

Antimicrobial resistance patterns among *E. coli* isolates from children presenting with diarrhoea at a cosmopolitan hospital in Kenya

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Abstract

Background: Diarrhoea is a serious infection that kills at least 2 million children globally. Children under the age of 5 years are particularly predisposed to diarrhoea especially if the surrounding environment is contaminated or polluted with human sewerage. Due to frequent episodes of diarrhoea, children may unjustifiably be subjected to antibiotics and this could in turn lead to emergence of multidrug resistant strains. In this study, we isolated and determined antimicrobial susceptibility profiles of 384 *E. coli* isolates from rural and urban children with chronic and acute diarrhoea.

Objective: To establish the antimicrobial resistance of diarrhoeagenic *Escherichia coli* in rural and urban population of children under the age of 5 years presenting with diarrhoea at Thika Level 5 Hospital in Kiambu County.

Methods: *E. coli* strains were isolated from stool specimen or anal swabs using conventional techniques and antibiotic susceptibilities determined using the disc diffusion technique. Questionnaires were used to collect data on history of hospitalization, antibiotic use history, access to clean drinking water and toilets.

Results: Resistance prevalence was high for ampicillin, sulphamethoxazole-trimethoprim combinations chloramphenicol (23%), amikacin (10%) and kanamycin (21%). Streptomycin (S) was the least effective aminoglycoside while gentamicin (CN) and amikacin (AK) were effective against at least 70% of all isolates. Close to 32% of all isolates were resistant to amoxicillin-clavulanic acid combinations (AMC) while 63% were resistant to ampicillin. At least 52% of the isolates were resistant to more than three classes of antimicrobials tested. Resistance was lowest for ciprofloxacin (4%), nalidixic acid (5%), cefepime (5%), ceftriaxone (7%) and ceftazidime (7%). A total of 47 (12%) of the 384 isolates exhibited the ESBL phenotype. Isolates recovered from children with acute

diarrhoea were more likely to exhibit resistance to co-trimoxazole than those with chronic diarrhoea ($p < 0.003$ CI=1.41-5.113, OR=2.68). In addition, isolates from acute diarrhoea were also more likely to exhibit combined resistance to SXT/FEP/NA or CIP/ and to at least one aminoglycoside than those from chronic diarrhoea ($p < 0.024$, CI=0.025-0.66, OR=0.13). Isolates from children who had used antibiotics 3 months prior to sampling were more likely to be resistant to multiple antimicrobials compared to those from children who had not (OR: 0.9; 95% CI=0.016-1.22; $p < \text{value}$: 0.01).

Conclusions: This study demonstrates that a significant proportion of *E. coli* strains obtained from children in urban and rural settings with acute and chronic diarrhoea are MDR. A significant proportion of these highly MDR strains are resistant to major classes of antimicrobials such as β -lactams, aminoglycosides and fluoroquinolones. The study therefore indicates a need to conduct culture and susceptibility testing with the view of determining aetiologic agents of diarrhoea and their effectiveness in situations where antimicrobial use is justified.

Key words: Antimicrobial resistance, Hygiene, Diarrhoea, Antimicrobials, *E. coli*

Introduction

Diarrhoeal diseases are leading causes of morbidity and mortality worldwide, especially in the developing countries. There are an estimated 2 million deaths per year among children globally, majority of whom are in developing countries [1]. Antibiotic treatment is recommended for use in amoebic and *Shigella* dysentery, but not for watery diarrhoea associated with *E. coli* infections [2]. This recommendation is frequently flouted in most developing countries because many health centers are not equipped to diagnose the aetiologic agent associated with diarrhoea. In such countries therefore, treatment of diarrhoea

is generally syndromic and includes use of antibiotics besides nutritional rehabilitation and replacement of fluid and electrolytes in the patient [3]. Use of antimicrobials in the absence of Culture and Susceptibility Testing (C&ST) exposes patients to two main dangers: - first, there is a high chance of inappropriate antimicrobials use, a phenomenon that promotes emergence and spread of resistant strains. Secondly, treatment of *E. coli* infections can result in adverse reactions especially if Enterohemorrhagic *E. coli* (EHEC) strains are implicated in the illness. The lysis of EHEC strains in blood after antimicrobial treatment can release toxins that trigger a potentially fatal immune response [4].

Young children, especially those under the age of 5 years are particularly vulnerable to diarrhoea and therefore represent a group frequently subjected to antimicrobial treatment. It is thus not surprising that studies conducted in many developing countries indicate that pathogens recovered from stool from children are resistant to multiple classes of antimicrobials. Since children who suffer frequent diarrhoea episodes may have other underlying conditions including malnutrition, they are likely to suffer frequent bouts of diarrhoea and they are particularly susceptible to invasive *E. coli* infections. Resistance to antimicrobials among intestinal *E. coli* is particularly important because, strains from the gut may find their way into other sites such as blood and urethra where they cause septicemia and urinary tract infections. Such infections may therefore necessitate the use of antimicrobials. It is therefore prudent to determine resistance profiles of intestinal *E. coli* regardless of whether they are implicated in diarrhoea or not. The aim of this study was to determine resistance profiles among *E. coli* strains recovered from children with diarrhoea under the age of 5 years seeking treatment at a large hospital in a cosmopolitan setting. We also investigated if there is a relationship between access to clean drinking water, toilets, family composition and carriage of multidrug resistant (MDR) strains.

Materials and Methods

Study population: This cross-section study obtained stool specimen from children younger than five years of age presenting with diarrhoea at a Thika Level 5 Hospital in Kiambu County.

The hospital has a bed capacity of 245 in the general wards and 51 in the paediatric ward but patients may share beds. The outpatient department treats an average of 4200 children per month. Stool or rectal swabs specimens were taken from diarrhoeic children with laboratory request. Information was obtained for each patient regarding age, sex, onset of diarrhoea, history of treatment and hospitalization, access to clean drinking water, composition of the family and availability and type of toilet facilities at the home of the child. Improved toilets were defined as in door flush toilets connected to a sealed septic tank or to the municipal sewerage system. For consistency, diarrhoea was defined as defecation of liquid or semi-liquid stool three or more times a day.

Acute diarrhoea was defined as an abnormally frequent discharge of semisolid or fluid faecal matter from the bowel, lasting less than 14 days. Chronic diarrhoea was defined as diarrhoea lasting for more than two weeks. Specimens were only collected from children whose parents or guardians gave a written consent. In addition, specimens were only obtained from patients who had not taken antibiotics at least one week before the onset and during the period of diarrhoea. Using this criterion, we recruited 384 children.

Specimen collection and processing: Stool specimen were collected from patients in sterile plastic containers and processed immediately. For children who could not readily produce stool specimen, faecal material was collected from around the anal opening using sterile moist cotton swabs. The specimens were plated on MacConkey agar and incubated at 37°C for up to 24 hours. A maximum of 3 colonies were randomly selected per plate for each patient and purified further before confirmation of species identity using standard biochemical methods as previously described [5]. The biochemical tests included gram staining, oxidase test, citrate utilization and growth characteristic on Lysine Iron Agar (LIA), Triple Sugar Iron agar (TSI), and motility test on indole ornithine media (Oxoid LTD, Basingstoke, Hampshire, England).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was done on Mueller-Hinton agar using the disc diffusion method according to the recommendation of Clinical and Laboratory Standards Institute [6]. The following antimicrobials were used: - ampicillin (10 µg), ceftazidime (30 µg), and cefepime ((30 µg). Sulphamethoxazole-trimethoprim combination is the ration of 30:5.2 µg, amoxicillin-clavulanic acid (in the ratios of 20:10 µg) respectively, ceftriaxone (30 µg), tetracyclines (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), kanamycin (30 µg), streptomycin (30 µg), amikacin (30 µg) and gentamicin (10 µg). All the antibiotic discs were obtained from Oxoid (Basingstoke, Hampshire, England). In order to identify potential β-lactamase-producers, one plate was inoculated with only β-lactam antibiotics while the second plate contained all other antimicrobials. The β-lactam antibiotics were placed adjacent to the amoxicillin/clavulanic (AMC) disc at inter-disc distances (centre to centre) of 20 mm. A clear extension of the edge of a cephalosporin disc zone towards the AMC (also described as an appearance of a ghost zone) was interpreted as positive for Extended Spectrum Beta Lactamase (ESBL) production.

Statistical analysis: For the purpose of analysis, both intermediate and resistant results for antibiotic susceptibility testing were grouped together as “resistant”. Differences in proportion of isolates bearing different elements was analyzed using the Chi (χ^2) tests while Fisher’s exact test were used for smaller sample sizes. The Odds Ratios (OR) and the 95% Confidence Intervals (CIs) accompanying the χ^2 tests were determined using the approximation of Woolf. The null hypothesis was rejected for values of $p \geq 0.05$. Statistical analysis was performed using Statgraphics plus version 5 (StatPoint Technologies, INC, Warrenton, VA, USA).

Results

Resistance to antimicrobials: Table 1 shows antimicrobial-susceptibility profiles of 384 isolates analyzed in this study. Resistance to ampicillin, sulfonamides, tetracyclines, streptomycin and amoxicillin-clavulanic acid (AMC) was noted in over 30% of all isolates (Figure 1). Resistance was also high for chloramphenicol (23%), amikacin (10%) and kanamycin (21%). At least 52% of the isolates were resistant to more than three different classes of antimicrobials tested and were thus classified as Multi-Drug Resistant (MDR). Resistance was lowest for ciprofloxacin (4%), nalidixic acid (5%), cefepime (5%), ceftriaxone (7%) and ceftazidime (7%).

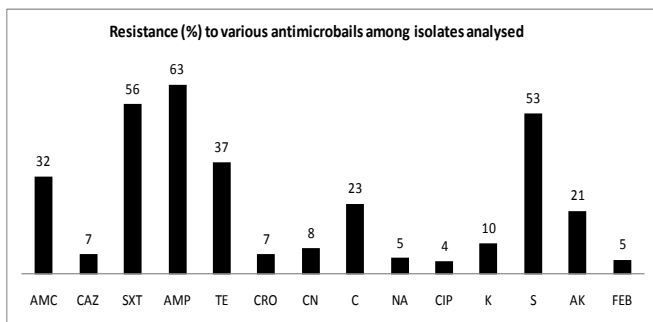


Figure 1: Percentage resistance among the 384 *E. coli* strains isolates

Key: Amoxicillin-Clavulanic Acid (AMC), Ceftazidime (CAZ) Sulphamethoxazole-Trimethoprim (SXT), Ampicillin (AMP), Tetracyclines (TET), Ceftriaxone (CRO), Gentamicin (CN), Chloramphenicol (C), Nalidixic Acid (NA), Ciprofloxacin (CIP), Kanamycin (K), Streptomycin (S), Amikacin (AK), and Cefepime (FEP).

Resistance to β -lactams in relation to other antimicrobials: This study also revealed that 32% of all isolates were resistant to amoxicillin-clavulanic acid combinations (AMC) while 63% were resistant to ampicillin. Resistance to other β -lactam antibiotics was much lower and only 47 (12%) of the 384 isolates exhibited the ESBL phenotype. This study also showed that 5% of isolates were resistant to cefepime (FEP). Majority of isolates resistant to β -lactams were also resistant to sulphamethoxazole and trimethoprim (SXT) and to at least one more class of antimicrobial especially tetracycline (TET) and chloramphenicol (C). Streptomycin (S) was the least effective aminoglycoside while gentamicin (CN) and amikacin (AK) were effective against at least 70% of all isolates. In addition, all isolates resistant to either nalidixic acid (NA) or ciprofloxacin (CIP) were also resistant to at least a 3rd generation cephalosporin. Such multi-drug resistant isolates were from both children who had taken antibiotics than those who had not, and resident in both urban and rural settings (Table 1).

Table 1: Number (%) of strains from different clinical backgrounds exhibiting combined resistance to multiple classes of antimicrobials

	Types of diarrhoea		History of hospitalization last 6 months		Antibiotic use last 3 months	
	Acute	Chronic	Yes. Last 6 months	No. Last 3 months	Yes	No
Number of isolates resistant to	338 (%)	46 (%)	158 (%)	226 (%)	174 (%)	210 (%)
SXT	59*	35	91*	31	75*	40
4th generation β -lactams (FEB)	5	0	9*	1	7	2
NA/ CIP	4	7	20*	7	18*	7
≥ 1 aminoglycosides	66*	29	87*	44	75*	50
SXT/FEP/ NA/ CIP/ ≥ 1 aminoglycoside	1*	7	3	<1	3	<1
ESBL phenotype	14*	0	18*	8	21*	5

Key: (*) indicates X^2 values for dichotomous variables (e.g. chronic vs. acute diarrhoea) that are statistically significant ($p < 0.05$)

Resistance pattern for acute or chronic diarrhoea, and exposure to antibiotics: This study did not seek to establish if indeed the isolates analyzed were the aetiologic agents of diarrhoea. However, our data indicates that isolates obtained from acute diarrhoea stools were significantly resistant to a broader spectrum of antimicrobials than those obtained from children with chronic diarrhoea. Isolates recovered from children with acute diarrhoea were more likely to exhibit resistance to co-trimoxazole than those with chronic diarrhoea ($p < 0.003$ CI=1.41- 5.113, OR=2.68 (Table 2) but no significant difference was recorded in the resistance to fourth generation cephalosporins (e.g. cefepime) or to nalidixic acid and ciprofloxacin among isolates from urban and rural patients. Strains obtained from children with acute diarrhoea were more likely to exhibit resistance to at least one or more aminoglycosides than those from children with chronic diarrhoea. Similarly all the 47 ESBL isolates identified were from stool specimens obtained from children with acute diarrhoea and so were the isolates from acute diarrhoea. Combined resistance to SXT/FEP/NA or CIP/ and to at least one aminoglycoside than those from chronic diarrhoea ($p < 0.024$, CI=0.025-0.66, OR=0.13).

Data on isolates from children who were hospitalized one time or another in the last 6 months were likely to be more resistant than those from children who

were not previously hospitalized (Table 2). Similarly, isolates from children who had used antibiotics in the last 3 months before sampling were more likely to be resistant to multiple antimicrobials compared to those from children who did not. Our observations indicate that majority of the children presenting with acute diarrhoea received β -lactam antibiotics including AMC while others received co-trimoxazole syrup.

Access to a toilet is important in reducing environmental contamination with intestinal tract pathogens that cause diarrhoea and other illnesses. However, this study shows that resistance patterns of isolates from children whose family use a pit latrine were almost similar to those of isolates from children whose family use improved latrines. This study also shows that there were no significant differences in resistance patterns of isolates obtained from children from rural or urban settings. Similarly, the resistance patterns of isolates from children living in single rooms were not significantly different from those living in multiple-room houses. Practice of treating or boiling water for drinking was higher in the rural areas (66%) than in the urban areas (13%). However, over 66% of families indicated that they treated drinking water by boiling or chlorination while only 13% from urban areas did the same. This study also shows that the number of family members was not related to carriage of MDR strains or episodes of diarrhoea.

Table 2: Number (%) of strains from different socio-demographic backgrounds exhibiting combined resistance

Resistance	Toilet type		Family members		Type of house		Social setting	
	Pit latrine	Improved	≤ 4	> 4	Single room	Multiple room	Rural	Urban
Number of isolates	238	146	234	150	171	213	167	217
SXT	57*	30	55	57	46*	64	59	54
4th generation β -lactam (FEB)	5	3	3*	7	5	4		
NA or CIP	13	10	5*	23	13	11	10	13
One aminoglycosides	68	51	70*	48	53	68	61	62
SXT/FEP/ NA or CIP/ \geq aminoglycoside	2*	1	1	2	1	2	1	2
ESBL phenotype	12	12	5*	23	16	15	11	13

Key: The symbol (*) statistically significant ($p < 0.05$)

Discussion

From the findings in this study a high proportion of isolates from children with diarrhoea are significantly resistant to older generation of antimicrobials such as sulfonamides, tetracyclines, chloramphenicol, trimethoprim and streptomycin but these resistances were lower than those reported from other developing countries [7,8]. Other studies have also reported high resistances among strains from children and it is therefore apparent that young children, especially those with diarrhoea may serve as reservoirs of MDR strains [9, 10]. Although antibiotics are not recommended for treatment of *E. coli* diarrhoea, empiric antimicrobials interventions are frequently applied in the management of diarrhoea in most developing countries. A previous study by Brooks *et al* [11] also conducted locally, showed that over 70% of patients with diarrhoea are likely to receive an antimicrobial to which the aetiologic agent is not susceptible. Such practices are likely to contribute to the emergence of MDR strains such as those observed in this study. Identification of strains with combined resistance to β -lactams, fluoroquinolones and aminoglycosides, especially in children without a history of antibiotic use or hospitalization is worrying. Such strain may seriously limit the treatment options especially if infections become invasive. We did not record any evidence that any of the children had been treated using nalidixic acid or ciprofloxacin yet 4% of the isolates were resistant to these antimicrobials. Co-resistance to multiple antimicrobials suggests that use of a single class of antimicrobials against these isolates could co-select for resistance to other unrelated antimicrobials as previously reported in a related study [12].

Contrary to related studies, the current study suggests a positive correlation between use of antibiotics and carriage of MDR strains [13,14]. It was also observed that strains from acute diarrhoea are more resistant than those from chronic cases. It would be expected that children who suffer acute episodes of diarrhoea are more likely to use antibiotics than those with mild diarrhoea that is not seen as potentially life-threatening. It is also possible that mothers are more likely to seek hospital treatment for children with acute diarrhoea than those with non-severe chronic diarrhoea.

A past study by Seidman *et al* [15] showed that susceptibilities profiles of common gut pathogens from children in rural and urban settings are generally similar and that in both settings, antibiotics are prescribed for diarrhoea cases. Similarly, the current study shows that there were no significant differences between the resistances of isolates from rural children and those from urban settings or between those living in single and multiple-roomed houses. This study also shows that the number of family members in a house was not related to carriage of MDR strains or episodes of diarrhoea. Contrary to our findings, other studies reported high episodes of diarrhoea among children living in large families, those attended by care-givers of low education level, those from parents with high responsibility

burden and those from houses where hygiene is poor [13, 14]. Same studies also indicate that young children were likely to acquire MDR strains from their siblings, especially from those who attend school [13, 14]. Another study found that children from crowded houses have lower odds for MDR [15]. One possible explanation to our observations is that families with fewer members (majority of these being children) are more likely to seek treatment and may afford to purchase antibiotics for their children than those with many children. If this is true, it is plausible to assume that children from families with few members may be significantly more exposed to antimicrobials, hence the probabilities of their misuse are high. There was no significant difference in the level of antimicrobial resistance between the urban and the rural children, unlike the findings of a recent study that urban health practitioners prescribed fewer, more effective antibiotics, and were more likely to prescribe oral rehydration therapy for bloody diarrhoea [16] hence deduced probabilities of acquiring antimicrobial resistance. The difference between the two surveys could be in the study setting. All the patients in our study came from the same hospital. The catchment area for Thika level 5 Hospital extends to the adjoining rural communities where urban lifestyle may influence the lifestyle in the adjoining rural communities. On the other hand, the second study was conducted in two different settings, one in the urban area hospital and the other one in the rural hospital setting. Our observations may be interpreted to mean that antibiotic use patterns and other factors that govern emergence and spread of resistance are similar in rural and urban settings.

A study conducted in Kenya by cruz *et al* [17] identified several factors that contribute to disease transmission in relation to home and toilet hygiene including, not boiling drinking water, not washing hands after using a toilet, not using soap to wash hands after using the toilet, use of dirty and inappropriate toilets and lack of toilets,. This study showed that majority of rural families are likely to treat drinking water using chlorination method or boiling than those in urban areas. This is partially because most people living in urban areas had access to piped municipal water. This water is assumed to be potable and palatable and many urban dwellers do not bother chlorinating or boiling it. However, the water pipes burst frequently and sewerage and other contaminants may reach the consumers thereby exposing them to potential pathogens [17]. It was also noted that majority of urban families had access to a flush toilet while most of the rural families use pit latrines that are deemed less safe for containment of human sewerage due to seepage especially during floods that disperse human waste exposing people to diarrhoea [17].

Conclusions

This study provided data that indirectly link carriage of MDR strains to various socio-demographic factors and antibiotic use patterns. While the study does not suggest

possible interventions for reducing diarrhoea and for reducing carriage of MDR strains, the data provided here can be vital in formulation of antimicrobial use policies for children and for identifying several issues that can be addressed with a view to reducing diarrhoea cases in young children. Some of these factors include: proper use of antimicrobials, access to proper and clean toilets and provision of clean water for domestic use and potable water for drinking.

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