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Bacterial Aetiologies of Diarrhea Among Children Seeking Treastment in Nairobi City, Kenya

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ABSTRACT :

Diarrhea remains to be a public healtb burden globally including Kenya. Therefore, this cross-sectional study characterized enteric bacteria and antibiotic resistance among diarrheic children younger than five years seeking treatment at Mbagathi hospital, Nairobi city, Kenya. Stool samples were cultured and identified via gram staining and biochemical tests. Resistant phenotypes were determined by disk diffusion method. Polymerase chain amplification was used for genotyping. A total of 136 (36.4%) children were infected with diarrheagenic *E. coli* harbouring enteroaggregative *Escherichia coli* (EAEC), enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC) or enteroinvasive *Escherichia coli* (EIEC) single and hybrid virulent gene. *Salmonella* species harbouring *invA, hilA, sopB* or *stn* virulent genes was detected in 9 (2.4%) children. There were 12 (3.2%) children infected with *Shigella* species which harboured *ipaH* virulent gene. *Campylobacter* and *Yersinia* species which harboured *cadF* and *Inv* virulent genes, was isolated from 6 (1.6%) and 5 (1.3%) children, respectively. *Aeromonas* species which harboured *aerA, hlyA, alt,* or *ast* virulent genes was detected in 4 (1.1%) children. There were 9 (2.4%) children co-infected with *Shigella* species and *E. coli*. Prevalence of *E. coli* in particular EAEC and ETEC virulent genotypes and that of *Shigella/E. coli* co-infection was higher during the dry season ($P<0.05$). Most of the phenotypic resistant isolates harboured *citm, bla CMY, aadA1, aac(3)-IV, qnr, catA1, ereA and tetA* genes associated with ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline resistance. Bacteria remaims to be an important cause of diarrhea in children in Nairobi. Therefore, children in Nairobi should be provided with safe water, sanitation and hygiene.

INTRODUCTION

Globally, the burden of child deaths due to diarrhoea has already halved since 2000, reducing from 1.5 million to 0.9 million deaths, owing to improvements in coverage of preventive and treatment interventions [1]. Majority of these deaths occur in resource-limited continents including Africa [1, 2]. While diarrhoea death rate in children younger than 5 years in Sub-Saharan Africa has been declining yearly by approximately 4% from 2000, morbidity and mortality is still high [1, 2] with nearly 300 billion diarrhoea episodes and half million diarrhoea-associated deaths occurring in Sub-Saharan Africa [2].

Enteric bacterial pathogens accounts for more than 300 million episodes of diarrhoea and one million deaths among children globally, majority of which is caused by *Escherichia coli* and the remainder being *Campylobacter, Shigella*, *Vibrio* and *Salmonella species* [2]. In Kenya, previous study demonstrated that *Shigella*, *E. coli*, and *Salmonella* species caused diarrhoea in Kilifi county [3], while another study in Kisii, Kisumu and Kericho counties found an association between *Shigella* but not *E. Coli* and Salmonella with diarrhoea [4] suggesting geographic uniqueness of gut microbiota in immunity towards enteric infection patterns [5-7]. Moreover, previous study found an association between *Campylobacter* and *Shigella* with HIV exposed infected in Kisumu [8] while no association was found in Kisii and Homabay counties [9] suggesting that HIV infection alters the gut microbiota profile hence immunity functional role towards enteric bacteria [10, 11]. There is inconclusive information on enteric pathogens circulating among children in Nairobi. A more recent study in Nairobi city identified *Yersinia enterocolitica* as an aetiology of childhood diarrhoea [3] while previous a study did not [12, 13] indicating emergence and spread of new strains driven by poor water, sanitation and hygiene (WASH) practices in the city [3].

The discovery of antibiotics led to optimism that enteric bacterial infections can be controlled and prevented. However, antibiotic resistant enteric bacteria are still the leading cause of death in developing world including Kenya [14]. Information on enteric pathogens antimicrobial resistant phenotypes and genotypes among diarrheic children in Nairobi city, Kenya, is inconclusive. For instance, previous study reported resistance of *Shigella* and pathogenic *E. coli* isolates to chloramphenicol, streptomycin, trimethoprim/sulfamethoxazole, tetracycline and ampicillin [3] while a recent study showed changing patterns with increasing resistance [15]. The increasing resistance has been linked to inappropriate antibiotic use in in the country whereby clinicians over-prescribe or under-prescribe antibiotics for non-dysentery in children while drug stores dispense antibiotics without prescription [16, 17]. This selective pressure of antibiotics drives the emergence of antibiotics resistant genes (Munita and Arias, 2016). Therefore, this study characterised bactria as well as their antimicrobial resistance among diarrheci children seeking treatment in Nairobi city, kenya.

METHODOLOGY

Study site, study design and study population

This cross-sectional study was conducted at Mbagathi county Referral Hospital, Nairobi city, Kenya. Mbagathi hospital was chosen because of the high prevalence of diarrhoea of 17% among children [18]. The high prevalence of diarrhoea in children visiting Mbagathi hospital is due to poor water, sanitation and hygiene practices in Nairobi city [3]. Diarrhogenic children under five years of age seeking treatment for diarrheal illness at Mbagathi county Hospital, Nairobi County, Kenya and whose parents or guardians gave consent were recruited in the study. Demeographic information was copllected using a questionnaire. Clinical features such as body temperature, nutritional status, and dehydration signs were collected by clinicians.

Bacterialogical analysis

Stool microbiology analysis was performed according WHO recommendations. All stool samples were cultured in alkaline peptone water, selenite broth and blood agar enrichment media, followed by sub-culture on selective and differential media. Isolates were then subjected to Gram staining and biochemical tests[45]. Bacterial isolates were polymerase chain reaction tested for the presence of virulence gens using primers in table 1.

Table 1: Genotyping primers

Primer sequences	Genotype	Species	Fragment size
F: CCCGAATTCGGCACAAGCATAAGC R: CCCGGATCCGTCTCGCCAGTATTCG	EPEC	*E. coli*	881bp
F: GAGCGAAATAATTTATTATGTG R: TGATGATGGCAATTCAGTAT	EHEC	*E. coli*	518bp
F: TTAATAGCACCCGGTACAAGCAGG R: CCTGACTCTTCAAAAGAGAAAATTAC	ETEC	*E. coli*	147bp
F: TCTCTATGTGCATACGGAGC R: CCATACTGATTGCCCGCAAT	ETEC	*E. coli*	322bp
F: GTTCCCTTGACCGCCTTTCCGATACCGTC R: GCCGGTCAGCCACCCTCTGAGAGTAC	EIEC,	*E. coli*	600bp
F: GTATACACAAAAGAAGGAAGC R: ACAGAATCGTCAGCATCAGC	EAEC	*E. coli*	254bp
F: AGACTCTGGCGAAAGACTGTATC R: ATGGCTGTCTGTAATAGATGAGAAC	EAEC	*E. coli*	194 bp
F: GCCTTTGCGGGTGGTAGCGG R: AACCCATTCGGTTAGAGCAC	EAEC	*E. coli*	282 bp
F: GTTCCCTTGACCGCCTTTCCGATACCGTC R: GCCGGTCAGCCACCCTCTGAGAGTAC	*IpaH*	*Shigella*	600bp
F: ACCACGCTCTTTCGTCTGG R: GAACTGACTACGTAGACGCTC	*invA,*	*Salmonella*	942bp
F: TGTTTCCGGGCTTGTGCT R: CAGGGCATTTGCTGATTCTTCC	*hilA,*	*Salmonella*	854bp
F: -AGCATCTCTAAACGCTACTG R: GCTTCTATCACTCAGCTTCA	*sopB,*	*Salmonella*	470bp
F: ATTGAGCGCTTTAATCTCCT R: GCTGTTGAATCTGTACCTGA	*Stn*	*Salmonella*	543bp
F: TGGAGGGTAATTTAGATATG R: CTAATACCTAAAGTTGAAAC	*cadF*	*Campylobacter*	400bp
F: CTGTGGGGAGAGTGGGGAAGTTTGG R: GAACTGCTTGAATCCCTGAAAACCG	*Inv*	*Yersinia*	570bp
F: CCTATGGCCTGAGCGAGAAG R: 59-CCAGTTCCAGTCCCACCACT	*aerA,*	*Aeromonas*	309bp
F: GGCCGGTGGCCCGAAGATACGGG R: GGCGGCGCCGGACGAGACGGG	*hlyA,*	*Aeromonas*	900bp
F: TGACCCAGTCCTGGCACGGC R: GGTGATCGATCACCACCAGC	*alt*	*Aeromonas*	148bp
F: TCTCCATGCTTCCCTTCCACT R: GTGTAGGGATTGAAGAAGCCG	*Ast*	*Aeromonas*	331bp

EPEC, enteropathogenic *E. coli*. ETEC, enterotoxigenic *E. coli*. EAEC, enteroaggregative *E. coli*. EIEC, enteroinvasive *E. coli*. EHEC, enterohaemoragic *E. coli*. Bp, base pairs.

Antimicrobial Resistance

Antimicrobial resistance tests of bacterial isolates were done on Muller-Hinton agar (Sigma-Aldrich chemical companies steinheim, Germany) with antibiotic discs by the disc diffusion method based on guidelines adopted from Clinical and Laboratory Standards Institute (CLSI). Antibiotics discs of ampicillin, trimethoprim/sulfamethoxazole, ceftriaxone, streptomycin, amoxicillin/clavulanic acid, gentamycin, kanamycin, ciprofloxacin, chloramphenicol, erythromycin, nalidixic acid and tetracycline were tested. Phenotypic resistant isolates were genotyped for the presence of antibmicrobial resistant genes using primers shown in table 2 .

Table 2 : Antimicrobial resistance primers and amplicon size

Antibiotic type	Antibiotic resistant gene	Primer sequence	Amplicon size
Ampicillin	*Citm*	F: TGG CCAGAACTGACAGGCAAA R: TTT CTC CTG AAC GTG GCT GGC	462bp
Ceftriaxone	*bla CMY*	F: CAATGTGTGAGAAGCAGTC R: CGCATGGGATTTTCCTTGCTG	462bp
Streptomycin	*aadA1*	F: TATCCAGCTAAGCGCGAACT R: ATTTGCCGACTACCTTGGTC	447bp
Gentamycin	*aac(3)-IV*	F: CTTCAGGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	286bp
Ciprofloxacin	*Qnr*	R: GGGTATGGATATTATTGATAAAG R: CTAATCCGGCAGCACTATTTA	670bp
Chloramphenicol	*catA1*	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547bp
Erythromycin	*(ere(A))*	F: GCCGGTGCTCATGAACTTGAG R: CGACTCTATTCGATCAGAGGC	419bp
Tetracycline	*(tet(A))*	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577bp

A, adenine. T, thymine. C, cytosine. G,guanosine. Bp, base pair.

Data analysis

Data collected was entered into excel, cleaned, coded and exported into SPSS. Statistical analyses was performed using SPSS version 19.0 for Windows (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.). Descriptive statistics, namely frequencies and percentages, was used to present demographic and clinical data, phenotypes and genotypes of enteric bacterial pathogens and antimicrobial resistance.

Ethical consideration

This study was ethically approved by Masinde Muliro University of Science and Technology Institutional Ethical Review Committee. A consent form was read and signed by either parent or guardian of each child. Study participants benefitted from free health edition and bacterial diagnosis. The findings of this research were disseminated through publications and presentations to the Ministry of health. All children were attended to by qualified health practitioners. Diarrheic children were treated by clinicians according to World Health Organization guidelines for treatment of diarrhea in children [19]. All study participants' information and test results remained confidentially throughout the study period.

RESULTS

Demographic and clinical information of diarrheic children

The demographic and clinical information of the diarrheic children under five years of age in Nairobi, Kenya, is presented in table 3. A total of 374 children were recruited, 218 and 156, were recruited during the dry and rainy season, respectively. The age distribution showed that, out of the total study subjects, 242 (70.0%) were within the age group between 1 and 36 months and 112 (30.0%) children were between 37 and 60 months. The overall gender distribution was 181 (48%) females and 193 (52%) males. Guardians of 371 (99.2%) and 3 (0.8%) reported using piped and borehole water, respectively. In addition, 220 (58.8%) reported treating drinking water. Occupation distribution showed that 2 (0.5%), 17 (4.5%), 5 (1.3%), 14 (3.7%), and 178 (47.6%) of the guardians were in the health care practitioner, office administrative support, construction/installation/repair, education/training, and sales, respectively, while 158 (42.2%) were unemployed.

Temperature of <38.0°C and ≥ 38.0°C was recorded in 58 (15.5%) and 316 (84.5%) children, respectively. In this study, 308 (82.4%), 35 (9.4%) and 31 (8.3%), respectively, reported having diarrhea for 1-3, 4-6 and ≥7 days. Vomiting was evidenced in 298 (79.7%) patients, fever in 310 (82.9%), abdominal cramp in 251 (67.1%), headache in 12 (3.2%), nausea in 50 (13.4%), and appetite loss in 345 (92.2%) children. Clinical diagnosis of dehydration revealed that 311 (83.2%) had sunken eyeballs, 117 (31.3%) children had dry tongue and 192 (51.3%) had reduced skin elasticity.

Table 3 : Demographic and clinical information of study participant

Characteristics	Number (%)
Diarrheal cases	
Dry season	218 (58.3)
Rainy Season	156 (41.7)
Age in months	
1-36	242 (70.0)
37-60	112 (30.0)
Gender	
Female	181 (48)
Male	193 (52)
Source of water	
Piped water	371 (99.2)

Borehole	3 (0.8)
Water treatment	220 (58.8)
Occupation of guardian	
Health care practitioner	2 (0.5)
Office/administrative/support	17 (4.5)
Construction/installation/repair	5 (1.3)
Education/ training	14 (3.7)
Sales	178 (47.6)
Unemployed	158 (42.2)
Body temperature in °C	
<38.0	58 (15.5)
≥ 38.0	316 (84.5)
Duration of diarrhea in days	
1-3	308 (82.4)
4-6	35 (9.4)
≥7	31 (8.3)
Symptoms	
Vomiting	298 (79.7)
Fever	310 (82.9)
Abdominal cramp	251 (67.1)
Headache	12 (3.2)
Nausea	50 (13.4)
Appetite loss	345 (92.2)
Sunken eyeball	311 (83.2)
Dry tongue	117 (31.3)
Reduced skin elasticity	192 (51.3)

Data are presented as number and proportions (%) of study participants. ≤, less than or equal to. <, less than. ≥, greater than or equal to. >, greater than. Health care practitioner (Nurse, Clinical officer). Office administrative support (secretary, clerical officer, social worker, driver, househelp, caretaker). Construction/installation/repair (welder, carpenter, mason, tailor). Education/ training (teacher). Sales (saloonist, hawkers and small scale business, sales agents).

Enteric bacterial pathogens phenotypes and genotypes among diarrheic children in Nairobi City, Kenya

The prevalence of enteric bacterial pathogens phenotypes and genotypes is presented in table 4. A total of 136 (36.4%) children were infected with diarrheagenic *E. coli*. Pathogen genotyping showed that 78 (57.4%), 15 (11.0%), 38 (27.9%), and 2 (1.5%) of the diarrheagenic *E. coli* isolates harboured EAEC, EPEC, ETEC and EIEC virulent genes, respectively, while mixed virulent gene was detected in 1 (0.7%) for EAEC/EPEC/ETEC and 2 (1.5%) for EAEC/ETEC. *Salmonella* species was detected in stool samples of 9 (2.4%) children. Virulotyping showed that 9 (100.0%), 7 (77.8%), 9 (100.0%), and 6 (77.8%) of the *Salmonella* isolates harboured *invA, hilA, sopB,* and *stn* virulent genes, respectively. There were 12 (3.2%) children infected with *Shigella* species, of which 2 (0.5%), 1 (0.3%), 7 (1.9%), and 2 (0.5%) were infected with *Shigella boydii, Shigella dysentriae, Shigella flexneri* and *Shigella sonnei,* respectively. All (100%) the *Shigella* isolates harboured *ipaH* virulent gene. There were 6 (1.6%) and 5 (1.3%) children infected with *Campylobacter* and *Yersinia* species which all (100.0%) harboured *cadF* and *Inv* virulent genes, respectively. *Aeromonas* species was detected in stool samples of 4 (1.1%) children. Virulent gene typing revealed that 3 (75.0%), 1 (25.0%), 1 (25.0%) and 1 (25.0%) of the *Aeromonas* isolates harboured *aerA, hlyA, alt,* and *ast* virulent genes, respectively. There were 9 (2.4%) children infected with both *Shigella* species and *E. coli,* of which, 4 (44.4%) harboured EAEC/*ipaH* mixed virulent gene, 2 (22.2%) were EAEC/*ipaH* mixed virulent gene, while one (11.1%) case was reported for EAEC/*ipaH*, EPEC/*ipaH* and ETEC/*ipaH* mixed virulent genes.

Table 4: Phenotype and virulent genotype of bacterial pathogens among diarrheic children in Nairobi City, Kenya

Enteropathogenic bacteria	Phenotype number n (%)	Virulent genotype	Genotype number n (%)
Diarrheagenic *E. coli*	136 (36.4)	EAEC	78 (57.4)
		EPEC	15 (11.0)
		ETEC	38 (27.9)
		EIEC	2 (1.5)
		EAEC/EPEC/ETEC	1 (0.7)
		EAEC/ETEC	2 (1.5)
		EHEC	0 (0.0)
Salmonella species	9 (2.4)	*invA*	9 (100.0)
		Hila	7 (77.8)
		sopB	9 (100.0)
		Stn	6 (77.8)
Shigella species	12 (3.2)	*ipaH*	12 (100.0)
Shigella boydii	2 (0.5)	*ipaH*	2 (100.0)
Shigella dysentriae	1 (0.3)	*ipaH*	1 (100.0)
Shigella flexneri	7 (1.9)	*ipaH*	7 (100.0)
Shigella sonnei	2 (0.5)	*ipaH*	2 (100.0)

Enteropathogenic bacteria	Phenotype number n (%)	Virulent genotype	Genotype number n (%)
Campylobacter species	6 (1.6)	*cadF*	6 (100.0)
Yersinia species	5 (1.3)	*Inv*	5 (100.0)
Aeromonas species	4 (1.1)	*aerA*	3 (75.0)
		hlyA	1 (25.0)
		alt	1 (25.0)
		Ast	1 (25.0)
Shigella species/*E. coli* co-infection	9 (2.4)	EAEC/*ipaH*	4 (44.4)
		EAEC/*ipaH*	2 (22.2)
		EAEC/ *ipaH*	1 (11.1)
		EPEC/ *ipaH*	1 (11.1)
		ETEC/ *ipaH*	1 (11.1)

Data are presented as number and proportions (%) of enteric bacteria and virulent genotypes. *E. coli*, *Escherichia coli*. EPEC, enteropathogenic *E. coli*. ETEC, enterotoxigenic *E. coli*. EAEC, enteroaggregative E. coli. EIEC, enteroinvasive *E. coli*. EAEC (*aspU*, *aggR*, and *pcvd432* virulent genes). EPEC (*eae* virulent gene). ETEC (*est* and *elt* virulent genes). EIEC (*ipaH* virulent gene). EHEC (*stx* virulent gene).

Phenotypic and genotypic antibiotic resistance of enteric bacterial pathogens among diarrheic children in Nairobi City, Kenya

The phenotypic and genotypic antimicrobial resistance of the enteric bacterial pathogens isolated from diarrheic children in Nairobi city, Kenya, is presented in table 5. A total of 80 (55.2%), 18 (100.0%), 91 (76.6%), 69 (47.6%), 41 (28.3%), 90 (62.1%), 12 (8.3%) and 120 (82.8%) of diarrheagenic *E. coli* were resistant to ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline, respectively. Genotypic antimicrobial resistance analysis revealed that, 79 (98.8%), 16 (88.9%), 85 (93.4%), 69 (100.0%), 39 (95.1%), 87 (96.7%), 11 (91.7%) and 120 (100.0%) of *E. coli* phenotypic resistant to ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline harbored ampicillin *(citm)*, ceftriaxone *(bla CMY)*, streptomycin *(aadA1)*, gentamycin *(aac(3)-IV)*, ciprofloxacin *(qnr)*, chloramphenicol *(catA1)*, erythromycin (*ereA)* and tetracycline *(tetA)* resistant gene, respectively.

Although none of the *Salmonella* isolate was resistant to gentamycin, 7 (77.8%), 7 (77.8%), 9 (100.0%), 8 (88.9%), 7 (77.8%), 6 (66.7%) and 5 (55.6%) of *Salmonella* species were resistant to ampicillin, ceftriaxone, streptomycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline, respectively. Additionally, ampicillin *(citm)*, ceftriaxone *(bla CMY)*, streptomycin *(aadA1)*, gentamycin *(aac(3)-IV)*, ciprofloxacin *(qnr)*, chloramphenicol *(catA1)*, erythromycin (*ereA)* and tetracycline *(tetA)* resistant gene was detected in 6 (85.7%), 6 (85.7%), 9 (100.0%), 8 (100.0%), 6 (85.7%), 6 (100.0%), and 5 (100.0%) of *Salmonella* isolates that were phenotypic resistant to ampicillin, ceftriaxone, streptomycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline, respectively.

Phenotypic antibiotic analysis showed that 13 (28.6%), 10 (47.6%), 12 (57.1%), 2 (9.5%), 4 (19.0%), 12 (57.1%), 2 (9.5%) and 14 (66.7%) of *Shigella* isolates were resistant to ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline, respectively. Most importantly, 13 (100.0%), 10 (100.0%), 12 (100.0%), 2 (100.0%), 4 (100.0%), 11 (100.0%), 2 (91.7%) and 14 (100.0%) of *Shigella* isolates that were phenotypic resistant to ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline harbored ampicillin *(citm)*, ceftriaxone *(bla CMY)*, streptomycin *(aadA1)*, gentamycin *(aac(3)-IV)*, ciprofloxacin *(qnr)*, chloramphenicol *(catA1)*, erythromycin (*ereA)* and tetracycline *(tetA)* resistant gene, respectively.

While *Campylobacter* species was not resistant to streptomycin and ciprofloxacin, 3 (50.0%), 1 (16.7%), 1 (16.7%), 4 (66.7%), 3 (50.0%) and 5 (83.3%) of the *Campylobacter* isolates were resistant to ampicillin, ceftriaxone, gentamycin, chloramphenicol, erythromycin and tetracycline, respectively. Additionally, 3 (100.0%), 1 (100.0%), 1 (100.0%), 4 (100.0%), 2 (66.7%) and 5 (100.0%) of *Campylobacter* isolates resistant to ampicillin, ceftriaxone, gentamycin, chloramphenicol, erythromycin and tetracycline haboured ampicillin *(citm)*, ceftriaxone *(bla CMY)*, gentamycin *(aac(3)-IV)*, chloramphenicol *(catA1)*, erythromycin (*ereA)* and tetracycline *(tetA)* resistant gene, respectively .

Even though *Yersinia* was not resistant to streptomycin and ciprofloxacin, 5 (100.0%), 1 (20.0%), 5 (100.0%), 4 (80.0%), 1 (20.0%) and 3 (60.0%) of *Yersinia* species were resistant to ampicillin, ceftriaxone, gentamycin, chloramphenicol, erythromycin and tetracycline, respectively. Moreover, ceftriaxone *(bla CMY)*, gentamycin *(aac(3)-IV)* and erythromycin (*ereA)* resistant genes was not detected while ampicillin *(citm)*, chloramphenicol *(catA1)*, and tetracycline *(tetA)* resistant gene was detected in each case of phenotypic resistant *Yersinia* isolate. While *Aeromonas* isolates was not resistant to ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin and erythromycin, 2 (50.0%) and 3 (75.0%) of *Aeromonas* isolates were resistant to cchloramphenicol, and tetracycline, respectively. Most importantly, none of cchloramphenicol resistant *Aeromonas* isolate harbored chloramphenicol *(catA1)* resistant gene while tetracycline (*tetA*) resistant gene was detected in only 1 (33.3%) of the *Aeromonas that was* phenotypic resistant to tetracycline.

Table 5: Antimicrobial susceptibility patterns of enteric bacterial pathogens among diarrheic children in Nairobi city, Kenya

Enteropathogenic bacteria	Antibiotic	Phenotypic resistance			Genotypic resistance	
		Sensitive	Intermediate	Resistant	Genotype	Number (%)
Diarrheagenic *E. coli*	Ampicillin	22 (15.2)	43 (29.7)	80 (55.2)	*Citm*	79 (98.8)
	Ceftriaxone	106 (73.1)	21 (14.5)	18 (12.4)	*bla CMY*	18 (100.0)
	Streptomycin	34 (23.4)	20 (13.8)	91 (76.6)	*aadA1*	85 (93.4)
	Gentamycin	48 (33.1)	28 (19.3)	69 (47.6)	*aac(3)-IV*	69 (100.0)
	Ciprofloxacin	94 (64.8)	10 (6.9)	41 (28.3)	*Qnr*	39 (95.1)
	Chloramphenicol	14 (9.7)	41 (28.3)	90 (62.1)	*catA1*	87 (96.7)

Enteropathogenic bacteria	Antibiotic	Phenotypic resistance			Genotypic resistance	
		Sensitive	Intermediate	Resistant	Genotype	Number (%)
	Erythromycin	81 (55.9)	52 (35.9)	12 (8.3)	*ere(A)*	11 (91.7)
	Tetracycline	15 (10.3)	10 (6.9)	120 (82.8)	*tet(A)*	120 (100.0)
Salmonella species	Ampicillin	0 (0.0)	2 (22.2)	7 (77.8)	*Citm*	6 (85.7)
	Ceftriaxone	2 (22.2)	0 (0.0)	7 (77.8)	*bla CMY*	6 (85.7)
	Streptomycin	0 (0.0)	0 (0.0)	9 (100.0)	*aadA1*	9 (100.0)
	Gentamycin	9 (100.0)	0 (0.0)	0 (0.0)	*aac(3)-IV*	-
	Ciprofloxacin	0 (0.0)	1 (11.1)	8 (88.9)	*Qnr*	8 (100.0)
	Chloramphenicol	2 (22.2)	0 (0.0)	7 (77.8)	*catA1*	6 (85.7)
	Erythromycin	1 (11.1)	2 (22.2)	6 (66.7)	*ere(A)*	6 (100.0)
	Tetracycline	3 (33.3)	1 (11.1)	5 (55.6)	*tet(A)*	5 (100.0)
Shigella species	Ampicillin	2 (9.5)	6 (28.6)	13 (28.6)	*Citm*	13 (100.0)
	Ceftriaxone	11 (52.4)	0 (0.0)	10 (47.6)	*bla CMY*	10 (100.0)
	Streptomycin	2 (9.5)	7 (33.3)	12 (57.1)	*aadA1*	12 (100.0)
	Gentamycin	18 (85.7)	1 (4.8)	2 (9.5)	*aac(3)-IV*	2 (100.0)
	Ciprofloxacin	11 (52.4)	6 (28.6)	4 (19.0)	*Qnr*	4 (100.0)
	Chloramphenicol	5 (23.8)	4 (19.0)	12 (57.1)	*catA1*	11 (100.0)
	Erythromycin	5 (23.8)	14 (66.7)	2 (9.5)	*ere(A)*	2 (91.7)
	Tetracycline	4 (19.0)	3 (14.3)	14 (66.7)	*tet(A)*	14 (100.0)
Campylobacter	Ampicillin	2 (33.3)	1 (16.7)	3 (50.0)	*Citm*	3 (100.0)
	Ceftriaxone	4 (66.7)	1 (16.7)	1 (16.7)	*bla CMY*	1 (100.0)
	Streptomycin	5 (83.3)	1 (16.7)	0 (0.0)	*aadA1*	-
	Gentamycin	5 (83.3)	0 (0.0)	1 (16.7)	*aac(3)-IV*	1 (100.0)
	Ciprofloxacin	6 (100.0)	0 (0.0)	0 (0.0)	*Qnr*	-
	Chloramphenicol	0 (0.0)	1 (33.3)	4 (66.7)	*catA1*	4 (100.0)
	Erythromycin	1 (16.7)	2 (33.3)	3 (50.0)	*ere(A)*	2 (66.7)
	Tetracycline	1 (16.7)	0 (0.0)	5 (83.3)	*tet(A)*	5 (100.0)
Yersinia	Ampicillin	0 (0.0)	0 (0.0)	5 (100.0)	*Citm*	1 (20.0)
	Ceftriaxone	0 (0.0)	4 (80.0)	1 (20.0)	*bla CMY*	0 (0.0)
	Streptomycin	0 (0.0)	5 (100.0)	0 (0.0)	*aadA1*	-
	Gentamycin	0 (0.0)	0 (0.0)	5 (100.0)	*aac(3)-IV*	0 (0.0)
	Ciprofloxacin	5 (100.0)	0 (0.0)	0 (0.0)	*Qnr*	-
	Chloramphenicol	0 (0.0)	1 (20.0)	4 (80.0)	*catA1*	1 (25.0)
	Erythromycin	0 (0.0)	4 (80.0)	1 (20.0)	*ere(A)*	0 (0.0)
	Tetracycline	1 (20.0)	1 (20.0)	3 (60.0)	*tet(A)*	1 (33.0)
Aeromonas	Ampicillin	0 (0.0)	4 (100.0)	0 (0.0)	*Citm*	-
	Ceftriaxone	4 (100.0)	0 (0.0)	0 (0.0)	*bla CMY*	-
	Streptomycin	2 (50.0)	2 (50.0)	0 (0.0)	*aadA1*	-
	Gentamycin	4 (100.0)	0 (0.0)	0 (0.0)	*aac(3)-IV*	-
	Ciprofloxacin	4 (100.0)	0 (0.0)	0 (0.0)	*Qnr*	-
	Chloramphenicol	0 (0.0)	2 (50.0)	2 (50.0)	*catA1*	0 (0.0)
	Erythromycin	1 (25.0)	3 (75.0)	0 (0.0)	*ere(A)*	-
	Tetracycline	0 (0.0)	1 (25.0)	3 (75.0)	*tet(A)*	1 (33.3)

Data are presented as number and proportions (%) of sensitive, intermediate and phnotypic resistant bacterial isolates and antibiotic resistant genes.

DISCUSSION

Diarrheagenic *E. coli* predominates in this study with the remainder being *Shigella, Salmonella, Campylobacter, Yersinia* and *Aeromonus* species, in that order, highlighting the prominent role of *diarrhegenic E. coli* in enterobacteria associated diarrhoea among children in Nairobi city, Kenya. These findings are consistent with previous studies in involving diarrheic children in Kiambu [20] and Homa Bay counties [21] in Kenya. As reviewed by [22], diarrhegenic *E. coli* persist longer in natural ecological niches like soil and water supporting the hypothesis of *E. coli* transmission via contaminated child hands as children frequently touch soil and water [23, 24]. However, the findings of this study are inconsistent with previous studies that demonstrated the dominancy of *Shigella* species, followed by diarrhegenic *E. coli, Salmonella* and *Yersinia* species among diarrheic children in Nairobi city, Kenya [3]. Maybe because of proved diarrhegenic *E. coli* in particular EAEC genome heterogeneity [25], using two virulence genes for EAEC detection decreased the rate of isolation in this previous study [3]. Most importantly, this study detected higher prevalence of *Shigella* mono-infection (3.2%) than *Shigella/E. coli* co-infection (2.4%) suggesting that co-infections alters epidemiological dynamics due pathogen synergism worsening disease severity and consequently mortality [26], and this observation is consistent with previous studies among diarrheic children in Kiambu county (Kenya) [21], China [27], Zanzibar and Rwanda [28]. Therefore, diarrhegenic *E. coli, Shigella, Salmonella, Campylobacter, Yersinia* and *Aeromonas* are important causes of childhood bacteria associated diarrhoea justifying access to safe water, sanitation and hygiene among children in Nairobi city, Kenya.

This study also determined the frequency of virulence factors of enteric bacteria isolated from children with diarrhoea. It was found that EAEC, EPEC, ETEC, EIEC, and EHEC virulent genotypes were frequently detected in diarrheagenic *E. coli*. This is consistent with previous studies that detected

EAEC, EPEC, ETEC, EIEC, and EHEC in diarrheagenic *E. coli* isolated from children with diarrhoea in Kiambu County, Kenya [20]. However, a study in Homa Bay county could not detect EIEC and EHEC in diarrheic children [21], indicating that there could be possible ecological barriers in the transmission of these virulent genotypes [29, 30]. Combinations of EAEC/EPEC/ETEC and EAEC/ETEC virulent genotypes were also detected in diarrheagenic *E. coli,* which is in agreement with a previous study that detected hybrids of EAEC/EPEC/ETEC and EAEC/ETEC in DEC among diarrheic children seeking treatment at Mbagathi Hospital in Nairobi, Malindi District Hospital in Malindi, New Nyanza Provincial Hospital in Kisumu, and Alupe Leprosy Hospital in Busia, Kenya [31]. Increasing trends in the hybrid phenomenon between EAEC and other virulent genotypes has been documented in Kiambu and Hombay counties of Kenya [21] implying that hybrid strains which acquire mixed virulence genes via horizontal gene transfer [32] are emerging as potential outbreak agents in the country and may cause high morbidity and mortalities [33]. All *Salmonella* virulence genes (*InvA, hilA, sopB* and *Stn*) studied here were detected with varying frequencies, which is consistent with previous studies that analysed and detected *invA, stn, sopB* and *hilA* in *Salmonella* species isolated from stool samples of children in China [34]. All *Shigella, Campylobacter* and *Yersinia* isolates harboured *ipaH, CadF* and *Inv* virulence genes, respectively, a finding that is consistent with previous study that reported 100% detection of *IpaH* [35] and *CadF* gene [36] in isolates from Iranian diarrheic children and *Inv* gene in isolates from Chinese adult and children with diarrhoea [37]. Genes such as *inv*A, *sop*B, and *hilA* of *Salmonella [38], ipaH* of *Shigella* [39], *CadF* of *Campylobacter* [40] and *Inv* of *Yersinia [41]* are involved in epithelial cell adhesion and invasion, a prerequisite for infection, may explain the abundance of these genes observed in this study. The occurrence of genes encoding hemolytic, cytotonic, cytotoxic, and enterotoxic activities (*aerA*, *hlyA*, *alt*, *ast*) may contribute to diarrheal-related virulence in *Aeromanas* infection [42]. In the present study, *aerA, hlyA, alt* and *Ast* were detected and *aerA* was the most prevalent. *Aeromonas aerA* gene is essential for adherence and invasion of intestinal mucosa [42]. Results from a study in Canada targeting patients with sporadic enteritis [43] support the distribution of *Aeromonas* virulence genes observed in this study. Therefore, there is diversity in virulence profiles of enteric bacterial pathogen circulating in diarrheic children in Nairobi city, Kenya, indicating that the pathogenic strains may vary in their degree of virulence.
in Nairobi city, Kenya.

This study observed high antimicrobial resistance rates indicating rapid and ongoing spread of antimicrobial-resistant organisms. Specifically, more than half of diarrheagenic *E. coli* isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, streptomycin, kanamycin, chloramphenicol and tetracycline which is in agreement with studies in Nairobi city [13] and Meru county [44] but inconsistent with previous study among the Maasai community of Narok and Kajiado counties that reported increased susceptibility to these antibiotics [45], possibly due to reduced antimicrobials use among the Maasai community who mostly practice traditional medicine [46]. Over half of the *Shigella* isolates were resistant to Streptomycin, Chloramphenicol and Tetracycline while resistance to ampicillin, ceftriaxone, amoxicillin-clavulanic acid, kanamycin, ciprofloxacin, and erythromycin occurred in more than half *Salmonella* isolates, which partly agrees with a multisite study conducted in Kisii, Homa Bay and Migori counties, Kenya [47] but conflicts a study in Zambia [48]. Over half of *Campylobacter* isolates were resistant to ampicillin, chloramphenicol, and erythromycin which is consistent with findings in Ethiopia [49] and disagrees with study in China [50]. *Yersinia* were resistant to ampicillin, streptomycin, gentamycin and tetracycline which mirrors a study in Ethiopia [51] but disagrees with a study in Mexico [52]. At least half of *Aeromonas* isolates were resistant to tetracycline and chloramphenicol which is in agreement with a study in Brazil [53] and disagrees with study in China [50]. Variations in resistance profiles worldwide indicate that some countries are overusing and misusing antibiotics facilitated by absence or inadequacies in laws, policies and regulations pertaining to antibiotics use [54]. For instance, even though Kenya has guidelines in place to control the use of antibiotics in animals [55], antibiotics are used as growth promoters and not for treatment of infections of farm animals [56]. At the same time, although the purchase of antibiotics from retail pharmacies without a prescription is forbidden by Kenya's Pharmacy and Poisons Board [57], over-the-counter sale of antimicrobials without a prescription is possible [16]. Such overuse and misuse of antibiotics drives the development of drug resistant pathogens in Kenya.

Molecular investigations on the underlying resistance mechanisms showed that most resistant phenotypes harboured *citm, blaCMY, aadA1, aac(3)-IV, Qnr, catA1, ere(A),* and *tet(A)* associated with ampicillin, ceftriaxone, streptomycin, gentamycin, ciprofloxacin, chloramphenicol, erythromycin and tetracycline resistance, respectively. However, some isolates were fully resistant but did not show the presence of these resistant genes indicating that resistance might be due to intrinsic resistance or different antibiotic resistant genes that were not evaluated in this study. Therefore, antimicrobial stewardship programs have to be developed to influence antibiotic use and prescribing behaviour to ensure long-term availability of effective treatment for bacterial infections in Kenya.

Conclusion

E. coli, Shigella, Salmonella, Campylobacter, Yersinia and *Aeromonas* species habouring virulent genes were aetiologies of diarrhea in children Most of the bacterial isolates habour resistant genes to commonly prescribed antibiotics.

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