Host and Environmental Correlates of Multi-Drug Resistance in Kenyan Children with Acute Bacterial Diarrhea

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Abstract

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Bacterial diarrhea results in significant morbidity and mortality in children in sub-Saharan Africa. Antibiotic treatment can be a life-saving intervention, but the emergence of antibiotic resistance limits its clinical efficacy. Data on the burden and risk factors for antibiotic resistance in enteric pathogens are needed to inform diarrhea management recommendations and resistance control interventions. Stool/rectal swab samples of children aged 6 mos - 15 yrs presenting with acute diarrhea in western Kenya were cultured for bacterial pathogens. HIV-uninfected children with identified *Shigella* or *Salmonella* species, or enteropathogenic [EPEC], enterotoxigenic [ETEC], enteroaggregative [EAEC], or enteroinvasive *Escherichia coli* [EIEC] were included in this substudy. Resistance to ampicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, and tetracycline was determined using MicroScan Walkaway40 Plus. To evaluate correlates of multi-

drug resistance (MDR [resistance to ≥ 3 classes of antibiotics]), we used multivariable log-binomial regression to estimate prevalence ratios (PRs) and 95% confidence intervals (CIs). Of 292 children in the analysis, median age was 22.5 mos (interquartile range: 10.5-41.5 mos), 60.6% used pit latrines and 8.6% were HIV-exposed. Resistance to cotrimoxazole (96.2%) was most common among all pathogens, followed by ampicillin (79.1%) and tetracycline (73.0%). Phenotypic MDR was identified in 60.3% of children; and in 38.2% of *Shigella*, 40.0% of *Salmonella*, 73.0% of EPEC, 54.1% of ETEC, 76.0% of EAEC, and 72.2% of EIEC isolates. Children 6-24 mos were more likely to have MDR infections identified than those 24-59 mos (PR = 1.51 [95% CI: 1.19, 1.90]) whereas there was no difference in MDR prevalence between children in the two older age categories, >59m vs. 24-59m (PR = 1.30 [95% CI: 0.91, 1.87]). Children in households with a shared pit latrine were more likely to have MDR (aPR = 1.92 [95% CI: 1.08, 3.38]), than those with flush toilets, as were children in households that practiced open defecation (aPR = 1.91 [95% CI: 1.11, 3.30]). Children living in a household with 2 or more persons per room were 22% more likely to have an MDR pathogen than children living with fewer than 2 persons per room (PR = 1.22 [95% CI: 1.04, 1.43]). Duration of exclusive breastfeeding, malnutrition, maternal HIV, and water source were not associated with MDR infections in this study. Young children and those living in contaminated environments may be at higher risk for infection by antibiotic resistant enteric pathogens.

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2.1 INTRODUCTION

The burden of diarrheal diseases remains high in sub-Saharan Africa, accounting for an estimated 10% of childhood deaths in the region.[1,2] Bacterial causes of diarrhea - including enteropathogenic and enterotoxigenic *Escherichia coli* (*E. coli*, EPEC and ETEC*), Shigella* species (spp.), and *Salmonella* spp. - account for nearly 30% of deaths from diarrhea worldwide.[3] These pathogenic bacteria are associated with linear growth faltering, a condition with consequences extending well beyond childhood.[4,5] In addition to causing diarrhea, some of these bacteria (such as non-typhoidal *Salmonella)* are important causes of bacteremia among children in sub-Saharan Africa.[6,7]

Current World Health Organization (WHO) guidelines recommend antibiotic treatment in cases of bloody diarrhea (suspected shigellosis) or suspected cholera.[8–10] Some evidence suggests that antibiotics may also provide benefit in the treatment of other diarrheal pathogens, such as EPEC and ETEC.[11,12] However, enteric bacteria may develop antibiotic resistance which limits effectiveness of these antibiotics. There is concern that guidelines encouraging wider use of antibiotics may increase antibiotic resistance and limit efficacy of available treatment options.[13] This may be a particular concern in Sub-Saharan Africa, where second-line antibiotics may be less accessible. In addition to concerns about reduced treatment effectiveness, antibiotic resistance has been found to be associated with increased out-of-pocket costs to the individual[14] and increased cost to health systems, because patients with resistant infections experience longer hospitalizations.[15]

There are multiple mechanisms driving the emergence of antibiotic resistant diarrheal pathogens. Previously susceptible organisms may undergo spontaneous gene mutations in the presence of selective pressure that confer resistance.[16] Antibiotic resistance can also develop in

an individual or in the environment as a result of genetic transfer from an organism carrying resistance genes to a susceptible organism.[16–18] Antibiotic use directly alters the gut flora by eliminating susceptible bacteria, favoring the propagation of resistant species. These resistant organisms can then be shed into the environment where they serve as a reservoir of infection or colonization of other individuals. In addition, antibiotics and their metabolites are also excreted directly into the environment[18–20] and even low levels of these excreted antibiotics drive resistance among environmental bacteria.[19,21,22] Bacteria such as *Salmonella* spp. can persist for 10-15 days in non-host environments,[23] and settings with limited access to improved sanitation may offer increased opportunity for the acquisition, persistence, and transmission of antibiotic resistant bacteria.[16,18]

Despite the high prevalence, morbidity, and mortality of bacterial diarrheal disease in sub-Saharan Africa, data on the burden and risk factors for antibiotic resistance in enteric pathogens from this region are limited.[13] Further, there is a paucity of data describing characteristics of children that are associated with antibiotic resistance in pathogenic enteric infections. A recent study in Western Kenya found high frequencies of antibiotic resistance in *Shigella* isolates from children with moderate to severe diarrhea[24] but did not evaluate resistance patterns in other clinically important enteric bacteria, many of which are more common in younger children, or evaluate correlates of resistance.

We conducted a cross-sectional study in western Kenya, using previously collected data from HIV-uninfected children 6 months to 15 years old presenting to hospital with acute diarrhea, in whom *Shigella* spp, *Salmonella* spp, or one of four *E. coli* pathotypes (enteropathogenic- [EPEC], enterotoxigenic [ETEC], enteroaggregative [EAEC], or enteroinvasive [EIEC] *E. coli*) were isolated. We determined the proportion of *Shigella,*

Salmonella, EPEC, ETEC, EIEC, and EAEC isolates that were resistant to ampicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, and tetracycline, as well as the prevalence and correlates of multi-drug resistant (MDR, phenotypic resistance to 3 or more antibiotics from different classes) bacteria. Understanding host and environmental factors associated with antibiotic resistance in pathogenic enteric bacteria may help identify groups of children who may not respond to commonly used antibiotics. Further, this may shed light into mechanisms of antibiotic resistance in high diarrhea burden settings, highlighting opportunities for intervention.

2.2 METHODS

Study population and setting

We conducted a cross-sectional study nested in a hospital-based surveillance study of diarrhea in the Nyanza region of western Kenya. The parent study was conducted in hospitals in Kisii, Homa Bay, and Migori. We utilized previously collected data from approximately 2000 children between the ages of 6 months and 15 years old presenting to the health care facility with acute diarrhea (defined as three or more loose stools passed per day, for fewer than 14 consecutive days) between 2011 and 2014. Children were excluded from the parent study if they had a diagnosis of chronic non-infectious diarrhea, were not accompanied by a primary caregiver, or were unable to provide a stool sample or rectal swab. Children were included in this secondary analysis if one of the pathogenic enteric bacteria of interest were isolated from their stool or rectal swab sample (*Shigella spp*, *Salmonella spp*, EPEC, ETEC, EAEC, or EIEC), and if they were HIV uninfected.

Data collection

Potentially eligible children were identified in outpatient and inpatient departments of the three study hospitals. After the caregiver gave informed consent on behalf of the child, a stool

sample (or rectal swab if the child is unable to produce stool) was collected, and height/length and weight were measured. A study nurse conducted an interview with the primary caregiver accompanying the child, collecting information on water sanitation and hygiene practices, socio-demographic characteristics, breastfeeding, vaccinations, and other possible exposures of interest. Caregivers underwent HIV counseling and testing if willing to do so.

Stool specimen processing

The stool samples were first evaluated for consistency and appearance by a laboratory technician. Stool samples and rectal swabs were then transferred into Cary-Blair transport media and shipped at 4-8ºC within 24 hours of collection to the Kenya Medical Research Institute / United States Army Medical Research Unit Microbiology Hub Laboratory in Kericho, Kenya. Samples were plated on selective media as follows: blood agar plate for haemolysis and oxidase test, Sorbitol-MacConkey agar to select for nonsorbitol fermenting (NSF) *Escherichia coli* (*E. coli*), MacConkey agar for *E. coli* colonies, Hektoen or xylose lysine deoxycholate agar for *Salmonella* and *Shigella* spp.

Salmonella and *Shigella* colonies exhibiting the proper characteristics on the various media above were further processed using MicroScan WalkAway 40 Plus (Siemens, Erlangen, Germany) automated platform and were serologically typed using their respective commercially available typing sera. *E. coli* isolates were batch tested using multiplex PCR to identify virulent *E. col* forms: heat labile enterotoxin (elt) and/or heat- stable enterotoxin (est); enteroaggregative E. coli (EAEC), aatA; enteroinvasive E. coli (EIEC), invasion plasmid antigen H (ipaH); enterohemmorhagic E. coli (EHEC), Shiga toxin 1, 2 and variants (stx); enteropathogenic *E. coli* (EPEC), bundle forming pilus (bfpA). Starting in March 2013, additional gene targets for EPEC, intimin (eae), and for EAEC, aaiC, were incorporated in the PCR. A classification of EAEC was

therefore the identification of aatA and/or aaiC. EPEC was disaggregated into two categories: typical EPEC (bfpA with or without eae) and atypical EPEC (eae without bfpA or stx).

Pathogenic bacteria isolated from stool underwent antibiotic susceptibility testing by Minimum Inhibitory Concentration (MIC) testing using the automated Microscan Walkaway 40 Plus System using conventional gram-negative panels which includes extended spectrum beta-lactamases (ESBL) testing. Interpretations of antibiotic susceptibility testing were based on standard Clinical and Laboratory Standards Institute (CLSI) guidelines M100-S19. Antibiotic resistance was defined as demonstrated phenotypic resistance to one or more of the following: ampicillin, ciprofloxacin, cotrimoxazole, tetracycline, and ceftriaxone. Multi-drug resistance (MDR) was defined as resistance to 3 or more antibiotics from different classes, per expert recommendations.[25]

Statistical analysis and variable definitions

We calculated the proportion of isolated strains resistant to the antibiotics of interest, both stratified by bacterial genus and overall. The proportion of isolates with an ESBL were reported, as well as proportions of each pathogen that are MDR. We used χ^2 tests to evaluate statistical significance of MDR proportions between pathogens. To determine correlates of MDR enteric infections, we compared MDR prevalences in children with and without the characteristic of interest. The correlates of interest included: age, HIV-exposure (defined as having an HIV infected biological mother); acute and chronic malnutrition (defined as wasting [< -2 weight for height Z-score {WHZ}] or stunting [< -2 height for age Z-score {HAZ}], respectively, determined using WHO reference standards), toilet type (shared pit latrine, open defecation, unshared pit latrine, or unshared flush toilet); reported protected water source (protected well, protected spring, piped water to household, yard, or public tap), reported frequency and type of

water treatment (use of a water filter, adding bleach or chlorine, or boiling water in the household); household crowding (number of people per room in the household); and frequency of reported hand-washing.

Prevalence ratios (PRs) and 95% confidence intervals (95% Cis) were estimated using log-binomial regression with robust standard errors. In addition to univariate models for each correlate of interest, we constructed a multivariable model for each correlate of interest. For the multivariable model, age and indicators of socioeconomic status (SES) were considered to be *a priori* confounders based on the association of these factors with antimicrobial resistance in prior literature.[26–29] These covariates were retained in the multivariable model if their inclusion changed the PR estimate by approximately 10% or more. The SES factors evaluated were level of caregiver educational attainment (primary school or no school, secondary school, vocational school, or university and above), and estimated monthly income above or below 5000 ksh (50 USD) per month. Children with more than 1 pathogen of interest isolated were classified as MDR if at least 1 of the pathogens was MDR.

2.3 RESULTS

Of 1758 children enrolled in the parent study, 1444 did not have a bacteria of interest isolated in their stool and 22 were HIV infected, leaving 292 children included in the current study (Figure 1). Median age of included subjects was 22.5 months (interquartile range: 10.5 – 41.5 months), and 56.5% were male (Table 1). Twenty-two children were HEU (8.6%). The majority of the children were enrolled at Kisii or Homa Bay hospitals, with 6 children (2.1%) enrolled at Migori. All children in the study were reported to be exclusively breastfed, with the reported median length of exclusive breastfeeding of 6.0 months (IQR: 4.0 – 6.0 months).

Young children were more likely to have used antibiotics in the last 7 days and been hospitalized in the last year, though the association was not statistically significant. Those 6-24 months were more likely to have used antibiotics in the last 7 days (PR: 1.21 [95% CI: 0.59, 2.50], p = 0.604) and more likely to have been hospitalized in the last year than children 24 – 59 months (PR = 2.04 [95% CI: 0.77, 5.40], p = 0.151).

EAEC was the most commonly identified enteric infection (36.4%) followed by *Shigella* spp. (24.7%), EPEC (13.3%), ETEC (12.0%) EIEC (7.1%), and *Salmonella* spp. (6.5%). No child had more than one bacterial genus identified and 16 had two of the *E. coli* pathotypes of interest. The age distribution differed by pathogen (Table 4). The mean age of children with a *Shigella* infection was 42 months (standard deviation [SD] = 32 months), compared to a mean age of 29 months (SD = 32 months) among children without (p =0.042). Similarly, the mean age of children in whom *Salmonella* spp. were isolated was 46 months (SD = 40 months) compared to 31 months (SD = 32 months) (p = 0.003). The mean age of children in whom any of the 4 *E. coli* pathotypes were isolated was 27 months (SD = 31 months); the mean ages of children with EPEC, ETEC, EIEC, and EAEC were 22, 34, 46, and 22 months, respectively.

Almost all children (97.6%) had an enteric organism resistant to at least 1 of the antibiotics of interest, and 60.3% had MDR. Resistance to ampicillin, cotrimoxazole, and tetracycline was frequently observed among all pathogens (Table 2) – 80.2% of all bacteria were resistant to ampicillin, 96.4% to cotrimoxazole, and 74.4% to tetracycline. Resistance to ciprofloxacin and ceftriaxone was rare (in 3.6% and 1.6%, respectively,) as was presence of ESBL (3.2%). There were notable differences in MDR prevalence between bacterial groups; *Shigella* spp. and *Salmonella* spp. were less likely to have MDR compared to any *E. coli*

pathotype ($p<0.0001$ and $p = 0.002$, respectively), whereas EAEC isolates were most likely compared to any other bacteria, with 77.7% of isolates MDR ($p < 0.0001$).

Young age was the only host factor that emerged as a significant correlate of MDR risk. Compared to older children aged between 24-59 months, children aged 6 to 24 months were 51% more likely to have a MDR enteric bacteria isolated (PR = 1.51 [95% CI: 1.19, 1.90]). This association remained when stratifying by pathogen (Table 4), although the effect was attenuated among children with infection by any of the *E. coli* pathotypes.

HEU children and children with wasting tended to have higher prevalence of MDR. Wasted children had a trend for being more likely to have an MDR pathogen (PR = 1.21 [95% CI: 0.99, 1.49]) than children who were neither stunted nor wasted. Similarly, HEU children were more likely to have an MDR pathogen than HIV unexposed, uninfected (HUU) children. (PR: 1.21 [95% CI: 0.92, 1.60]). All 22 HEU children had pathogenic enteric infections that were resistant to cotrimoxazole, as compared to 91.9% of HUU children (Fisher's exact test p-value = 0.402).

Several environmental factors were significantly associated with MDR: use of unimproved sanitation facilities, lack of hand-washing by the caregiver, and household crowding. After adjustment for age and SES indicators, children in households with a shared pit latrine were nearly twice as likely to have an MDR bacteria isolated than those with a flush toilet, (aPR: 1.91 [95% CI: 1.11, 3.30]). The results were similar for children whose caregivers reported use of a non-shared pit latrine or open defecation (aPR= 1.92 [1.08, 3.38] and aPR = 1.93 [0.88, 4.20], respectively). Children whose caregivers reported "sometimes" or "never" washing their hands after defecating were 52% more likely to have an MDR enteric bacteria isolated than those whose caregivers reported "always" or "usually" washing their hands (aPR:

1.52 [95% CI: 1.21, 1.91]). Results were similar for children whose caregivers reported "sometimes" or "never" washing their hands before eating (aPR: 1.54 [95% CI: 1.03, 2.29]). Household crowding was associated with a higher prevalence of MDR enteric infections. Children who lived with >2 persons per room in the household were significantly more likely to have an MDR enteric pathogen identified than those in a household with 2 or fewer persons per room (PR = 1.22 [95% CI: 1.04, 1.43]). The association was unchanged by adjustment by for socioeconomic factors and age. For each additional personal person per room in a child's household, there was a 6% higher prevalence of MDR (PR = 1.06 [95% CI: 0.99, 1.14]).

2.4 DISCUSSION

Among children under age 15 presenting to clinic with acute diarrhea in Western Kenya, resistance to ampicillin, cotrimoxazole, and tetracycline was common among pathogenic enteric bacteria. These three antibiotics are on the WHO Essential Medicines List (2010)[30], are widely available and low cost. Cotrimoxazole is also used prophylactically in HIV-infected adults and children and in HIV-exposed children until infection is ruled out. Tetracycline, and cotrimoxazole to a lesser extent, are also widely used in livestock husbandry in Kenya,[31] which may further contribute to bacterial resistance to these antibiotics in the region. Resistance to antibiotics that are less commonly available due to cost or availability, such as ciprofloxacin and ceftriaxone, was lower among all pathogens. The pattern of lower resistance to ciprofloxacin and ceftriaxone, and higher resistance to ampicillin, cotrimoxazole, and tetracycline, is consistent with trends reported by other recent studies of these pathogens in children with diarrhea in Sub-Saharan Africa.[13,32–40]

Despite WHO recommendations indicating ciprofloxacin for treatment of *Shigella*-associated diarrhea, all *Shigella* isolates were susceptible to ciprofloxacin.[9] This could be due to

lack of availability of this fluoroquinolone or its high cost in the communities where this study took place, resulting in antibiotics that are more affordable or commonly available used instead (such as ampicillin, cotrimoxazole, or tetracycline). Further, the guideline used to empirically identify *Shigella* infections (presence of dysentery) has been found to be a poor predictor of *Shigella* infection in this setting,[41] so ciprofloxacin may be indicated in only a few children. As a result, ciprofloxacin may not be widely used enough to drive selective drug pressure. In our study, there were 11 isolates resistant to Ciprofloxacin (2 EIEC, 2 EPEC, and 7 EAEC isolates) from 10 children. Since *Shigella* and EIEC share the virulence gene *ipaH,* resistance in these 2 EIEC may be indicative of the prevalence of ciprofloxacin resistance if molecular techniques were used to detect *Shigella* in these patients rather than empiric guidelines. Given that inter-genus transfer of resistant plasmids can occur among gut bacteria, the ciprofloxacin resistance in the *E.coli* pathotypes may represent a reservoir for ciprofloxacin resistance in future pathogen infections in the same children.

We found age under 24 months to be associated with a higher prevalence of MDR in enteric pathogens. Young age has previously been described as a risk factor for resistance in commensal enteric bacteria among children in resource-limited countries,[42–45] and our analysis suggests this may be true of enteric pathogenic bacteria as well. It has been hypothesized that the association between young age and resistance may due to high incidence of infections and subsequent antibiotic use in this population.[42–44,46] In our data, children under 24 months were more likely to have used antibiotics recently, and the selective pressure exerted by frequent antibiotic use may have contributed to the increased prevalence of MDR pathogens in this group. Younger children were also more likely to be infected with EPEC or EAEC, the two pathogens with the highest prevalence of MDR. Adjusting for the bacterial type of infection reduced the

magnitude of, but did not remove, the inverse association between age and MDR prevalence, suggesting that infection with enteric bacteria that are most likely to be MDR may not fully explain the mechanisms why young children are at higher risk of MDR enteric infections. Younger children may be exposed to a wider diversity of pathogens in their environment through oral investigation (the process of mouthing nearby objects), resulting in frequent ingestion of pathogens in their environment. By the sheer volume of pathogen exposure, younger children may have a higher burden of resistant infections that either remain in the gut or pass their resistant genetic material to commensal bacteria leading to a higher burden of resistant gut bacteria. Additionally, young age has been reported to be a risk factor for antimicrobial resistance and MDR enteric bacteria among young of livestock animals,[47–50] though this is not well understood.

While age was the only host characteristic in our study that was significantly associated with MDR, our data do show a trend toward wasted children and HEU children having a higher prevalence of resistant enteric pathogens. The immunocompromise that results from HIV exposure or malnutrition may place these children at higher risk of MDR enteric infections, as both systemic and local intestinal immunity appear important in the development of resistance.[51–53] Further, these children often receive antibiotics for prophylaxis or treatment, further increasing the selective pressure for resistance to emerge.[54–56] HEU children typically live with HIV-infected household members, which may increase exposure to resistant enteric pathogens as a result of the increased use of antibiotics by the HIV-infected household member.

Several factors pertaining to living in close proximity to other people and to a contaminated environment were significantly associated with MDR in our study. First, children whose caregivers used an unimproved defecation facility, or who infrequently washed their

hands after defecation or before eating, were more likely to have MDR enteric infections. Our data are consistent with evidence that suggests that exposure to environmental fecal contamination may play an important role in acquiring antibacterial resistance in enteric bacteria.[46,57] Unimproved sanitation increases exposure to enteric pathogens, which may have been subject to selective pressures causing them to be more likely to be resistant than susceptible. Antibiotic use in the community could lead to selection pressure in the environment by eliminating susceptible bacteria in the intestines prior to excretion[18,58] and the excretion of the antibiotics themselves.[19,20] Animal waste contamination, naturally occurring minerals, and agricultural pollutants can also co-select for resistant bacteria in the environment.[16,18] Much like the gut where bacteria are in close proximity to one-another and thus easily able to share genetic material, settings of poor sanitation may similarly offer opportunity for inter-species transfer of resistance genes, resulting in the widespread dissemination of resistance in these settings.[18,27]

Household crowding was associated with MDR pathogenic enteric infections in children, with a trend toward higher prevalence of resistance with greater numbers of persons per room in the household. Exposure to household members may increase likelihood of resistant pathogenic enteric infections through person-to-person transmission of resistant bacteria. Prior research evaluating close contact with other people as a risk factor for resistance in children in low-income countries is inconclusive. Attendance at a large primary school[42] and sharing a bed with another child[36] were found to be risk factors for carriage of resistant commensal *E. coli* but two studies reported no association between sharing a home with 2 or more children and resistance.[36,45] Both studies that reported no association measured only the absolute number of children in the household, without incorporating a measure of household size, suggesting that the

degree of actual contact with other household members may be a more important predictor of risk of acquiring a resistant infection than the absolute number of household members.

The strengths of this study include its setting in Kenya, as there is a need for more data on resistance in Sub-Saharan Africa. Studies are scarce that identify risk factors for carriage of antibiotic resistance in enteric bacteria among children in low-resource settings, and even fewer are available that examine risk factors for resistance in pathogenic enteric infections, a clinically important topic for children in Sub-Saharan Africa. There are several limitations to our study as well. The small sample size may limit the strength of our conclusions, since this may have resulted in low statistical power, particularly for correlate variables that were rare in this dataset and for analyses stratified by bacteria pathotype. While combining all the bacteria in the regression analysis created larger strata and more stable estimates, this may have created a heterogeneous category since these bacterial genuses have different mechanisms of resistance acquisition and different age distributions. Due to the cross-sectional design of this study, we can only draw conclusions about associations between these factors and MDR at the single time point of data collection, and we have no data on longitudinal outcomes (including virulence) associated with MDR in these organisms. We also cannot ascertain from this data whether the children's acute diarrhea at presentation could be attributed to these pathogenic bacteria isolated from the children's stool as many of these infections have been found to be asymptomatically carried in children in similar settings.[2] Our adjustments of caregiver education and monthly income may not fully capture the effects of socioeconomic status, and there may still be residual confounding. Accurate measures of weight, and therefore wasting, are difficult to ascertain in children with diarrhea due to dehydration, though this is expected to be non-differential between the MDR and non-MDR children. Further, we were not able to ascertain from this data whether a

child was truly exposed to HIV, and our definition of HEU (an HIV uninfected child born to an HIV infected mother) was used as a proxy. However, we expect misclassification to be minimal, and we expect this definition to capture other risks associated with living with an HIV infected caregiver.

There are important public health implications of our findings. We report widespread resistance to commonly used, inexpensive antibiotics (ampicillin, tetracycline, and cotrimoxazole), which may present challenges to management of childhood infections including bacterial diarrhea. The fact that MDR prevalence was higher in children under 24 months is concerning given that this age group is at highest risk of death from diarrheal disease.[2] The higher prevalences of MDR associated with unimproved sanitation, household crowding, and infrequent hand-washing suggests that living in a contaminated environment not only increases risk of pathogen exposure but also poses risk of acquisition of antibiotic resistant infections since enteric bacteria in environmental fecal contamination may be more likely to be resistant than not. Public health interventions that target improved sanitation may be contribute to control of antibiotic resistance in enteric pathogens.

2.5 CONCLUSION

Resistance to commonly available antibiotics (ampicillin, tetracycline, and cotrimoxazole) among enteric infections in children presenting with acute diarrhea is highly prevalent in Western Kenya. Children under 24 months of age and those who live in settings with poor sanitation may be at particularly high risk of having MDR enteric infections. Exposure to fecal contamination in a child's environment may increase transmission of fecal bacteria resistant to antibiotics commonly used in the community.

CHAPTER 3

3.1 TABLES AND FIGURES

Figure 1. Flowchart of inclusion of study subjects, for a secondary analysis of data on children aged 6 mo – 15 yrs presenting with acute diarrhea to clinics in Western Kenya, between 2011 and 2014

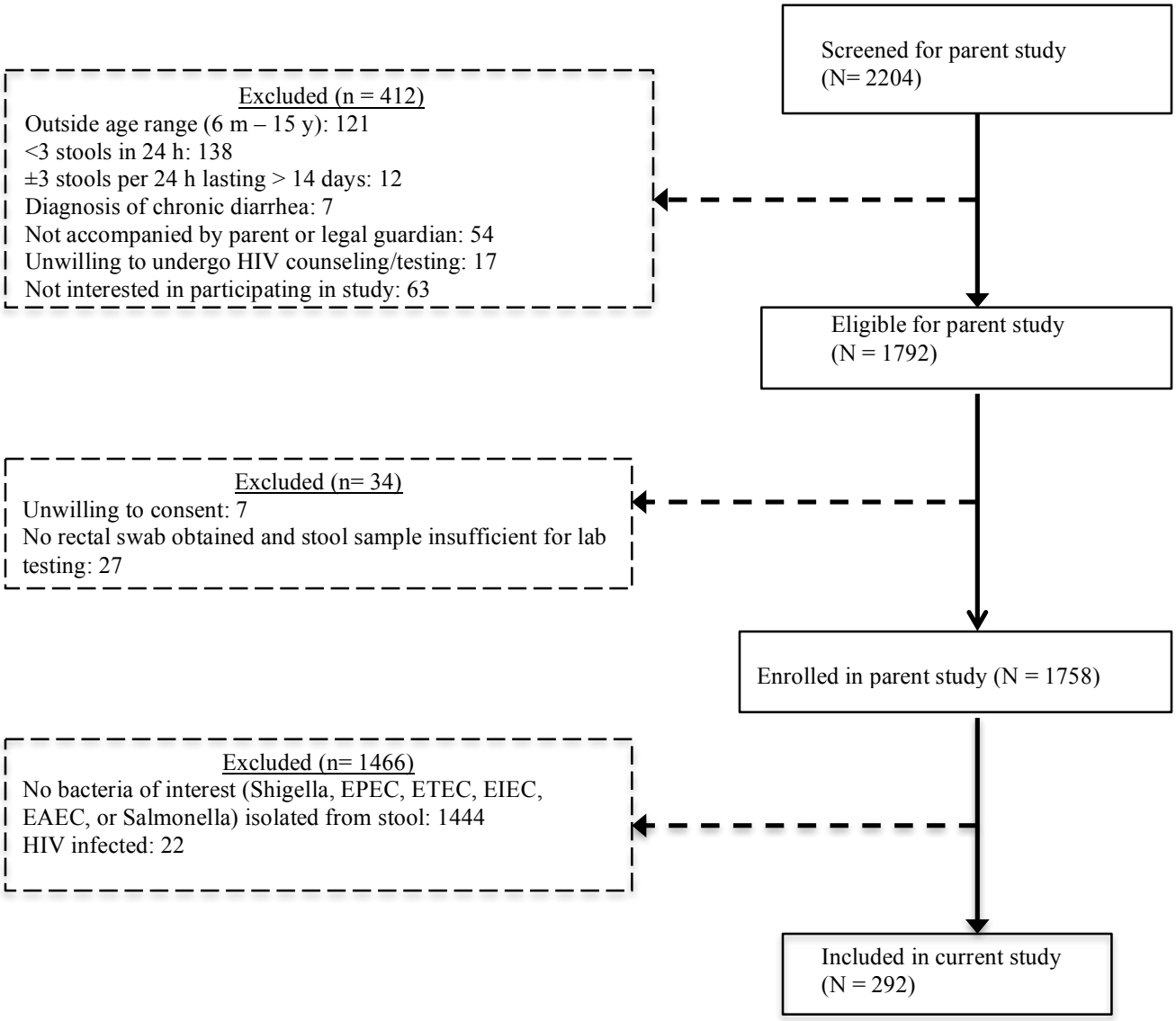

Table 1. Characteristics of children enrolled in a sub-study of multi-drug resistance, among HIV-uninfected children 6 mos – 15 yrs presenting with acute diarrhea in Western Kenya

	Children with MDR[i] enteric infections N = 176 (60.3%)	**Children with non-MDR enteric infections** N = 116 (39.7%)	**Total** N = 292
Sociodemographic Characteristics			
Age, mo (median [IQR])	16 (10.0 – 35.0)	27.5 (13 - 48.5)	22.5 (10.5 – 41.5)
Age category			
6 – 12 mo	69 (39.20%)	29 (25.0%)	98 (33.6%)
>12 – 24 mo	42 (23.9%)	20 (17.2%)	62 (21.2%)
>24 – 59 mo	47 (26.7%)	55 (47.4%)	102 (34.9%)
59 mo – 15 yr	18 (10.2%)	12 (10.3%)	30 (10.3%)
Male (n [%])	116 (54.7%)	70 (56.5%)	165 (56.5%)
Monthly income <5000 KSH	75 (35.4%)	35 (30.2%)	93 (31.9%)
Site			
Kisii	93 (52.8%)	70 (60.3%)	163 (55.8%)
Homa Bay	80 (45.5%)	43 (37.1%)	123 (42.12%)
Migori	3 (1.7%)	3 (2.6%)	6 (2.1%)
Travel time to clinic greater than 1 hour	25 (14.2%)	21 (18.1%)	46 (15.8%)
Crowding (≥2 persons per room in home)	80 (45.5%)	38 (32.8%)	118 (40.4%)
Own land	123 (69.9%)	83 (71.6%)	206 (70.6%)
Caregiver education			
Primary school or less	86 (48.9%)	62 (53.5%)	148 (50.7%)
Secondary school	50 (28.4%)	34 (29.3%)	84 (28.8%)
Vocational school	33 (18.8%)	15 (12.9%)	48 (16.4%)
University or above	7 (4.0%)	5 (4.3%)	12 (4.1%)
Clinical Characteristics			
Months exclusive breastfeeding (median [IQR])	6.0 (4.0 – 6.0)	6.0 (4.0 – 6.0)	6.0 (4.0 – 6.0)
Currently breastfeeding (among children <24 mo)	89 of 111 (80.2%)	35 of 49 (71.4%)	124 of 160 (77.5%)
Any antibiotic use in the last 7 days[ii]	19 (10.8%)	14 (12.1%)	33 (11.3%)
Hospitalized in the last year	13 (7.4%)	9 (7.8%)	22 (7.5%)
HIV exposed	16 (10.2%)	6 (6.0%)	22 (8.6%)[iii]
Malnourished[iv]			
Stunted	24 (13.6%)	18 (15.5%)	42 (14.4%)
Wasted	35 (19.9%)	15 (12.9%)	50 (17.1%)
Water, Sanitation, and Hygiene Characteristics			
Water source			
Use of water from an improved source[v]	60 (34.1%)	41 (35.3%)	101 (34.6%)
Use of treated water[vi]	45 (25.6%)	35 (30.2%)	80 (27.4%)
Use of both treated and untreated water[vii]	51 (29.0%)	31 (26.7%)	82 (28.1%)
Use of water from an unimproved source[viii]	20 (11.4%)	9 (7.8%)	29 (9.9%)
Sanitation facility			

[i] Multi-drug resistant (resistance to 3 or more antibiotics from different classes)
[ii] Includes cotrimoxazole prophylaxis use
[iii] Data on HIV exposure status are missing for 35 subjects (12.0% of total). HIV status was missing for 4 child subjects, and 31 biological mothers.
[iv] Stunting defined as < -2 weight for height Z-score (WHZ); wasting defined as < -2 height for age Z-score (HAZ). HAZ and WHZ were calculated using 2006 and 2007 WHO reference population
[v] Defined as use of a protected well, protected spring, piped water to household, yard or public tap, with no treatment method, or a reported water treatment method is missing or is removal of particulate matter of any frequency.
[vi] Defined as "always" using a water filter, adding bleach or chlorine, or boiling water in the household on water from any source.
[vii] Defined as "usually or sometimes" using a water filter, adding bleach or chlorine, or boiling water in the household on water from any source.
[viii] Defined as use of an unprotected well, spring, or surface water source, and no treatment method or treatment method is removal of particulate matter (letting water settle or straining through a cloth) of any frequency.

Flushing toilet	8 (4.7%)	13 (11.2%)	21 (7.2%)
Pit latrine for household use only	57 (32.8%)	37 (31.9%)	94 (32.2%)
Shared pit latrine	106 (60.2%)	63 (54.3%)	169 (57.9%)
Open defecation	5 (2.8%)	3 (2.6%)	8 (2.7%)
Hand-washing			
"Sometimes or never" wash hands before eating	5 (2.8%)	1 (0.9%)	6 (2.1%)
"Sometimes or never" wash hands after defecating	9 (5.1%)	1 (0.9%)	10 (3.4%)

Table 2. Resistance patterns of 308 Shigella, Salmonella, and select E. coli strains isolated from stool of 292 children presenting with acute diarrhea to clinics in Western Kenya, between 2011 and 2014. Abbreviations: CLSI = Clinical and Laboratory Standards Institute; MIC = Minimum Inhibitory Concentration; ESBL = extended spectrum beta lactamase; EPEC = enteropathogenic *E. coli*; ETEC = enterotoxigenic *E. coli*; EAEC = enteraggregative *E. coli;* EIEC = enteroinvasive *E. coli*; MDR = multi-drug resistance (resistance to 3 or more antibiotics of different classes)

	MIC Break-point (µg/ml) [i]	***Shigella* spp. N = 76 (24.7% of total)** (n resistant (% resistant))	***Salmonella* spp. N = 20 (6.5% of total)** (n resistant (% resistant))	**EPEC N = 41 (13.3% of total)** (n resistant (% resistant))	**ETEC N = 37 (12.0% of total)** (n resistant (% resistant))	**EIEC N = 22 (7.1% of total)** (n resistant (% resistant))	**EAEC N = 112 (36.4% of total)** (n resistant (% resistant))	**All *E. coli* pathotypes N = 212 (68.8% of total)** (n resistant (% resistant))	**Total N = 308** (n resistant (% resistant))
Ampicillin	≥32	35 (46.1%)	16 (80.0%)	37 (90.2%)	35 (94.6%)	21 (95.5%)	103 (92.0%)	196 (92.5%)	247 (80.2%)
Ceftriaxone	≥4	0[ii]	0	2 (5.4%)	0	0	3 (2.7%)	5 (2.4%)	5 (1.6%)
		0 ESBL	4 ESBL (20.0%)[iii]	0 ESBL	1 ESBL (2.7%)	1 ESBL (4.6%)	4 ESBL (3.6%)	6 (2.8%)	10 (3.2%)
Ciprofloxacin	≥4	0	0	2 (4.9%)	0	2 (9.1%)	7 (6.3%)	11 (5.2%)	11 (3.6%)
Cotrimoxazole	≥4/76	72 (94.7%)	16 (80.0%)	39 (95.1%)	37 (100.0%)	22 (100.0%)	111 (99.1%)	209 (98.6%)	297 (96.4%)
Tetracycline	≥16	59 (81.9%)[iv]	8 (40.0%)[v]	34 (82.9%)	20 (54.1%)	17 (77.3%)	91 (81.3%)	162 (76.4%)	229 (74.4%)
MDR (p-value)[vi]	--	29 (38.2%) ($p < 0.0001$)	8 (40.0%) ($p = 0.001$)	31 (75.6%) --	20 (54.1%) --	17 (77.3%) --	87 (77.7%) --	155 (73.1%) Referent	176 (60.3%) --

[i] Breakpoints are for the "resistant" interpretation, based on CLSI 2009 standards (M100-S19).
[ii] 6 Shigella isolates are missing ceftriaxone data. Percentages are out of 70 isolates
[iii] 7 Salmonella isolates are missing ceftriaxone data. Percentages are out of 13 isolates
[iv] 4 Shigella isolates are missing tetracycline data. Percentages are out of 72 isolates
[v] 6 Salmonella isolates are missing tetracycline data. Percentages are out of 14 isolates
[vi] P-values are from χ^2 tests comparing proportion of a *Shigella* spp or *Salmonella* spp that are MDR to the proportion of all *E. coli* pathotypes that are MDR.

*Table 3. Correlates of multi-drug resistance in enteric bacterial infections among Kenyan children 6 mos – 15 yrs with acute diarrhea. Statistically significant results are **bolded.*** Abbreviations: PR = prevalence ratio; 95% CI = 95% confidence interval

	Crude estimates		Adjusted estimates	
	PR (95%CI)	p-value	aPR (95% CI)[i]	p-value
Host Characteristics				
Age				
6 – 24 mos	**1.51 (1.19, 1.90)**	**p = 0.001**	**1.51 (1.19, 1.90)**	**p = 0.001**
>24 – 59 mos	Referent		Referent	
>59 mos – 15 yrs	1.30 (0.91, 1.87)	p = 0.151	1.30 (0.91, 1.87)	p = 0.151
HIV exposure				
HIV unexposed, uninfected	Referent			
HIV exposed, uninfected	1.21 (0.92, 1.60)	p = 0.173	1.21 (0.92, 1.60)	p = 0.173
Malnutrition[ii]				
Neither wasted nor stunted	Referent		Referent	
Stunted[iii]	0.88 (0.61, 1.27)	p = 0.496	0.88 (0.61, 1.27)	p = 0.496
Wasted	1.21 (0.99, 1.49)	p = 0.070	1.21 (0.99, 1.49)	p = 0.070
Both wasted and stunted	1.04 (0.71, 1.54)	p = 0.826	1.04 (0.71, 1.54)	p = 0.826
Duration of exclusive breastfeeding				
6 mos or more	Referent		Referent	
< 6 mos	1.03 (0.97, 1.10)		p = 0.277	
Environmental Factors				
Sanitation facility				
Flush toilet	Referent		Referent	
Non-shared pit latrine	1.59 (0.90, 2.81)	p = 0.109	**1.92 (1.08, 3.38)**	**p = 0.025**
Shared pit latrine	1.65 (0.94, 2.88)	p = 0.080	**1.91 (1.11, 3.30)**	**p = 0.020**
Open defection	1.64 (0.76, 3.53)	p = 0.205	1.93 (0.88, 4.20)	p = 0.099
Water source				
Use of treated or improved water	Referent		Referent	
Any use of untreated or unimproved water	1.10 (0.91, 1.33)	p = 0.306	1.10 (0.91, 1.33)	p = 0.306
Household crowding				
<2 persons per room	Referent		Referent	
2 or more persons per room	**1.22 (1.04, 1.43)**	**p = 0.016**	**1.22 (1.04, 1.43)**	**p = 0.016**
Caregiver-reported hand-washing before eating				
Always or usually	Referent		Referent	
Sometimes or never	1.39 (0.96, 2.02)	p = 0.079	**1.54 (1.03, 2.29)**	**p = 0.035**
Caregiver-reported and-washing after using the toilet				
Always or usually	Referent		Referent	
Sometimes or never	**1.52 (1.21, 1.91)**	**p < 0.0001**	**1.52 (1.21, 1.91)**	**p < 0.0001**

[i] All estimates were adjusted for socioeconomic indicators (monthly income above/ below 5000 ksh, and caregiver education). Estimates were also adjusted for age, except estimates for the association of MDR with age (since age was the correlate of interest), and hand-washing and crowding (due to model convergence failure)

[ii] Wasting = < -2 weight for height Z-score (WHZ); stunting = < -2 height for age Z-score (HAZ)

[iii] The "wasting" and "both wasting and stunting" categories include only children 6 months – 10 years of age. WAZ data are not available for children older than age 10 because this indicator does not distinguish between height and body mass during the pubertal growth spurt[59]

Table 4. The association of age with multi-drug resistant pathogenic enteric infections in children under age 15 years presenting with acute diarrhea to hospitals in western Kenya, stratified by isolated pathogen.

	Children with *Shigella* infections N = 76		**Children with *Salmonella* infections** N = 20		**Children with EPEC infections** N = 37		**Children with ETEC infections** N = 37		**Children with EIEC infections** N = 18		**Children with EAEC infections** N = 104	
	n(%)	PR (95% CI)	n(%)	PR (95% CI)	n(%)	PR (95% CI)	n(%)	PR (95% CI)	n(%)	PR (95% CI)	n(%)	PR (95% CI)
Age category												
6-24 mo	23 (30.3%)	1.50 (0.82, 2.76)	6 (30.0%)	1.83 (0.52, 6.42)	28 (75.7%)	1.13 (0.61, 2.06)	21 (56.8%)	1.13 (0.60, 2.14)	6 (33.3%)	1.50 (0.76, 2.97)	76 (73.1%)	1.02 (0.79, 1.30)
>24-59 mo	44 (57.9%)	Referent	11 (55.0%)	Referent	6 (16.2%)	Referent	11 (29.7%)	Referent	9 (50.0%)	Referent	21 (20.2%)	Referent
>59 mo - 15 yr	9 (11.8%)	1.40 (0.60, 3.26)	3 (15.0%)	2.44 (0.70, 8.56)	3 (8.1%)	1.00 (0.38, 2.66)	5 (13.5%)	0.37 (0.06, 2.30)	3 (16.7%)	Undefined	7 (6.7%)	[i]
Mean age (p-value[ii])	41.6 mo (p = 0.042)		46.4 mo (p = 0.003)		21.4 mo (p = 0.037)		34.1 mo (p = 0.683)		43.8 mo (p = 0.033)		23.2 mo (p < 0.0001)	

[i] Due to failure in model convergence, this analysis used binary age categories, comparing children 6 – 24 mos to 24 – 59 mos.
[ii] P-values correspond to linear regression coefficients estimating mean difference in age between children with a given pathogen and children without

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