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Prevalence of Enteric Bacteria Pathogens among HIV Infected and Uninfected Children in Dandora, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSR drafted the concept paper of her work and with the help of authors WS, AN and EM she came up with a full proposal. Author WS played a key role in providing consistent guidance and corrections while doing the lab work. Authors IH and TM provided technical advice. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diarrhoea is the second disease killer after respiratory diseases in children. Globally, there are nearly 1.7 billion cases of diarrheal disease every year. In developing countries, enteric bacterial pathogens are most common causes of morbidity and mortality in children especially

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under 5 years. Most of the studies done on enteric bacteria pathogens and HIV co-infection have focused on the children less than 5 years of age but not above.

Objective: This study aimed at evaluating the distribution of common circulating enteric bacterial pathogens; *Escherichia (E).coli, Shigella,* and *Salmonella* among HIV infected (n=79) and uninfected (n=78) children aged 5-12 years from Dandora slums of Nairobi.

Methods: This was analytic cross-sectional study of HIV positive children enrolled at Nyumbani Lea Toto HIV/AIDS outreach program in Dandora, while HIV negative are children from same area (preferably sibling). Stool samples were collected from consenting participants and sent to Microbiology laboratory in Kenya Medical Research institute for processing. The samples were cultured using differential media for enteric bacteria. Suspected isolates were further identified using conventional biochemical methods and serology. Multiplex PCR was done on *E. coli* isolates to detect virulence factors responsible for different *E. coli* pathotypes.

Results: The overall prevalence of pathogenic *Escherichia coli, Shigella* and *Salmonella* was 44 (28%), 31 (19.7%) and 0 (0.0%) respectively. Enteroaggregative *E.coli* (43.2%) was the main *E. coli* pathotypes observed. Distribution of pathogenic *E. coli* in HIV infected and uninfected was 12.7% and 15.3%, respectively (p = 0.30), while that of *Shigella* was 6.4% and 13.4% (*p* = 0.03).

Conclusion: From this study HIV infected children had less infestation of *Shigella species* as compare to HIV uninfected children, which could be due to constant treatment for any infections thus interferes with bacteria grow.

Keywords: Pathogenic E. coli; Shigella; Salmonella; HIV infected and uninfected.

1. INTRODUCTION

Human digestive tract represents a very attractive environment for bacteria to colonize and it is therefore not surprising that most of the bacteria live in the gut. Although majority of these gut bacteria are harmless, gastrointestinal mucosal repair and regeneration is decreased in HIV populations allowing the pathogens that could have been controlled by mucosal barrier to cause disease. Previous study indicates that 1 in 9 deaths that take place in children around the world are due to diarrhoea, this is even worse in HIV-infected children [1]. It is generally estimated that about 100% of HIV positive patients in the developing world may suffer from chronic diarrhoea, as estimated on a cumulative life-time incidence, but the situation in the developed world is better, where a lower percentage of HIV-positive patients suffer from diarrhoea [2]. In Africa, human immunodeficiency virus (HIV) epidemic has aggravated diarrheal illness which is the main cause of morbidity and mortality among HIV-infected patients. A broad range of etiologic agents are responsible for acute and chronic diarrheal disease, and the prevalence of such agents varies greatly by geographical region, season, patient age, immune status, and socioeconomic conditions. A large proportion of the infections, about 36%, take place through contaminated food and water [1]. In the developing world, over 80% of foodborne illness attributable to non-typhoid S*almonella*, *Escherichia coli*, *Shigella*, and *Campylobacter*. Up to 63% of children in low and middle income countries who suffer from persistent diarrhea have been found to harbor *Escherichia coli* infection, often a marker of poor hygiene [3]. Majority of the enteric bacterial pathogens are transmitted through the faecal-oral route especially in developing countries where access to clean water and proper sanitation are lacking.

They are six *Escherichia coli (E. coli)* pathotypes with different mechanisms of action which have been established. These are: enterotoxigenic *E. coli* (ETEC), which is associated with travellers' diarrhoea; enteropathogenic *E. coli* (EPEC), which causes childhood diarrhoea; enteroinvasive *E. coli* (EIEC), that causes dysentery; Enterohemorrhagic *E. coli* (EHEC), which leads to haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) renal failure ; enteroaggregative *E. coli* (EAggEC), which is typically associated with persistent diarrhoea in children, especially in developing countries, enteroadherent *E. coli* (EAEC) which is a key cause of traveller's diarrhoea in North America; and Shiga toxin-producing *E. coli* (STEC), commonly associated with foodborne diseases. *Salmonella* is the most common enteric pathogen known to cause bacterial foodborne diseases. It has also been implicated in several conditions, including typhoid or enteric fever (*Salmonella typhi* and *Salmonella paratyphi*), and enterocolitis (*Salmonella typhimurium*, *Salmonella heidelberg*, and *Salmonella enteritidis*). The genus *Shigella* is divided into four O antigenic groups, including *S. dysenteriae, S. flexneri*, *S. boydii,* and *S. sonnei*. *Shigella*

spp. are invasive bacteria that cause shigellosis that can be spread from person to person. Shigellosis can vary from mild to severe, depending on several factors such as, immunity, age and an individual's HIV status. Symptoms range from diarrhea (watery and sometimes bloody), fever and nausea. Cases of bacterial diarrhea due to *Shigella spp*. occur worldwide but are more prevalent in developing countries. *Shigella spp* are the major cause of bacterial dysentery, accounting for an estimated 165 million cases and up to 1 million deaths each year around the world [4].

Most of the studies done on common enteric bacteria pathogens and HIV co-infection focus on children less than 5 years and not above 5 years [5,6]. However, this study was able to capture the distribution of these enteric bacteria pathogens and virulence factors associated with *E. coli* infection in children above 5 years of age who are more exposed to infections due to their diverse interactions with children from other homes and environment. This will aid in establishing the distribution of these causative agents in order to ensure appropriate treatment and control of infection.

2. MATERIALS AND METHODS

2.1 Study Design and Population

Analytic cross-sectional study design was adapted to compare the prevalence of common enteric bacterial pathogens: *E. coli, Salmonella* and *Shigella*, among HIV positive and HIV negative children aged between 5 and 12 years, residing at Dandora slum of Nairobi. The HIV positive children included in this study were randomly selected from those enrolled in Nyumbani Lea Toto HIV/AIDS outreach program and receiving free ART, while HIV negative are children from same area (preferably sibling) with no history of antibiotic use for at least three months prior to the study. A total of 157 participants with a mean age of 10.5 years, median of 10 years and interquartile range (IQR) of 6 were recruited after obtaining inform consent/assent from parents/guardians. Among these 79 were HIV uninfected (38 male & 41 female) and 78 were HIV infected (40 male & 38 female).

2.2 Bacteria Isolation and Identification

For detection of *E. coli, Salmonella* and *Shigella* species all stool samples were placed on differential and selective media: MacConkey for *E. coli spp*, Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* and *Shigella spp* and Selenite F enrichment broth within two hours of collection. All inoculated media were incubated at 35ºC - 37ºC for 18-24 hours, after overnight incubation subculture was done from enrichment broth to primary media to improve recovery of the isolates which were previously negative on primary media. Suspect colonies identified as *E. coli, Salmonella* and *Shigella* isolates by their colony morphology were subjected to biochemical tests and serotype identification using O antigen and H antigen antisera (Denka Seiken Co LTD, Tokyo-Japan) by slide agglutination assay.

2.3 Polymerase Chain Reaction (PCR)

Multiplex PCR was performed to detect virulence factors that characterize *E. coli* pathotypes. DNA standards were extracted from bacteria containing ATCC 35401 (LT/ST), pEWD299 (LT) pDAS100 (STp), pDAS101 (STh), ATCC 43893 (EIEC), ATCC43887 (BfpA/EAE), 933J (SLTI), 933W (SLTII), ATCC1175 negative control and pCVD432 (Eagg) were obtained from the Armed Forces Research Institute of Medical Sciences in Bangkok. These isolates were culture on MacConkey agar plates to check purity and later sub-cultured on Mueller –Hinton agar plates for PCR analysis. Primers for amplifying segments of Cytotoxin necrotising factors (CNFI and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanism (Eagg), enteroinvasive mechanism (Einv), heat-labile (LT) and heat-stable (ST1 ad ST2) toxins were tested using the method [7]. Vero toxin assay were carried out according to Konowalchuk method [8].

2.3.1 Colony PCR

A colony of *E.coli* isolate was picked from Muller-Hinton Agar plate and suspended in 20 µl of nuclease free water and vortex. From this suspension a 2 µl (DNA template) was added to a 25 µl reaction mixture containing 2.0 µl of 10 mM mix deoxynucleiotide triphosphate (dNTPs), 2.5 µl of $MgCl_2$ (25 mM), 2.5 µl 10X buffer solution and 1.25 µl of each of the PCR primer with concentration of (0.5 pmol/µl) (Bioserve Biotechnologies, Laurel, MD.USA). 0.3 µl of Taq Polymerase (5U/µl), (Applied Biosystems, Roche Molecular, Inc, and Branchbury, New Jersey, USA) was added to this reaction mix. Base sequences and predicted sizes of amplified

products for the specific oligonucleotide primers were used in this study are as shown in Table 1. The PCR assay was set as follows: PCR program consists of an initial denaturation cycle at 95°C for 30 s, followed by 20 cycles each at 95°C for 30 s (denaturation), 63°C for 30 s (annealing), 72°C for 30 s (polymerization) and a final extension of 72°C for 5 mins. Reaction products were separated by agarose gel electrophoresis on a 2% (Sigma) high-resolution agarose stained using A-Z in gel vision dye in Tris Borate (TBE) buffer at 100 V for one and half hours . A molecular size marker (100 bp DNA; Promega, Madison, Wisconsin, USA) was added to every agarose gel to estimate the size of amplicons. DNA in the gel were visualized on a UV trans illuminator and photographed using a black/white instant Polaroid film.

2.4 Data Analysis

Data collected was entered, cleaned and analysed using Microsoft excel 2010 (Microsoft corporation, USA).Using Stata version 14, Chi - square test was used in computing the *p*-value for the distribution of enteric bacteria pathogens, and differences were considered significant at $p < 0.05$. Binary logistic regression model was used to compute the odds ratios, and CI's.

3. RESULTS

3.1 Prevalence of Enteric Bacterial Pathogens among HIV Infected and Uninfected Children

Out of 157 participants surveyed, diarrheagenic bacteria pathogens were observed in (n=75) children. The pathogens comprised of *E. coli* n= 44 (28.0%) and *Shigella spp* n= 31 (19.7%). No *Salmonella spp.*was detected. Distribution of pathogenic *E. coli* was random in gender groups (male 29.5% vs. female 26.6%) similar to *Shigella spp* (male 16.7%vs 22.8%). On the other hand, children aged less than 10 years had lower risk of getting shigellosis (OD =1.55,95% CI = 0.76-3.16, = 0.23) and *E. coli* infection (OD =1.56, 95% CI= 0.83-2.94, *P* =0.166) compare to aged greater than 10 years, no statistical significant association was observed. Distribution of diarrheagenic bacteria was high among HIV uninfected n=45 (57.0%) as compared to infected n=30 (38.46%) children (Table 2).

Table 1. Sequences of multiplex (m) PCR primers; forward (fp) and reverse (bp), product and sizes

Primers	Amplicon	Target gene	Sequence (5' to 3')
MEinv a	140	Invasive	fp: TGG AAA AAC TCA GTG CCT CTG CGG
MEinv b			bp: TTC TGA TGC CTG ATG GAC CAG GAG
mVT1 a	121	Verotoxin-1	fp: ACG TTA CAG CGT GTT GCA GGG ATC
mVT1 b			bp: TTG CCA CAG ACT GCG TCA GTG AGG
mVT2a	102	Verotoxin-2	fp: TGT GGC TGG GTT CGT TAA TAC GGC
mVT2b			bp: TCC GTT GTC ATG GAA ACC GTT GTC
mVT2ea	322	Verotoxin	fp: CCA GAA TGT CAG TAT ACT GGC GAC
mVT2eb		-animal	bp: GCT GAG GAC TTT GTA ACA ATG GCT G
MEagga	194	aggregative	fp: AGA CTC TGG CGA AAG ACT GTA TC
mEaggb			bp: ATG GCT GTC TGT AAT AGA TGA GAA C
mST1a	160	Heat-stable	fp: TTT CCC CTC TTT TAG TCA GTC AAC TG
mST1b		toxin 1	bp: GGC AGG ATT ACA ACA AAG TTC ACA G
mST2a	423	Heat-stable	fp: CCC CCT CTC TTT TGC ACT TCT TTC C
mST2b		toxin 2	bp: TGC TCC AGC AGT ACC ATC TCT AAC CC
MEaeA	241	Attaching	fp: TGA GCG GCT GGC ATG AGT CAT AC
mEAEAb		and effacing	bp: TCG ATC CCC ATC GTC ACC AGA GG
mLT1a	360	Heat-labile toxin 1	fp: TGG ATT CAT CAT GCA CCA CAA GG
mLT1b			bp: CCA TTT CTC TTT TGC CTG CCA TC
mCNF1a	552	Cytotoxic	fp: GGC GAC AAA TGC AGT ATT GCT TGG
mCNF1b		necrotizing-1	bp: GAC GTT GGT TGC GGT AAT TTT GGG
mCNF2a	839	Cytotoxic	fp: GTG AGG CTC AAC GAG ATT ATG CAC TG
mCNF2b		necrotizing-2	bp: CCA CGC TTC TTC TTC AGT TGT TCC TC

Table 2. Distribution of enteric bacteria pathogens among HIV infected and uninfected children

Bacterial species	Pathotype	HIV negative (N=79)	HIV positive (N=78)	P-values	95% confidence intervals
Shigella		21(26.6%)	10 (12.8%)	0.033	0.17 - 0.93
E. coli	ETEC	1	0	0.300	0.32 - 1.38
	EPEC	3	2		
	STEC	1	1		
	EIEC	8	9		
	EAggEC	11	8		
	Sub total	24 (30.4%)	20 (25.6%)		
Salmonella		0	0	-	-

Key: ETEC = Enterotoxigenic E. coli; EPEC =Enteropathogenic E.coli; STEC =Shiga toxin-producing E. coli; EIEC = Enteroinvasive E. coli; EAggEC = Enteroaggregative E. coli

EAggEC strain harbouring Eagg was the most detected 17 (43.2%). Seventeen (38.6%) isolates that harboured both cnf2 and invasive genes were grouped as EIEC. Five (11.4%) isolates with intimin genes (eae) and without Vt genes were grouped as EPEC. STEC stains harbouring vt1, vt2, vt1vt2 and with or without intimin (eae) and ETEC producing either ST or LT was the least detected (table 3).

4. DISCUSSION

Diarrheagenic enteric bacteria especially *E. coli, Salmonella* and *Shigella* species are a potential public health threat causing persistence diarrhoea in children in developing countries. In this study the overall prevalence of diarrheagenic bacteria was 47.8%; 28.7% in HIV uninfected and 19.1% in HIV infected children. This agrees with earlier findings by Rono *et al.,* in Kenya [5], where most of diarrheagenic bacterial pathogens cases were from HIV negative as compared to HIV positive participants. Moreover, a study done on parasites reported high prevalence of *Entamoeba* species among HIV negative than HIV positive children [9].

In sub-Saharan Africa, *Shigella spp* predominated as a cause of bacterial diarrhoeal illness [10]. However, in our findings pathogenic *E. coli spp* was the most dominant (28.0%) which were followed by *Shigella spp* (19.7%) and no cases of *Salmonella* spp was found. HIV negative was associated with *Shigella* infection (p=0.033) but not *E.coli* infection (p=0.300).

Table 3. Distribution of virulence factors associated with *E. coli* pathotypes

Enteric pathogen	HIV negative		HIV positive		Totals		P-values
	Freq.	%	Freq.	%	Freq.	%	
STEC							
vt1alone	0	0.0%	0	0.0%	0	0.0%	0.993
vt2 alone	1	1.3%	1	1.3%	2	1.3%	
vt1vt2	0	0.0%	0	0.0%	0	0.0%	
vt2eaeA	0	0.0%	0	0.0%	0	0.0%	
ETEC							
st1	0	0.0%	0	0.0%	0	0.0%	-
st2	0	0.0%	0	0.0%	0	0.0%	
lt1	1	1.3%	0	0.0%	1	0.6%	
EIEC							
Einvisive	8	10.1%	7	9.0%	15	9.6%	0.776
cnf2	0	0.0%	2	2.6%	2	1.3%	
EAEC							
cnf1	0	0.0%	0	0.0%	0	0.0%	0.230
Eagg	12	15.2%	7	9.0%	19	12.1%	
EPEC							
eaeA	3	3.8%	2	2.6%	5	3.2%	0.659

This may due to frequent administration of antibiotics for treatment of other infections among HIV infected children, which affects the growth of these pathogens during isolation. In Kenya, HIV infected children are managed by local health sectors, which constantly observed their health condition [11] thus, strengthen their awareness of hygiene and control their daily activities.

In our study, children above 10 years are 1.56 times more likely to get infection compared to those aged between 5-10 years. Younger children aged less than 10 years old have less risk of getting bacterial diarrhoeal illness. This may be because these children are closely monitored by their parents/guardians on the type of food they eat and water they drink thus reducing the rate of acquiring infection, this is strongly supported by previous studies [12]. The frequency of infection was high in female compared to male children. This is unlike previous studies which reported a higher prevalence in males than in females [13,14]. However, another study done [9] that agrees with our finding reported high prevalence of *Entamoeba spp* in female than in male children, this may be due to young female are mostly involve in helping their parents/guardians in doing house work and they may not be keen on observing personal hygiene like proper hands washing before eating anything and this can contribute a lot to diarrheagenic bacterial infection among them.

Diarrheagenic *E. coli* (DEC) is major public health risk in children in developing countries causing persistent diarrhoea [15].These have been classified into pathotypes based on their virulence factors that are associated with diarrhoea. EAggEC has a global distribution and is associated with diarrhoea both in young children and adults, similarly in this study; EAggEC (43.18%) was the main Pathotype of DEC isolated, followed by EIEC (38.64%), EPEC (11.36%), STEC (4.55%) and ETEC (2.27%).This was consistent with several studies showing that EAggEC was the main *E.coli* pathotypes commonly associated with persistent diarrhoea in children in Kenya [5,16,17].

5. CONCLUSION

Diarrheagenic *E. coli* and *Shigella* were the main cause of diarrheal illnesses in children aged five to twelve years in Dandora slums of Nairobi. From this study, there is a steady positive correlation between HIV status and the prevalence of diarrheagenic bacteria. HIV positive children had less infestation of enteric bacterial pathogens as compare to HIV negative children. Therefore, it could be speculated that HIV negative children maybe reservoir of these organisms. More studies should be done in a bigger population to bring a true picture of the distribution of common circulating enteric bacteria pathogens in HIV infected and uninfected children.

CONSENT

As per international standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

This study was reviewed and approval by Kenya Medical research Institute (KEMRI) Scientific Ethical Review Unit (SERU) on 19/02/2018.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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