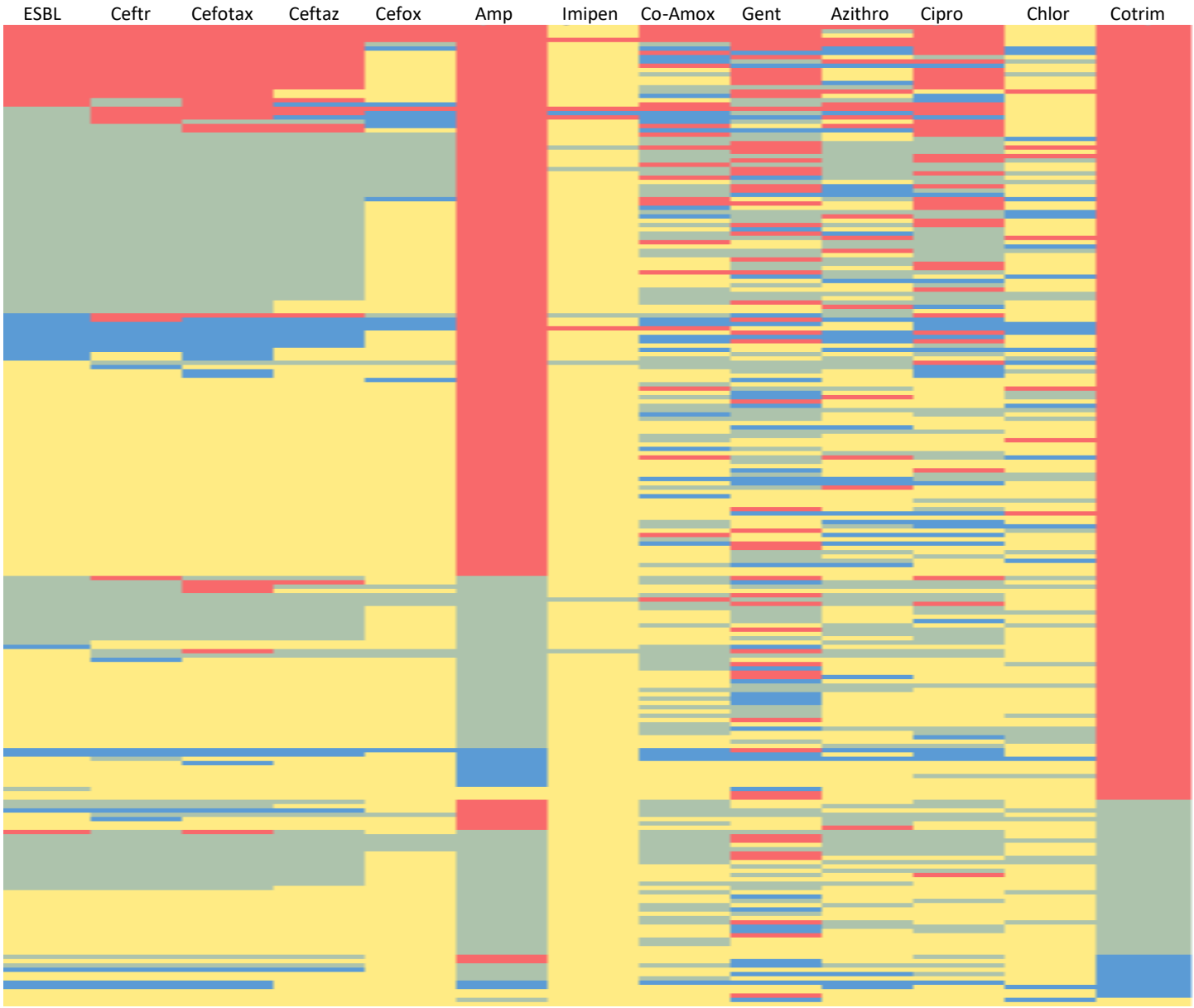


Figure 2: Proportion of *E. coli* isolates lacking susceptibility to commonly used antibiotics in *E. coli* isolates from caregivers (n = 229) and their children (n=229) at hospital discharge. Non-susceptible isolates are determined by the sizes of clearings around antibiotics measured using disc diffusion antimicrobial susceptibility testing and classified as “resistant” or “intermediate” by CLSI-2020

Table 2. Agreement of ESBL *E. coli* Isolated from Children at Hospital Discharge and their Caregivers

		Caregiver Susceptibility		% Agreement	Positive Likelihood Ratio	Negative Likelihood Ratio
		Not Susceptible	Susceptible			
Child Susceptibility	ESBL					
	Producing	20	82	56.3%	1.4	0.9
	Non-Producing	18	109			
	Cephalosporins					
	Ceftriaxone					
	Non-Susceptible	24	83	64.6%	1.7	0.9
	Susceptible	16	106			
	Cefotaxime					
	Non-Susceptible	30	75	59.0%	1.9	0.8
	Susceptible	19	105			
	Ceftazidime					
	Non-Susceptible	22	73	62.5%	2.4	0.9
	Susceptible	13	121			
	Cefoxitin					
	Non-Susceptible	5	30	81.7%	2.3	0.9
	Susceptible	12	182			
	Penicillins					
	Ampicillin					
	Non-Susceptible	137	73	62.9%	1.0	0.9
	Susceptible	12	7			
	Penicillin/beta-lactamase inhibitor combination					
	Amoxicillin/clavulanic acid					
	Non-Susceptible	22	82	53.7%	1.1	1.0
	Susceptible	24	101			
	Carbapenem					
	Imipenem					
	Non-Susceptible	4	6	96.9%	87.6	0.6
	Susceptible	1	218			
	Aminoglycoside					
	Gentamicin					
	Non-Susceptible	55	64	55.8%	1.2	0.9
	Susceptible	41	69			
	Macrolide					
	Azithromycin					
	Non-Susceptible	17	67	57.6%	1.0	1.0
	Susceptible	30	115			
	Fluoroquinolone					
	Ciprofloxacin					
	Non-Susceptible	40	71	56.8%	1.5	0.8
	Susceptible	28	90			
	Phenicol					
	Chloramphenicol					
	Non-Susceptible	7	38	74.7%	1.4	0.9
	Susceptible	20	164			
	Antifolate/sulfonamide combination					
	Trimethoprim/sulfamethoxazole					
	Non-Susceptible	180	36	79.9%	1.1	0.7
	Susceptible	10	3			



Antibiotic Abbreviations

ESBL:	extended-spectrum beta-lactamase
ceftr:	ceftriaxone
cefotax:	cefotaxime
ceftaz:	ceftazidime
cefox:	cefoxitin
amp:	ampicillin
imipen:	imipenem
co-amox:	co-amoxiclav
gent:	gentamicin
azithro:	azithromycin
cipro:	ciprofloxacin
chlor:	chloramphenicol
cotrim:	cotrimoxazole

Legend

- Concordant resistant
- Concordant susceptible
- Discordant (child resistant/caregiver susceptible)
- Discordant (child susceptible/caregiver resistant)

Figure 3. Heatmap demonstrating concordance of antimicrobial resistance to select drugs in *E. coli* isolated from child-caregiver pairs

Table 3. Associations of risk factors and concordance of ESBL-producing *E. coli* isolated from children and their caregivers

	Concordant ESBL Production (N = 20)		All Other Pairs[i] (N = 209)		Chi^2 or Fisher's Exact p-value
	N	(%)[ii]	N	(%)[ii]	
Facility					
Kisii	13	(65.0%)	125	(59.8%)	0.65
Homa Bay	7	(35.0%)	84	(40.2%)	
Caregiver and Household Information					
Sex[i]					
Male	0	(0.0%)	8	(3.8%)	1.00
Female	20	(100.0%)	201	(96.2%)	
Age (years)					
≤ 24	11	(55.0%)	86	(41.4%)	
25 - 35	8	(40.0%)	87	(41.8%)	0.32
36 and over	1	(5.0%)	35	(16.8%)	
HIV[iii]					
HIV Uninfected	17	(84.7%)	172	(86.0%)	
HIV Infected	3	(15.3%)	28	(14.0%)	1.00
BMI					
18.6 – 24.9 (normal)	8	(40.0%)	130	(62.2%)	
≤18.5 (underweight)	3	(15.0%)	10	(4.8%)	0.06
≥25.0 (overweight – obese)	9	(45.0%)	69	(33.0%)	
Marital Status					
Married – Monogamous	16	(80.0%)	143	(68.4%)	
Married – Polygamous	0	(10.1%)	25	(12.0%)	0.31
Not Married/Widowed/Separated	4	(20.0%)	41	(19.6%)	
Total Children Under Care					
One	9	(45.0%)	42	(20.1%)	
Two	3	(15.0%)	62	(29.7%)	0.05*
Three or More	8	(40.0%)	105	(50.2%)	
Employment					
Student or Employed	11	(55.0%)	158	(78.6%)	0.02*
Not Employed or a Student[iv]	9	(45.0%)	43	(21.4%)	
Education Completed[v]					
Secondary or Higher	9	(45.0%)	98	(46.9%)	0.87
Primary or Less	11	(55.0%)	111	(53.1%)	
Income (Kenyan Shillings) Household[vi]					
≥ 5,000	20	(100.0%)	163	(86.7%)	0.14
< 5,000	0	(0.0%)	25	(13.3%)	
Crowding					
No	15	(75.0%)	108	(51.7%)	0.06
Yes	5	(25.0%)	101	(48.3%)	
Livestock Ownership					
No	7	(35.0%)	60	(28.7%)	0.56
Yes	13	(65.0%)	149	(71.3%)	
Improved Water Source					
No	3	(15.0%)	34	(16.3%)	1.00
Yes	17	(85.0%)	175	(83.7%)	
Toilet[vii]					
Household Only	9	(45.0%)	96	(48.5%)	0.77
Shared	11	(55.0%)	102	(51.5%)	
Open Defecation					
No	20	(100%)	199	(95.2%)	1.00
Yes	0	(0.0%)	10	(4.8%)	
Child Characteristics					
Sex					
Male	10	(50.0%)	129	(61.7%)	0.31
Female	10	(50.0%)	80	(38.3%)	
Age (months)					
24 and over	6	(30.0%)	87	(41.6%)	
12 – 23	8	(40.0%)	55	(26.3%)	
6 – 11	5	(25.0%)	44	(21.1%)	0.50
1 – 5	1	(5.0%)	23	(11.0%)	

HIV[viii]			
HIV Uninfected	17 (89.5%)	172 (86.4%)	
HIV Uninfected, Exposed	2 (10.5%)	23 (11.6%)	1.00
HIV Infected	0 (0.0%)	4 (2.0%)	
Nutritional Characteristics			
Neither stunted nor wasted	16 (80.0%)	133 (63.6%)	
Wasted, not Stunted	0 (0.0%)	24 (11.5%)	0.21
Stunted, not Wasted	3 (15.0%)	47 (22.5%)	
Stunted and Wasted	1 (5.0%)	5 (2.4%)	
Child's Hospitalization Information			
Referred from another healthcare facility			
No	15 (75.0%)	146 (69.9%)	0.63
Yes	5 (28.7%)	63 (30.1%)	
Length of Hospital Stay (in days)			
< 4	9 (45.0%)	102 (48.8%)	0.75
≥ 4	11 (55.0%)	107 (51.2%)	
Received Antibiotic			
No	3 (15.0%)	30 (14.4%)	1.00
Yes	17 (85.0%)	179 (85.7%)	
Antibiotic Received[ix]			
Ceftriaxone	12 (80.0%)	68 (69.4%)	0.40
Penicillin(s)	8 (72.7%)	124 (80.5%)	0.53
Gentamicin	8 (72.7%)	112 (78.9%)	0.63

[i] Discordant pairs, having a child with ESBL-producing *E. coli* and a caregiver without or vice versa, and pairs not without isolates producing *E. coli* are included in the comparison group

[ii] Column percentages shown

[iii] Of those with HIV infection status information data available (n = 220)

[iv] Of those with employment or student status known (n =221)

[v] Of those with education attainment information provided (n =221)

[vi] Of those with reporting household information data (n = 220)

[vii] Of those who do not practice open defecation (n = 219). Shared Toilets are those used by more than 1 household and did not include open defecation.

[viii] Uninfected, exposure Status unknown (n = 9), Exposed, infection status unknown (n = 2); Column percentages of children with exposure and infection status known (n = 218)

[ix][ix] Compared to those who did not receive antibiotics (n = 33). The total number of children who received ceftriaxone was n = 80, 132 received a penicillin antibiotic, and 120 were given gentamicin.

* Significantly associated with concordant isolated ESBL-producing *E. coli* at an alpha of 0.05

Table 4. Risk factors for ESBL Producing *E. coli* in caregivers with a child at hospital discharge

	ESBL + (N = 38) N (%)[i]	ESBL - (N = 191) N (%)[i]	PR (95% CI)	p-value	Adjusted PR (95% CI)[ii]	p-value
Facility						
Kisii	24 (63.2%)	114 (59.7%)	REF.		REF.	
Homa Bay	14 (36.8%)	77 (40.3%)	0.88 (048 – 1.62)	0.69	0.99 (0.55 – 1.81)	0.98
Caregiver and Household Information						
Gender						
Male	2 (5.3%)	6 (3.1%)	REF.		REF.	
Female	36 (94.7%)	185 (96.9%)	0.65 (0.19 – 2.25)	0.50	0.68 (0.20 – 2.37)	0.55
Age (years)						
≤ 24	15 (39.5%)	82 (43.2%)	REF.		REF.	
25 - 35	17 (44.7%)	78 (41.1%)	1.16 (0.61 – 2.18)	0.65	1.12 (0.60 – 2.13)	0.73
36 and over	6 (15.8%)	30 (15.8%)	1.08 (0.45 – 2.57)	0.87	1.01 (0.44 – 2.47)	0.93
HIV[iii]						
HIV Uninfected	29 (78.4%)	160 (87.4%)	REF		REF.	
HIV Infected	8 (21.6%)	23 (12.6%)	1.68 (0.85 – 3.34)	0.14	1.86 (0.94 – 3.69)	0.08
BMI						
18.6 – 24.9 (normal)	21 (55.3%)	117 (61.3%)	REF		REF.	
≤18.5 (underweight)	3 (7.9%)	10 (5.2%)	1.52 (0.52 – 4.42)	0.45	1.43 (0.48 – 4.28)	0.52
≥25.0 (overweight – obese)	14 (36.8%)	64 (33.5%)	1.18 (0.60 – 2.19)	0.60	1.14 (0.61 – 2.11)	0.68
Marital Status						
Married – Monogamous	26 (78.8%)	133 (77.8%)	REF.		REF.	
Married – Polygamous	2 (6.1%)	23 (13.5%)	0.49 (0.12 – 1.94)	0.31	0.52 (0.13 – 2.10)	0.36
Not Married/Widowed/Separated	5 (15.2%)	15 (8.8%)	1.53 (0.66 – 3.54)	0.32	1.58 (0.68 – 3.67)	0.29
Total Children Under Care						
One	12 (31.6%)	39 (20.4%)	REF.		REF.	
Two	10 (26.3%)	55 (28.8%)	0.65 (0.31 – 1.39)	0.27	0.71 (0.33 – 1.51)	0.37
Three or More	16 (42.1%)	97 (50.8%)	0.60 (0.31 – 1.18)	0.14	0.63 (0.32 – 1.23)	0.17
Employment[iv]						
Student or Employed	26 (70.3%)	143 (77.7%)	REF.		REF.	
Not Employed or a Student	11 (29.7%)	41 (22.3%)	1.38 (0.73 – 2.59)	0.33	1.40 (0.74 – 2.64)	0.30
Education Completed[v]						
Secondary or Higher	15 (39.5%)	92 (48.2%)	REF.		REF.	
Primary or Less	23 (60.5%)	99 (51.8%)	1.34 (0.74 – 2.44)	0.33	1.39 (0.77 – 2.52)	0.28
Income (Kenyan Shillings) Household[vi]						
≥ 5,000	32 (86.5%)	151 (88.3%)	REF.		REF.	
< 5,000	5 (13.5%)	20 (11.7%)	1.14 (0.49 – 2.67)	0.76	1.15 (0.50 – 2.67)	0.74
Crowding						
No	21 (55.3%)	102 (53.4%)	REF.		REF.	
Yes	17 (44.7%)	89 (46.6%)	0.94 (0.94 – 1.69)	0.83	0.96 (0.54 – 1.72)	0.90
Livestock Ownership						
No	13 (34.2%)	54 (28.3%)	REF.		REF.	
Yes	25 (65.8%)	137 (71.7%)	0.80 (0.43 – 1.46)	0.46	0.83 (0.45 – 1.52)	0.55
Improved Water Source						
No	7 (18.4%)	30 (15.7%)	REF.		REF.	
Yes	31 (81.6%)	161 (84.3%)	0.85 (0.41 – 1.79)	0.68	0.93 (0.45 – 1.95)	0.86
Toilet[vii]						
Household Only	19 (50.0%)	86 (47.8%)	REF.		REF.	
Shared	19 (50.0%)	94 (52.2%)	0.93 (0.52 – 1.66)	0.80	0.93 (0.52 – 1.63)	0.79
Open Defecation						
No	38 (100%)	181 (94.8%)	Not calculable	--	Not calculable	--
Yes	0 (0%)	10 (5.2%)				
Child's Hospitalization Information						
Referred from another healthcare facility						
No	27 (71.1%)	134 (70.2%)	REF.		REF.	
Yes	11 (29.0%)	57 (29.8%)	0.96 (0.51 – 1.83)	0.91	0.90 (0.47 – 1.72)	0.76
Length of Hospital Stay (in days)						
< 4	16 (42.1%)	95 (49.7%)	REF		REF.	
≥ 4	22 (57.9%)	96 (50.3%)	1.29 (0.72 – 2.34)	0.39	1.39 (0.77 – 2.51)	0.27
Received Antibiotic During Hospitalization						
No	7 (18.4%)	26 (13.6%)	REF.		REF.	
Yes	31 (81.6%)	165 (86.4%)	0.75 (0.36 – 1.55)	0.43	0.73 (0.33 – 1.63)	0.45

Antibiotic Received[viii]				
Ceftriaxone	15 (68.2%)	65 (71.4%)	0.88 (0.40 – 1.97) 0.76	0.86 (0.37 – 2.02) 0.73
Penicillins	22 (75.9%)	110 (80.9%)	0.79 (0.37 – 1.68) 0.54	0.82 (0.33 – 2.06) 0.67
Gentamicin	21 (75.0%)	99 (79.2%)	0.83 (0.38 – 1.78) 0.62	0.85 (0.32 – 2.22) 0.74

[i] Column percentages shown
[ii] Adjusted for hospital center participant enrolled from and open defecation.
[iii] Of those with HIV infection status information data available (n = 220)
[iv] Of those with employment or student status known (n =221)
[v] Of those with educational attainment known (n =221)
[vi] Of those with reporting household information data (n = 220)
[vii] Among those who did not practice open defecation (n = 219). Shared toilets are those used by more than 1 household.
[viii] Reference category is the caregiver's child not having received an antibiotic.
* Significant at an alpha of ≤0.05

Appendix I. Associations of risk factors and concordant ESBL-producing *E. coli* isolated from children and their caregivers compared to discordant[i] pairs

	Concordant ESBL Production (N = 20)		Discordant for ESBL Production (N = 100)		Chi^2 or Fisher's Exact p-value
	N	(%)[ii]	N	(%)[ii]	
Facility					
Kisii	13	(65.0%)	62	(62.0%)	0.80
Homa Bay	7	(35.0%)	38	(38.0%)	
Caregiver and Household Information					
Sex					
Male	0	(0.0%)	6	(6.0%)	0.59
Female	20	(100.0%)	94	(94.0%)	
Age (years)					
≤ 24	11	(55.0%)	36	(36.0%)	
25 - 35	8	(40.0%)	47	(47.0%)	0.21
36 and over	1	(5.0%)	17	(17.0%)	
HIV[iii]					
HIV Uninfected	17	(84.7%)	84	(87.5%)	
HIV Infected	3	(15.3%)	12	(12.5%)	0.72
BMI					
≤18.5 (underweight)	3	(15.0%)	4	(4.0%)	
18.6 – 24.9 (normal)	8	(40.0%)	58	(58.0%)	0.06
≥25.0 (overweight – obese)	9	(45.0%)	38	(38.0%)	
Marital Status[iv]					
Married – Monogamous	16	(69.0%)	70	(79.6%)	
Married – Polygamous	0	(10.1%)	12	(11.4%)	0.32
Not Married/Widowed/Separated	4	(20.9%)	18	(9.1%)	
Total Children Under Care					
One	9	(45.0%)	18	(18.0%)	
Two	3	(15.0%)	32	(32.0%)	0.03*
Three or More	8	(40.0%)	50	(50.0%)	
Employment					
Student or Employed	11	(55.0%)	75	(76.5%)	0.05*
Not Employed or a Student[v]	9	(45.0%)	23	(23.5%)	
Education Completed[vi]					
Primary or Less	11	(55.0%)	56	(44.0%)	0.93
Secondary or Higher	9	(45.0%)	44	(56.0%)	
Income (Kenyan Shillings) Household[vii]					
≥ 5,000	20	(100.0%)	77	(85.6%)	0.12
< 5,000	0	(0.0%)	13	(14.4%)	
Crowding					
No	15	(75.0%)	49	(49.0%)	0.05*
Yes	5	(25.0%)	51	(51.0%)	
Livestock Ownership					
No	7	(35.0%)	32	(32.0%)	0.79
Yes	13	(65.0%)	68	(68.0%)	
Improved Water Source					
No	3	(15.0%)	20	(20.0%)	0.76
Yes	17	(85.0%)	80	(80.0%)	
Toilet[viii]					
Household Only	9	(45.0%)	44	(46.8%)	0.76
Shared	11	(55.0%)	50	(53.2%)	
Open Defecation					
No	20	(100%)	94	(94.0%)	0.59
Yes	0	(0.0%)	6	(6.0%)	
Child Characteristics					
Sex					
Male	10	(50.0%)	61	(61.0%)	0.36
Female	10	(50.0%)	39	(39.0%)	
Age (months)					
24 and over	6	(30.0%)	44	(44.0%)	
12 – 23	8	(40.0%)	26	(26.0%)	
6 – 11	5	(25.0%)	18	(18.0%)	0.41
1 – 5	1	(5.0%)	12	(11.0%)	

HIV[ix]			
HIV Uninfected	17 (89.5%)	84 (88.4%)	
HIV Uninfected, Exposed	2 (10.5%)	8 (8.4%)	0.82
HIV Infected	0 (0.0%)	3 (3.2%)	
Nutritional Characteristics			
Neither stunted nor wasted	16 (80.0%)	64 (64.0%)	
Wasted, not Stunted	0 (0.0%)	10 (10.0%)	0.44
Stunted, not Wasted	3 (15.0%)	21 (21.5%)	
Stunted and Wasted	1 (5.0%)	5 (5.0%)	
Child's Hospitalization Information			
Referred			
No	15 (75.0%)	69 (69.0%)	0.79
Yes	5 (28.7%)	31 (31.0%)	
Length of Hospital Stay (in days)			
< 4	9 (45.0%)	37 (37.0%)	0.50
≥ 4	11 (55.0%)	63 (63.0%)	
Received Antibiotic			
No	3 (15.0%)	8 (8.0%)	0.39
Yes	17 (85.0%)	92 (92.0%)	
Antibiotic Received[x]			
Ceftriaxone	12 (80.0%)	51 (86.4%)	0.68
Penicillin(s)	8 (72.7%)	55 (87.3%)	0.35
Gentamicin	8 (72.7%)	48 (78.9%)	0.37

[i] Pairs discordant for ESBL-producing E. coli had one person with ESBL-producing isolates and one without
[ii] Column Percentages shown
[iii] Of those with HIV infection status information data available (n = 108)
[iv] During interviews, n = 108 caregivers responded
[v] Of those with employment or student status known (n =118)
[vi] Of those with education attainment information provided (n =120)
[vii] Of those with reporting household information data (n = 110)
[viii] Of those who do not practice open defecation (n = 114). Shared Toilets are those used by more than 1 household and did not include open defecation.
[ix] Uninfected, exposure Status unknown (n = 4), Exposed, infection status unknown (n = 3); Column percentages of children with exposure and infection status known (n = 113)
[xx] Compared to those who did not receive antibiotics (n = 11). The total number of children who received ceftriaxone was n = 63, 63 received a penicillin antibiotic, and 56 were given gentamicin.
* Significantly associated with concordant isolated ESBL-producing *E. coli* at an alpha of 0.05.

Chapter 3: Concordance of antimicrobial resistance in enteric pathogens and *E. coli* isolated from hospitalized children in Kenya

INTRODUCTION

Antimicrobial resistance **(AMR)** is a major emerging global public health problem, responsible for substantial mortality and morbidity, particularly in low- and middle- income countries **(LMICs)**.[1] Many current World Health Organization antibiotic treatment guidelines suggest antibiotics which are often low-cost and readily accessible in LMICs.[10] However, widespread use of antibiotics, coupled with poor sanitation and environmental contamination, contribute to high rates of antibiotic resistance (AMR).[6,81] Treatment failure in settings where alternative antibiotic options are limited due to lack of availability and high cost are increasingly being documented and these treatment failures likely contribute to high rates of mortality and morbidity in LMICs.[14,15,82]

Bacteria belonging to the family *Enterobacteriaceae* are common commensal organisms as well as pathogens. These bacteria can serve as an important reservoir of AMR within individuals and communities. Two species of the *Enterobacteriaceae* family, *Salmonella* and *Shigella*, resulted in over 91,000 deaths in children under the age of 5 in Sub-Saharan Africa **(SSA)** in 2016.[82-85] Antimicrobial susceptibility testing **(AST)** of enteric pathogens, including *Shigella* and *Salmonella,* can inform management decisions for children with infections and may reduce overall antibiotic exposure by ensuring more appropriate targeted therapy. However, in many LMIC settings, capacity to conduct microbiological culture with speciation and AST of enteric pathogens is limited.[86]

Escherichia coli **(*E. coli*)** are commensal *Enterobacteriaceae* that often exist harmlessly in the human gastrointestinal **(GI)** tract. *E. coli* are easy to culture and may act as good proxy of AMR circulating among other *Enterobacteriaceae*. *E. coli* can exchange genetic elements with other bacteria conferring antibiotic resistance.[87,88] Commensal *E. coli* in the gut may serve as an important reservoir of AMR and these resistance determinants may be transferred to pathogens, including *Salmonella* and *Shigella*. Evidence of in vivo transfer of extended spectrum beta-lactamase **(ESBL)** genes between *E. coli* and *Salmonella enterica* has been documented and similar resistance has been observed between *Shigella*

and *E. coli*.[68,89] Inflammation, which is often present during episodes of diarrhea, has also been shown to further promote the exchange of genetic elements of AMR among *Enterobacteriaceae*.[33]

Given limitations in AMR testing of pathogens in LMIC settings, we explored the potential utility of using antibiotic resistance in *E. coli* as a proxy for resistance in other pathogenic enteric bacteria. We compared antibiotic susceptibility patterns in *Salmonella* and *Shigella* isolates with *E. coli* isolated from the same fecal sample collected from children in Western Kenya and evaluated the ability of AST results in *E. coli* to predict AST results in *Salmonella* and *Shigella*.

Methods

Parent Trial

This cross-sectional study was nested within a clinical trial examining the impact of giving azithromycin to children discharged from hospital in Kenya to reduce rehospitalizations and deaths.[4,41] Fecal samples were collected from children at discharge from hospital (prior to randomization or provision of investigational product) as well as at 3 and 6 months post-randomization for microbiologic culture and **AST.** The protocol and primary analysis of the parent trial have been published previously.[4,41]

Study Population

Children aged 1 – 59 months were identified and screened by study staff at the time of hospital discharge from Kisii and Homa Bay County Teaching and Referral hospitals in western Kenya. The purpose of the parent study was to determine whether a 5-day course of azithromycin reduces rehospitalization and/or death in the 6-month period following hospitalization.[41,4] Eligible children weighed at least 2kg, caregivers stated they planned to stay in the area for a at least 6 months, and consent from a legal guardian needed to be obtained.[4,41] Children hospitalized solely due to trauma, poisoning, or a congenital syndrome were not included in the study.[4,41] Having a twin of the same sex enrolled on the same day, contraindications to azithromycin, being discharged with a prescription to a macrolide drug, or taking a protease inhibitor were also reasons for exclusion.[4,41]

Population

Children who had either *Salmonella* or *Shigella*, as well as *E. coli,* isolated from the same fecal sample were selected for this study. Phenotypic AMR was determined using disc diffusion as described by the Clinical and Laboratory Standards Institute (**CLSI**).[44] Briefly, isolates were thawed and isolated using quadrant-streaking onto MAC and incubated in ambient air at 37°C. If more than one colony morphology was seen following the restoration of *E. coli* on MAC agar, isolates from up to 3 of each morphology were separately tested for AMR. If multiple morphologies of *Salmonella* /*Shigella* isolates were identified, one was randomly selected and underwent testing for antimicrobial susceptibility.

Collection and transport of fecal samples

Whole stool or rectal swabs (FLOQSwabs, Copan Diagnostics) were immediately placed in Cary-Blair media, which was kept between 2 - 8°C, which was shipped within 24 hours of collection to the Center for Microbiology at the Kenya Medical Research Institute **(KEMRI CMR)**. The fecal samples were first plated on Mueller-Hinton agar **(MHA)** and followed by MacConkey **(MAC),** Salmonella/Shigella agar **(SSA)**, and eosin methylene blue **(EMB)** agars and incubated for 18 hours in 37°C. Culture results from MAC, EMB, and SSA were recorded and isolated colonies suspected as being *E. coli*, *Salmonella*, or *Shigella* were confirmed using the API 20E system (bioMérieux, Inc) and oxidase reactions were performed as described previously.[43] Isolates were placed in tryptic soy broth with 15% glycerol and frozen at -80°C for later AST.

Antibiotic Susceptibility Testing

Cultures of *E. coli, Salmonella, and Shigella* were grown overnight and isolates were placed into 5ml of sterile normal saline. Uniformity was obtained by adjusting the saline/bacteria diluents with a 0.5 MacFarland standard. MH plates were covered homogenously with a sterile swab that had been placed into a bacterial diluent. A disc dispenser was used to apply antibiotic discs on top of the inoculated MH agar and the MH plates were placed into an incubator maintained at 37°C for 18 – 24 hours. The following antibiotics were used for AST: ampicillin, ceftriaxone, cefotaxime, ceftazidime, cefoxitin, imipenem, ciprofloxacin, gentamicin, azithromycin, chloramphenicol, combined amoxicillin and clavulanic acid, and

combined trimethoprim/sulfamethoxazole **(cotrimoxazole)**. Diameters of the zone sizes **(ZS)** around antibiotics were measured macroscopically in millimeters and interpretations of susceptibility, resistance, or an intermediate were determined using guidelines in the CLSI-2021 M-100 (Table 2A, Zone Diameter and MIC Breakpoints for *Enterobacteriaceae*).[44] Resistance was defined as either an intermediate or resistant determination. To date, there are no zone diameters established to determine phenotypic resistance to azithromycin for *E. coli.* Therefore, we used the 2021 guidelines for *Salmonella enterica* serovar Typhi as has been done in other studies.[4,44] When more than one *E. coli* isolate was tested, the result was determined to be resistant if any isolate demonstrated non-susceptibility to a given antibiotic.

Determination of ESBL-producing E. coli

Phenotypic designations of ESBL producers were determined using the double-disc synergy test. The ZS of ceftazidime and cefotaxime with and without clavulanic acid, a beta-lactamase inhibitor, were compared as previously described.[12,45] Quality control of the test was obtained by discerning appropriate differences in ZS between the two selected antibiotics with and without clavulanic acid for ESBL-producing (NCTC 13351) and ESBL-negative (ATCC 25922) strains of *E. coli*. If the difference in ZS between cefotaxime and cefotaxime with clavulanic acid and ceftazidime compared to ceftazidime with clavulanic acid was <5mm then it was determined the ESBL-negative QC strain performed adequately. On the contrary, if the difference in ZS between cefotaxime and the ZS of cefotaxime with clavulanic acid or the ZS of ceftazidime and ceftazidime with clavulanic acid was ≥5mm the ESBL positive QC strain was verified.

Ethical Considerations

The study was approved by the Institutional Review Boards of the Kenya Medical Research Institute (SERU 3086), the Kenyan Pharmacy and Poisons Board (Ref. No. PPB/ECCT/15/10/04), and the University of Washington (IRB# 49120). The parent trial was registered at ClinicalTrials.gov (Identifier: NCT02414399). Consent was obtained from the primary guardians of children prior to entry into the study.

Statistical Analysis

Characteristics pertaining to the children included in this study, their home environment, and the

hospitalization which immediately preceded their enrollment in the parent study were described using frequencies and percentages or median and interquartile ranges.

The number isolates susceptible to each antibiotic tested was determined for *Shigella*, *Salmonella,* and *E. coli* cultured from the same fecal sample. For the primary analyses, *Shigella* and *Salmonella* AST results were combined. The percent agreement was determined for each antibiotic (or ESBL-production) by including concordant susceptibility or resistance in both the pathogenic bacteria (either *Salmonella* or *Shigella*) and *E. coli* over the total number of samples included in the study. We evaluated the diagnostic accuracy (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of AST results from *E. coli* isolates to AST results from *Salmonella*/*Shigella* isolates. In secondary analyses we assessed the sensitivity, specificity, PPV, and NPV of *Salmonella spp.* and *E. coli* (Appendix I) and *Shigella spp.* and *E. coli* (Appendix II) separately. Two-sided 95% confidence intervals (CIs) were calculated assuming a binomial distribution, where possible. When a one-sided CI was appropriate an alpha of 2.5% was used.

We calculated 1-specificty, or the false positive rate, for susceptibility to the antibiotics tested and non-ESBL production. A plot was constructed using the sensitivity (true positives) on the y-axis and false positives on the x-axis to compare the ability for the AST results in enteric pathogens to act as a proxy to identify results in *E. coli*. The closer the point was to the top left corner, the better the performance of a given antibiotic to predict the true positive rate and least false positives in *E. coli*. Points which fell below the diagonal reference line performed worse than chance alone. Statistical analyses were performed in StataMP 17 (StataCorp, College Station, Texas).

RESULTS

Study Population

Among the 30 children included in this sub-study, nearly half were over 24 months of age at enrollment (n = 14; 46.7%). None of the included children were HIV-infected, although four (13.8%) were HIV exposed but uninfected. Slightly more than half (55.2%) were exclusively breastfed during their first

six months of life (Table 1). The majority of children had received antibiotics during their hospital stay (n = 25; 83.3%) (Table 1. Of those who received antibiotics, the most common included amoxicillin/ampicillin (n = 18; 72.0%), gentamicin (n = 16; 64.0%), and ceftriaxone (n = 12; 48.0%) (Table 3). Crowding, defined as more than 2 individuals per room, was reported by 13 (43.3%) caregivers. Most caregivers (22; 73.3%) reported using an improved water source and 16 (53.3%) reported using treated drinking water (Table 1).

Of the 1,400 children enrolled in the parent trial, 30 (2.1%) had at least one fecal sample in which both *E. coli* and either *Salmonella* or *Shigella* were isolated. Three of these children had two separate samples with both *E. coli* and either *Salmonella*/*Shigella* isolated, resulting in 33 total paired samples. From the enrollment visit samples, a total of 15 had either *Salmonella* or *Shigella* isolated and 13 (86.7%) of these had *E. coli* isolated (Table 2). Fourteen samples had an enteric pathogen isolated at the month 3 visit, and of these 13 (92.9%) also had *E. coli* isolated from the same sample. At the 6 month visit 7 samples had both an enteric pathogen and *E. coli* isolated (Table 2).

Antimicrobial susceptibility in *E. coli* predicting susceptibility in *Salmonella* and *Shigella*

Percent agreement in susceptibility between *E. coli* and either *Salmonella* or *Shigella* isolates was highest for imipenem, with all 33 pathogenic bacteria and *E. coli* susceptible to the antibiotic (Table 3). Cephalosporins showed agreement near or above 70% (ceftriaxone 69.7% [95% CI 51.3% - 84.4%], cefotaxime 72.7% [95% CI 54.5% - 86.7%], ceftazidime 78.8% [95% CI 61.1% - 91.0%], and cefoxitin 90.9% [95% CI 75.7% - 98.1%]). Agreement was lowest for ciprofloxacin (51.5% [95% 33.5 – 69.2%]) and combined amoxicillin and clavulanic acid (54.6% [95% CI 36.4% - 71.9%]) (Table 4).

Among the 20 children without ESBL-producing *Salmonella* or *Shigella*, 16 had *E. coli* that was also not ESBL-producing (Sensitivity: 80% [95% CI 56.3% - 86.7%]). Among the 13 children with ESBL-producing *Salmonella* or *Shigella,* 8 had *E. coli* that was also ESBL-producing (Specificity 61.5% [95% CI 31.6% - 86.1%]). (Table 2). Sensitivity was highest for imipenem (100% [97.2% single-sided CI 89.4%]), chloramphenicol (95.0% [95% CI 75.1% - 99.9%]), ceftazidime (90.0% [95% CI 68.3% - 98.8%]), cefoxitin 90.9% [95% CI 75.7% - 98.1%]), and gentamicin (90.0% [95% CI 68.3% - 98.8%]) (Table 2). Particularly low sensitivity was observed for ampicillin (27.3% [95% CI 6.0% - 61.0%]) and cotrimoxazole

(25.0% [95% CI 0.6% - 80.6%]), although specificity was highest for these two antibiotics (81.8% [95%CI 59.7% - 94.8%] and 86.2% [95% CI 68.3% - 96.1%], respectively). Specificity and was not able to be calculated for imipenem due to all isolates being susceptible to the antibiotic (Table 4).

When plotting the sensitivity (true positive rate) and 1-specificity (true negative rate) the area above the reference line, which represents chance, and to the left of the plot suggests better ability of susceptibility in *E. coli* to predict susceptibility to a given antibiotic in *Salmonella*/*Shigella* (Figure 2). AST results from the third generation cephalosporins tested (ceftazidime, cefotaxime, and ceftriaxone), gentamicin, and ESBL-production in *E. coli* performed best when predicting results in *Salmonella* and *Shigella* (Figure 2). Chloramphenicol susceptibility patterns in *E. coli* were able to predict most *Salmonella* and *Shigella* isolates susceptible to the antibiotic; however false positives were also high (Figure 2). The ability of AST results from ciprofloxacin and azithromycin in *Salmonella* and *Shigella* to predict the outcome in *E. coli* were below the reference line and therefore no better than chance (Figure 2).

DISCUSSION

In this study, we determined that the ability of AST results in *E. coli* to successfully predict AST results in *Salmonella* or *Shigella* varies by class of antibiotic. The ability to predict susceptibility was highest for cephalosporins, gentamicin, azithromycin, and cotrimoxazole and for imipenem, which had 100% concordance for susceptibility. These results suggest modest agreement in sensitivity patterns between *E. coli* and important enteric pathogens. We interpret these findings to mean that the frequency of discordant results observed at the individual level may suggest that using *E. coli* as a proxy for antibiotic susceptibility in enteric pathogens may only be useful at the population or facility level and may not perform adequately to inform individual treatment decisions.

The concept of using proxy organisms such as *E. coli* to monitor resistance is not novel. Macrolide resistance following mass drug administration of azithromycin has also been monitored using *E. coli* and *Staphylococcus aureus*.[90,91] A recent study examining whether *E. coli* could serve as an

indicator of resistance in *Salmonella* for surveillance in retail meat or slaughtered animals suggested some utility, although differences were observed between species.[92] However, this proxy approach has not been validated in humans. In this study, we found susceptibility to azithromycin in *E. coli* predicted, with reasonable accuracy, susceptibility to azithromycin in *Salmonella* and *Shigella*. However, because resistance to azithromycin was rare (~5%) in *Salmonella* and *Shigella,* as compared to resistance in *E. coli* (~20%), resistance was not as well predicted.

In this study, the susceptibility to some commonly used antibiotics, including cephalosporins, in *E. coli* were frequently in agreement with the susceptibility in *Salmonella*/*Shigella*. Third generation cephalosporins are recommended as second-line treatments in low resource settings for common infectious diseases in children.[10,93] Reasonable confidence in susceptibility of pathogens to these antibiotics is necessary as these drugs are given to children with greater severity of illness or those who do not respond to first-line therapies.[93,94] Our findings suggest an ability to approximate susceptibility to these antibiotics using easy to culture and ubiquitous *E. coli* as a proxy to inform whether these drugs should be used or if alternatives should be sought. As opposed to cephalosporins, susceptibility to ampicillin, a common first-line antibiotic, in *E. coli* did not accurately predict susceptibility in Salmonella/*Shigella*. However, given widespread resistance to penicillin drugs, such as ampicillin, in SSA, resistance to penicillin drugs may be assumed to be high and ineffective for many common illnesses.[95]

Failure to detect ESBL in *E. coli* reliably predicted lack of ESBL production in *Salmonella* and *Shigella*. ESBLs confer broad resistance to many first- and second-line antibiotics, including penicillins and cephalosporins. In addition, many ESBL determinants are able to spread between *Enterobacteriaceae,* as they are frequently located on mobile genetic elements.[36,72,96] ESBL also often co-occurs with resistance to other commonly used antibiotics, such as aminoglycosides and fluoroquinolones.[12,36] Therefore, the ability to reliably predict the absence of ESBL in enteric pathogens using *E. coli* may be useful in informing treatment options and may reduce the need for broader spectrum antibiotics, including carbapenems.

Susceptibility to ciprofloxacin in *E. coli* was not a good predictor of susceptibility to the antibiotic in *Salmonella* or *Shigella*. Ciprofloxacin is currently recommended by the World Health Organization as a first-line antibiotic for the treatment of dysentery, caused by *Shigella*, in children and is considered an essential drug.[10,97] While susceptibility to other essential antibiotics, such as ceftriaxone, in *E. coli* reliably predicted the outcome in *Salmonella*/*Shigella*, the poor performance of predicting susceptibility to ciprofloxacin demonstrates the importance of considering the utility of *E. coli* as a proxy as drug-dependent. Some conditions, such as dysentery, would still require empiric treatment or isolation and AST of isolates from patient samples, the latter of which is difficult in SSA due to laboratory constraints.

This study has several strengths. While the GI tract is hypothesized as a reservoir of AMR where *Enterobacteriaceae* exchange genetic elements, to our knowledge this is the first study to examine patterns of antimicrobial susceptibility in *E. coli* and *Salmonella*/*Shigella* isolates from the same human fecal sample. This study also examined AMR in children who had been recently hospitalized and subsequently discharged. These children often suffer poor outcomes in the post-discharge period, which may be due to incomplete clearance, reinfection, or a new infection. The initial choice of an effective antibiotic to treat high risk children may be important in improving outcomes and reducing morbidity and mortality. However, this study also had limitations, including a relatively small sample size. However, given that enteric pathogens are often more difficult to culture, this pilot study provides vital information suggesting additional investments examining the potential for AST in *E. coli* to predict AST in enteric pathogens in SSA may be of important public health value. It is also important to note that some *E. coli* are pathogenic and we did not test for virulence determinants in isolates recovered from children in this study.

CONCLUSION

While the exchange of AMR between commensal and pathogenic *Enterobacteriaceae* is well understood, there has been limited evidence comparing patterns of resistance in *E. coli* and enteric pathogens. In this study, we evaluated the diagnostic accuracy of using *E. coli* as a proxy for AMR in

enteric pathogens. The use of easy-to-culture *E. coli* as a marker of AMR risk at the population or facility level may be useful to guide clinical management in areas where resistance is increasing and where laboratory capacity is limited. Ultimately, access to improved information to guide antimicrobial therapy in SSA may improve treatment outcomes and reduce morbidity and mortality.

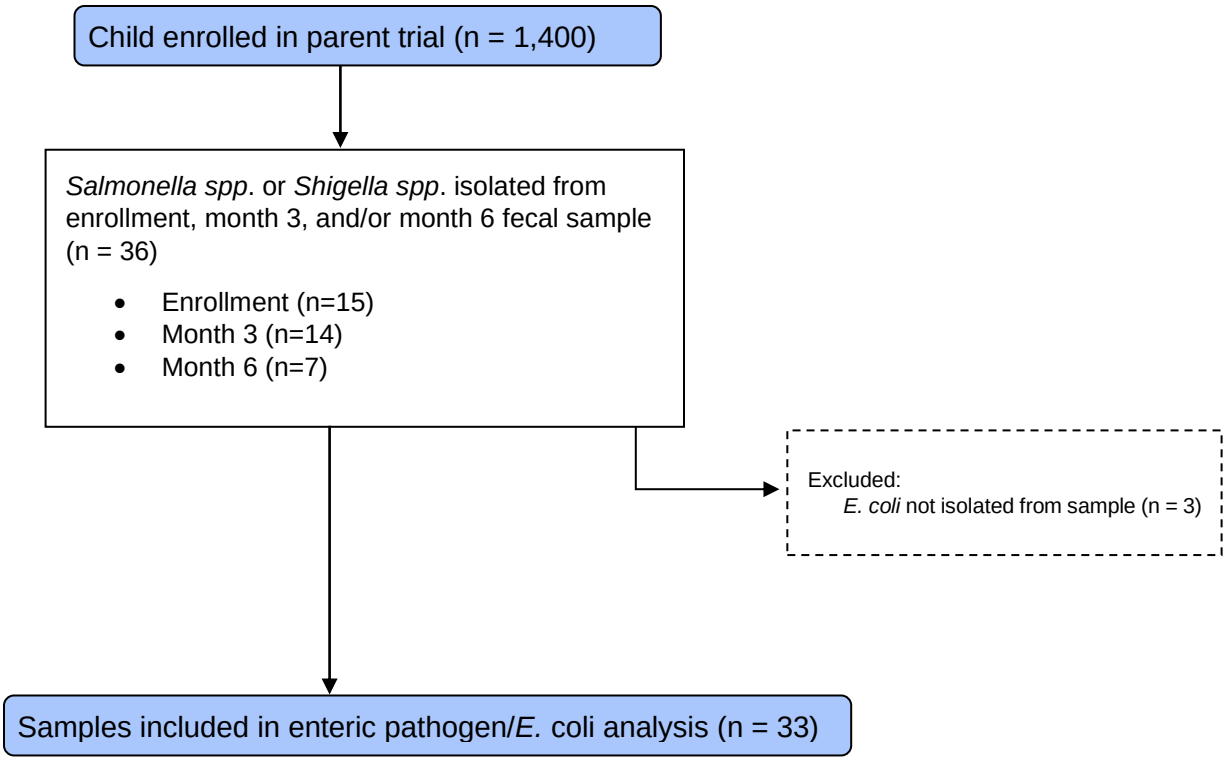


Figure 1. Sample flow chart. Three participants had *Salmonella spp*./*Shigella spp*. isolated at more than one visit for a total of 30.

Table 1. Participant characteristics of children with *E. coli* and either *Salmonella spp*. or *Shigella spp*. isolated from the same fecal sample

	Total (n = 30)[i] n (%)
Center	
Kisii	15 (50.0%)
Homa Bay	15 (50.0%)
Participant Characteristics	
Sex	
Male	17 (56.7%)
Female	13 (43.3%)
Age (months)	
1 – 5	3 (10.0%)
6 – 11	7 (23.3%)
12 – 23	6 (20.0%)
24 and over	14 (46.7%)
Breastfeeding[ii]	
Exclusively Breastfed	16 (55.2%)
Partially Breastfed	13 (44.8%)
HIV Status[iii]	
HIV Uninfected	25 (86.2%)
HIV Uninfected, Exposed	4 (13.8%)
Stunted (HAZ <-2)	6 (20.0%)
Underweight (WAZ <-2)	4 (13.3%)
Acute Malnutrition[iv]	
Severe (WHZ < -3 or MUAC <11.5cm or oedema)	2 (6.7%)
Moderate (WHZ ≥ -3 to < -2 or MUAC ≥11.5cm to <12.5cm)	3 (10.0%)
Hospitalization Information	
Referred from another Heath Facility	3 (0.0%)
Hospitalized ≤1 Year Prior to this Hospitalization	4 (13.3%)
Length of Hospital Stay (in days)[v]	3 (3 – 5)
Received Antibiotic during Hospitalization	25 (83.3%)
Antibiotic Received during Hospitalization[vi]	
Penicillins	18 (72.0%)
Ceftriaxone	12 (48.0%)
Gentamicin	16 (64.0%)
Other	4 (16.0%)
Discharge Diagnosis[vii]	
Anemia	4 (13.3%)
Gastroenteritis or Diarrhea	3 (10.0%)
Lower Respiratory Tract Infection	8 (26.7%)
Malaria	9 (30.0%)
Malnutrition	2 (6.7%)
Meningitis	1 (3.3%)
Sepsis	2 (6.7%)
Sickle Cell	5 (16.7%)
Other	3 (10.0%)
Prescribed Antibiotic at Discharge	18 (60.0%)
Household Information	
Crowding (>2 people/room)	13 (43.3%)
Livestock Ownership	22 (73.3%)
Improved Water Source	22 (73.3%)
Treated Drinking Water (use of filters, boiling, or chlorination)	16 (53.3%)
Shared Toilet (use by more than 1 household)[viii]	17 (58.6%)
Toilet Type	
Flushing	3 (10.0%)
Pit Latrine	26 (86.7%)
Open Defecation	1 (3.3%)

[i] A total of 33 samples were included in this study from 30 unique individuals

[ii] Of those with data available (n = 29) Current breastfeeding for children ≤6 months; breastfeeding practiced when children were under 6 months; n = 1 unknown

[iii] Uninfected, Exposure Status unknown (n = 1); Column percentages of children with exposure and infection status known (n = 29)

[iv] MUAC is only taken into consideration in children 6 months or older

[v] Median and interquartile range provided

[vi] Not mutually exclusive. Total n = 25 received antibiotics, column percentages are of these children. Other antibiotics given: cotrimoxazole (n = 1), chloramphenicol (n = 3), metronidazole (n = 1)

[vii] Not mutually exclusive. Other diagnoses at discharge include: upper respiratory tract infection (n = 3), convulsions (n = 2), skin/soft tissue infection (n = 1), unknown (n = 1)

[viii] Of those who do not practice open defecation (n = 1)

Table 2. Proportion of Samples with Isolated *E. coli*, *Salmonella spp.*, and *Shigella spp.*

	Visit 0 N = 1,400	%	Month 3 N = 1,261	%	Month 6 N = 1,210	%	Total N = 3,871	%
E. coli								
Isolated	1,222	(87.3%)	1,214	(96.3%)	1,150	(95.0%)	3,586	(92.6%)
Not Isolated	178	(12.7%)	47	(3.7%)	60	(5.0%)	285	(7.4%)
Salmonella spp.								
Isolated	11	(0.8%)	5	(0.4%)	4	(0.3%)	20	(0.5%)
Not Isolated	1,389	(99.2%)	1,256	(99.6%)	1,206	(99.7%)	3,851	(99.5%)
Shigella spp.								
Isolated	4	(0.3%)	9	(0.7%)	3	(0.3%)	16	(0.4%)
Not Isolated	1,396	(99.7%)	1,252	(99.3%)	1,207	(99.8%)	3,855	(99.6%)

Table 3. Agreement of susceptibility between *Shigella spp.* or *Salmonella spp.* with *E. coli* isolated from the same fecal sample[1]

		Salmonella spp./Shigella spp. susceptibility results						
		Susceptible	**Not Susceptible**	**Percent (%) Agreement**	**Sensitivity (%) 95% CI**	**Specificity (%) 95% CI**	**PPV[1] (%) 95% CI**	**NPV[1] (%) 95% CI**
E. coli. susceptibility results	ESBL							
	Producing	16	5	72.7%	80.0%	61.5%	76.2%	66.7%
	Non-Producing	4	8	55.5% - 86.7%	56.3 % - 94.3%	31.6% - 86.1%	52.8% - 91.8%	34.9% - 90.1%
	Cephalosporins							
	Cefoxitin							
	Susceptible	30	0	90.9%	90.9%	--	100%	0%
	Non-Susceptible	3	0	75.7% - 98.1%	75.7% - 98.1%		88.4%[1]	70.8%[iv]
	Cefotaxime							
	Susceptible	16	5	72.7%	80.0%	61.5%	76.2%	66.7%
	Non-Susceptible	4	8	54.5% - 86.7%	56.3% - 94.3%	31.6% - 86.1%	52.8% - 91.8%	34.9% - 90.1%
	Ceftazidime							
	Susceptible	18	5	78.8%	90.0%	61.5%	78.3%	80.0%
	Non-Susceptible	2	8	61.1% - 91.0%	68.3% - 98.8%	31.6% - 86.1%	56.3% - 92.5%	44.4% - 97.5%
	Ceftriaxone							
	Susceptible	15	4	69.7%	71.4%	66.7%	78.9%	57.1%
	Non-Susceptible	6	8	51.3% - 84.4%	47.8% - 88.7%	34.9% - 90.1%	54.4% - 94.0%	28.9% - 82.3%
	Penicillins							
	Ampicillin							
	Susceptible	3	4	63.6%	27.3%	81.8%	42.9%	69.2%
	Non-Susceptible	8	18	45.1% - 79.6%	6.0% - 61.0%	59.7% - 94.8%	9.9% - 81.6%	48.2% - 85.7%
	Carbapenems							
	Imipenem							
	Susceptible	33	0	100%	100%	--	100%	--
	Non-Susceptible	0	0	89.4%[iv]	89.4%[iv]		89.4%[iv]	
	Penicillin & β-lactamase inhibitor							
	Co-Amoxiclav							
	Susceptible	7	6	54.6%	43.8%	64.7%	53.8%	55.0%
	Non-Susceptible	9	11	36.4% - 71.9%	19.8% - 70.1%	29.8% - 74.3%	25.1% - 80.8%	31.5% - 76.9%
	Aminoglycoside							
	Gentamicin							
	Susceptible	18	6	75.8%	90.0%	53.8%	75.0%	77.8%
	Non-Susceptible	2	7	57.7% - 88.9%	68.3% - 98.8%	25.1% - 80.8%	53.3% - 90.2%	40.0% - 97.2%
	Macrolide							
	Azithromycin							
	Susceptible	24	2	72.7%	77.4%	0%	92.3%	0%
	Non-Susceptible	7	0	54.5% - 86.7%	58.9% - 90.4%	84.2%[iv]	74.9% - 99.1%	41.0%[iv]
	Fluoroquinolone							
	Ciprofloxacin							
	Susceptible	15	3	51.5%	53.6%	40.0%	83.3%	13.3%
	Non-Susceptible	13	2	33.5% - 69.2%	33.9% – 72.5%	5.3% - 85.3%	58.6% - 96.4%	1.7% - 40.5%
	Phenicol							
	Chloramphenicol							
	Susceptible	19	10	66.7%	95.0%	23.1%	65.5%	75.0%
	Non-Susceptible	1	3	48.2% - 82.0%	75.1% - 99.9%	5.0% - 53.8%	45.7% - 82.1%	19.4% - 99.4%
	Sulfonamide							
	Cotrimoxazole							
	Susceptible	1	4	78.8%	25.0%	86.2%	20.0%	89.3%
	Non-Susceptible	3	25	61.1% - 91.0%	0.6% - 80.6%	68.3% - 96.1%	0.5% - 71.6%	71.8% - 97.7%

CONCLUSION

This dissertation presents analyses that contribute to the understanding of antimicrobial resistance (AMR) in young children in Sub-Saharan Africa (SSA), specifically those who have been discharged from hospital following an acute illness. Children in SSA remain at high risk of mortality and morbidity in the post-discharge period. However, the pathways underpinning such risk are not fully understood. At the time of discharge, children have a high burden of AMR, which is supported by the work presented here, which may factor into the poor outcomes post-discharge. We determined that antibiotics provided during hospitalization, specifically receiving the antibiotic ceftriaxone, had the strongest association to carriage of extended beta-lactamase **(ESBL)** producing *Escherichia coli* **(*E. coli*)**. Additionally, we determined that resistance patterns to some antibiotics was similar in *E. coli* and *Salmonella* or *Shigella* isolated from the same children, suggesting the possibility of using commensal *E. coli* as a proxy to determine risk of resistance in settings where more complete AMR determination in pathogens is not feasible. Finally, primary caregivers and their children who were discharged from hospital did not always share similar patterns of AMR, with children frequently having a higher overall burden of AMR in these settings.

Carriage of AMR during the post-discharge period may negatively impact the health of children in SSA and may be associated with a higher risk of death and illness. In Chapter 1 we ascertained that *E. coli* isolated from children under the age of 5 in western Kenya harbored high rates of AMR to many antibiotic classes. Resistance to antibiotics recommended by international and national guidelines for first-and second-line therapies for common illnesses and syndromes, including ampicillin, ceftriaxone, and gentamicin was high. In addition, ESBL-producing *E. coli*, which confers broad resistance to most classes of beta-lactams, was frequently isolated. These findings suggest that common infections in children in the post-discharge period may not respond adequately to current treatment guidelines as a result of high rates of AMR.

In this analysis, prior receipt of an antibiotic more than doubled the likelihood of having ESBL-producing *E. coli* isolated. Additionally, longer hospital stays and being hospitalized previously within the

year were also associated with a higher risk of ESBL carriage. In addition, lack of access to adequate sanitation facilities in the home was also significantly associated with ESBL. Open defecation or having a toilet that was shared with at least one other household increased the likelihood of ESBL-producing *E. coli* being isolated. Although primary caregivers were exposed to the same hospital environment, we determined in Chapter 2 that their child's hospitalization was not associated with a greater likelihood of carriage of ESBL-producing bacteria being isolated.

Because of the ease with which ESBL can transfer between bacteria and people, some concordance between children and their caregivers was hypothesized. In general, however, we did not observe uniformly strong concordance between children and their caregivers. The exceptions occurred when there was an overall low prevalence of AMR to a drug (imipenem) or high resistance (ampicillin and cotrimoxazole) in both children and caregivers. We examined predictors of concordance for ESBL-producing E. coli in children and their caregivers. Of the risk factors examined, in Chapter 2 we determined that crowded living conditions were associated with greater concordance, while having other children and being employed or a student were negatively associated with such concordance. It is possible that these responsibilities reduced the time primary caregivers spend at the hospital, which in turn may have reduced the chance of acquiring AMR in the caregivers.

Agreement was more apparent when comparing *Salmonella* or *Shigella* with *E. coli* isolated from the same fecal sample in children in Chapter 3. While imipenem had perfect percent agreement due to no resistance in any of the isolates tested, other antibiotics also had higher percent agreements. These included the third generation cephalosporins, gentamicin, and azithromycin. ESBLs confer resistance to 3rd generation cephalosporins and often exist on plasmids with resistance to gentamicin or macrolide drugs such as azithromycin. Chapter 3 adds to the evidence that the gastrointestinal **(GI)** tract provides an environment for the exchange of resistance among bacteria, especially those from the same family. These results also highlight the importance of surveillance of AMR in bacteria, such as *E. coli*, that often exist harmlessly as commensals in the GI. Pathogens such as *Salmonella* and *Shigella* are more difficult to isolate and individual antibiotic susceptibility testing is usually not performed due laboratory infrastructure. We tested the diagnostic accuracy and found *E. coli* may serve as a reasonable proxy to

determine susceptibility to some antibiotics in *Salmonella* and *Shigella,* including 3rd generation cephalosporins, gentamicin, chloramphenicol, azithromycin, and imipenem. In addition, the lack of production of ESBLs was also reliably predicted. To date, this is the first study in humans that demonstrates that easily culturable and ubiquitous *E. coli* may be a useful proxy to determine antimicrobial resistance pattens in enteric pathogens, such as *Salmonella* and *Shigella*, in some settings.

In conclusion, children in SSA recently discharged from hospital have a high burden of AMR, which may contribute to the exceptionally high post-discharge morbidity and mortality observed in these settings. The high burden of AMR in these children suggests that interventions, including updated guidelines for alternative antibiotics to be administered to children requiring treatment during the post-discharge period, should be examined. Reductions in AMR in SSA should include interventions that reduce the spread of AMR from children returning to the home and community following a hospitalization. AMR is a global problem, and the burden of mortality is greatest in SSA. Reducing resistance in children at very high risk of acquiring AMR may reduce this burden and improve the outcomes in children who have been discharged from hospital in the region.

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