Diarrhoeagenic Escherichia coli isolated from children with acute diarrhoea at Rakai hospital, Southern Uganda

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Research

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Abstract

Background: Diarrhoeagenic Escherichia coli (DEC) is a leading cause of childhood diarrhoea in the developing countries. However, there is scanty information on DEC and its association with childhood diarrhoea in Uganda. This study aimed to estimate the prevalence of DEC and its pathotypes among children with acute diarrhoea in Southern Uganda.

Results: A cross-sectional study was conducted over a period of 3 months, May 2016 to July 2016. A total of 267 children less than 5 years with acute diarrhoea admitted to Rakai General Hospital in Southern Uganda were enrolled into the study. Stool was collected from all the children and processed for isolation of E. coli . Conventional polymerase chain reaction (PCR) was performed to determine the presence of DEC and its pathotypes . A total of 102 (38.2%, 102/267) children had DEC of various pathotypes –enteroaggregative E. coli (EAEC) (14.2%); enteropathogenic E. coli (EPEC) (6.7%); enterotoxigenic E. coli (ETEC) (6%); enteroinvasive E. coli (EIEC) (7.5%); enterohemorrhagic E. coli (EHEC) (3%); and cell-detaching E. coli (CDEC) (0.75%). The difference in overall prevalence of DEC was not significant regarding HIV status but individually, ETEC was associated with HIV-negative status while EAEC and CDEC were associated with HIV-positive status.

Conclusions: DEC is prevalent in Ugandan children with acute diarrhoea. Therefore, its identification should be considered among strategies for combatting childhood diarrhoea in Uganda.

Background

Diarrhoea is a leading cause of morbidity and mortality in children less than 5 years of age worldwide and it is also a common problem in HIV-infected individuals [1, 2]. While tried-and-tested interventions show that diarrheal deaths are preventable [3], diarrhoea remains a leading cause of death in Africa and it was responsible for ~ 330,000 deaths in children less than 5 years in 2015 [4].

Diarrhoeagenic *Escherichia coli* (DEC) is a major cause of gastrointestinal disorders worldwide [5–8]. DEC is classified into six major pathotypes (pathogenic variants) i.e. enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC also known as Shiga-toxin producing *E. coli*), and diffusely adherent *E. coli* (DAEC) [6, 9–11]. While *E. coli* is frequently associated with acute childhood diarrhoea in Uganda [12], the prevalence of DEC and its pathotypes in Uganda has not been documented. Therefore, the aim of this study was to determine the prevalence of DEC and the distribution of DEC pathotypes among children with acute diarrhoea admitted to Rakai General Hospital in Southern Uganda. We also compared the distribution of DEC between HIV-positive children and HIV-negative children with acute diarrhoea.

Results

Demographics

Table 1 summarizes the participants' demographic characteristics. A total of 267 children with acute diarrhoea were enrolled, of whom 68 (25.5%) were HIV-positive while 199 (74.5%) were HIV-negative. The children' age ranged from 6 to 59 months with a mean age of 29.622 ± 0.946 months and median age of 28 months; 154 (57.7%) children were male while 113 (42.3%) were female. Of the 68 HIV-positive children, 29 (42.6%) were male while 39 (57.4%) were female; 64 (94.1%) of the HIV-positive children were on HAART while 4 (5.9%) were not because they were newly diagnosed. Of the 199 HIV-negative children, 125 (62.8%) were male while 74 (37.2%) were female. The modal age group were 13–24 months for HIV negative and 25–36 months for HIV-positive children.

Table 1
Demographics of the children with acute diarrhoea in Southern Uganda (n = 267)

Age (months)	Sex		HIV serostatus		On HAART	
	Males (%)	Females (%)	Positive (%)	Negative (%)	Yes (%, n = 68)	No (%, n = 68)
6–12	20	24	2	42	1	0
13–24	35	37	13	59	11	0
25–36	38	20	20	38	18	1
37–48	32	19	16	35	14	2
49–59	29	13	17	25	20	1
Total	154 (57.7)	113 (42.3)	68 (25.5)	199 (74.5)	64 (94.1)	4 (5.9)

Furthermore, 78 samples were screened by PCR for HIV (for children who were less than 18 months of age), six of whom were positive (4 male, 2 female), Table 2. Most (66.7%, 4/6) of these children belonged to the age group 13–18 months.

Table 2
PCR screening of dried blood spots (DBS) for HIV-1

Age (months)	Sex				Total		
	Males		Females				
	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	Overall
6–12	01	19	01	23	02	42	44
13–18	03	16	01	14	04	30	34
Total	04	35	02	37	06	72	78

Most children (61.4%, 164/267) had taken antibiotics prior to visiting the hospital especially children in the 13–24 months age group. Relatedly, this age group had the highest number of children with body temperature of above 37.5 °C, Table 3. However, there was no association between use of antibiotics and elevated body temperature ($X^2 = 0.030$, df = 1, p = 0.862, $p \geq 0.05$).

Table 3
Previous antibiotic use, body temperature and age category

Age group (months)	Prior use of antibiotics		Body temperature	
	Yes (%)	No (%)	36-37.5°C (%)	Above 37.5°C (%)
6–12	32	12	28	16
13–24	47	25	42	30
25–36	31	27	37	21
37–48	30	21	29	22
49–59	24	18	28	14
Total	164 (61.4)	103 (38.6)	164 (61.4)	103 (38.6)

Prevalence of DEC pathotypes

Of the 267 children with acute diarrhoea, 38.2% (102/267) had a DEC pathotype. The prevalence of DEC in HIV-positive children and HIV-negative children was 45.6% (31/68) and 35.7% (71/199) respectively, and the difference in prevalence was not significant regarding HIV status (p = 0.2662), Table 4. A total of six pathotypes were detected; EAEC was the most frequent (14.2%) while CDEC was the least frequent (0.75%). Individually, ETEC was associated with HIV-negative status while EAEC and CDEC were associated with HIV-positive status, Table 4. Generally, virulence genes were more frequent in DEC pathotypes from HIV-negative children compared to pathotypes from HIV-positive children but the difference was not significant (p = 0.0662), Table 5. However, the CDEC *CNF1* gene entirely occurred in HIV-positive children (p = 0.015), Table 5. Parasites and other enteric bacteria were also detected, Table 6.

Table 4
Frequency of DEC pathotypes among children and association with HIV status

Pathotype	Prevalence among HIV+ (N = 68), n (%)	Prevalence among HIV- (N = 199), n (%)	Overall prevalence (N = 267), n (%)	P Value
EAEC	15 (22.1)	23 (11.6)	38 (14.2)	**0.0324**
EPEC	8 (11.8)	10 (0.5)	18 (6.7)	0.0557
ETEC	0 (0)	16 (8)	16 (6)	**0.0159**
EIEC	6 (8.8)	14 (7)	20 (7.5)	0.6286
EHEC	0 (0)	8 (4)	8 (3)	0.0932
CDEC	2 (2.9)	0 (0)	2 (0.75)	**0.0152**
Total	31 (45.6)	71 (35.7)	102 (38.2)	0.2662

Table 5
Frequencies of virulence genes among DEC pathotypes and relationship with HIV

DEC pathotype	Gene	Frequency		X^2 P- value
		HIV-positive (N = 68), n (%)	HIV-negative (N = 267), n (%)	
ETEC	LT1	0	9	0.744
	ST1	0	4	0.293
	ST2	0	3	0.309
EHEC	VT1	0	4	0.744
	VT2	0	6	0.148
	VT2e	0	0	-
CDEC	CNF1	2	0	**0.015**
	CNF2	0	0	-
Total		2 (3)	26 (10)	0.0662

Table 6
Other pathogens identified

Age (months)	DEC		Other pathogens detected			
	HIV+ (%, n = 68)	HIV- (%, n = 199)	Salmonella species (%)	*Shigella* species (%)	Hook worms (%)	*Trichomonas hominis* (%)
6–12	1	8	0	0	0	1
13–24	4	17	0	0	0	0
25–36	4	13	0	0	0	4
37–48	9	19	2	1	0	5
49–59	13	14	2	0	2	9
Total	31 (45.6)	71 (35.7)	4 (1.5)	1 (0.4)	2 (0.7)	19 (7.1)

Discussion

In this study, the prevalence of DEC was found to be high in Ugandan children with acute diarrhoea. As *E. coli* is a frequent cause of childhood diarrhoea in Uganda [12], DEC could be a significant problem that deserves more attention. The prevalence of DEC in Southern Uganda (38.2%) was higher than the reported rate in Tanzania (22.9%) [13] but lower than rates from Nigeria (73.8%) [6] and India (52%) [11]. Our findings regarding HIV status are comparable to findings from similar settings in Africa and beyond. For example, the exclusive detection of a DEC pathotype (i.e. CDEC) in HIV-positive cases has been reported before [14]. Similarly, studies on DEC in East Africa also showed that EAEC is the most frequent pathotype among children with diarrhoea [15–20]; in Western Kenya, ETEC was also associated with acute diarrhoea while EAEC was linked to persistent diarrhoea in HIV-positive children [17]. Overall, studies elsewhere have shown variations in prevalence of DEC pathotypes in children [21].

The distribution of virulence genes among DEC pathotypes in relation to type of diarrhoea has been evaluated before: In Kenya, acute diarrhoea among HIV-positive cases was found to be associated with EAEC's *aatA* and EPEC's *bfp*, while EIEC's *ipaH*, EHEC's *stx1*/*stx2* and ETEC's *elt*/*est* were associated with HIV-negative cases [17]. In our study, CDEC's *CNF1* was only detected in HIV-positive children but generally there was no significant association between detection of the genes and HIV status. Some of the differences observed between our findings and other works could be due to environmental factors, differences in levels of hygiene, sanitation/sewage management, host susceptibility or unknown underlying health conditions. Furthermore, Okeke *et al* [22] noted that *CNF1* and *CNF2* are frequently found in invasive *E. coli* isolates. Overall, dual occurrence of multiple virulence genes as opposed to a single gene raises a question to whether these genes act in synergy to produce acute disease [23]. Several studies indicate that *E. coli* pathotypes require multiple genes to be highly virulent [24]. For instance,

ETEC with heat-labile toxin *elt*, heat-stable toxin *est* and colonization factor antigens (CFAs); EPEC with *bfp* and *eae* genes and Shiga-toxin-producing *E. coli* (STEC or EHEC) with shiga-like toxin *stx* and *eaeA*, are considered the most virulent strains [25]. Although EAEC strains are genetically heterogeneous and contain various virulence genes including *aggR* [26], the presence of multiple genes has not been associated with EAEC pathogenesis. This may explain why EAEC is predominantly associated with persistent diarrhoea while strains with multiple genes (i.e. EPEC, ETEC and EHEC) are associated with acute diarrhoea [27]. In our study, we detected multiple genes in ETEC (*LT1, ST1*, *ST2*) and EHEC (*VT1, VT2*) but we did not evaluate their association with acute diarrhoea.

Lastly, in our study the 13–24 months age group had the highest number of children with body temperature above 37.5 °C however, we were not able to establish the cause of high body temperature though some children (38.6%, n = 103) had a positive blood smear for malaria. Relatedly, stool analysis showed coinfection with multiple pathogens in 10% (26/267) of the children, which complicates the aetiology of acute diarrhoea in these children. However, the coinfection rate (10%) in this study is lower than the 22.1% rate reported in India [11]. The finding of *Trichomonas hominis* at high frequency i.e. 7.1% (19/267) may not be surprising as it is frequently associated with diarrhoeic stools but generally non-pathogenic. However, further studies are necessary to investigate whether *Trichomonas hominis* is a significant cause of childhood diarrhoea in Uganda. One limitation of this study is that some children had consumed antibiotics in the preceding months prior to enrolling them and this could have influenced the identification of DEC and other pathogens.

Conclusions

DEC is prevalent in Ugandan children with acute diarrhoea hence, it should be considered for inclusion in diagnostics for acute childhood diarrhoea.

Methods

This was a cross-sectional study of children aged 6 months to 5 years admitted to Rakai General Hospital, Southern Uganda. Rakai Hospital is a 100-beds capacity hospital that admits approx. 200 sick children every week. The study recruited children with acute diarrhoea (passage of 3 or more loose stool over a 24 hour period lasting less than 2 weeks). The children were consecutively recruited over a period of 3 months (May 2016 to July 2016). HIV-serostatus was performed according to the algorithm of Uganda's Ministry of Health. The clinical and epidemiological data were collected from the children by using an interviewer-administered questionnaire.

Stool was obtained from each child by using a sterile plastic container labelled with a unique study number. Specimen containers and instructions pertaining specimen collection were given to caretakers of the recruited children. For younger children, mothers collected stool from diapers as soon as the children passed stool while for older children, stool was collected into a disposable plate and immediately transferred into a sterile container. As soon as specimens were obtained, the caretakers were instructed to

deliver them to Kakuuto Health Centre IV Microbiology Laboratory, Rakai district where they were processed for growth/identification of *E. coli*. In the laboratory, each sample was inoculated onto MacConkey agar plate within an hour of reception and incubated at 37 °C for 14 hours. Lactose fermenting colonies suggestive of *E. coli* were inoculated onto IMViCU (Indole, Methyl red, Voges Proskaeur, Citrate and Urease) media for confirmation as *E. coli*. Isolates that were positive with indole and methyl red tests but negative with Voges-Proskaeur, citrate and urea were classified as *E. coli*. To increase chances of detecting DEC, five *E.coli* colonies were sampled from each plate. Isolates were separately preserved in tryptone soy broth and transported to Makerere University for molecular analysis.

Crude and/or pure chromosomal DNA used in the PCRs was extracted as described previously [28]. Briefly, each sample was inoculated onto Nutrient agar and incubated at 37^{O}C for 24 hours: To extract the DNA, a swipe of colonies was suspended into 300 µl of sterile 0.25X Tris-EDTA (TE) buffer in 1.5 ml microcentrifuge tubes, vortexed for 10 seconds, centrifuged at 13,000 g for 2 minutes, and the supernatant discarded to retain the pellet of clean bacteria. To the cell pellet, we added 100 µl of 0.25X TE buffer, vortexed for 10 seconds, centrifuged at 13,000 g for 5 minutes and discarded the supernatant. Then, cells were suspended in 60 µl of 0.25X TE buffer and heated at 95^{O}C for 10 minutes in a Thermal mixer (Eppendorf, Germany). Then, samples were vortexed for 20 seconds and cooled to room temperature, centrifuged at 13,000 g for 5 minutes and the supernatant which contained the crude DNA was transferred into a sterile 1.5 ml microcentrifuge tube and used as template in PCRs.

Identification of DEC pathotypes

DEC pathotypes can be molecularly identified and/or classified based on the virulence genes specific to each pathotype [11]. To identify the pathotypes, we used previously published PCR primers [29] targeting eleven virulence genes specific to six DEC pathotypes i.e. ETEC, heat stable toxin (*ST1 & ST2*), heat labile toxin (*LT*); EHEC, verotoxins (*VT1, 2 & 2e*); EIEC (Einv); EAEC (Eagg); EPEC, attaching & effacing antigen (*eaeA*); CDEC (Cell-detaching *E. coli*), cytotoxic necrotising factors (CNF 1 & 2).

The PCRs were prepared by following the Taq 2X Taq Master Mix protocol (New England BioLabs, Inc.). Briefly, a 25 µL PCR assay was prepared by mixing 12.5 µL Taq 2X master mix, 1 µL each of the forward & reverse primers, 8 µL of nuclease free water, and 2.5 µL of crude DNA extract. Amplification was achieved in a thermocycler by using a programme reported by Todd *et al*. [30] with minor modifications i.e. Initial denaturation of 94^{O}C for 5 minutes followed by 30 cycles of denaturation at 94^{O}C for 60 seconds, annealing at 55^{O}C for 90 seconds, extension at 72^{O}C for 90 seconds, and a final extension step at 72^{O}C for 10 minutes. Approx. 5 µL each of the PCR product was analysed by agarose gel electrophoresis (2% w/v agarose) in Tris-acetate EDTA (TAE) buffer stained with Ethidium Bromide (5 mg/ml) (Sigma-Aldrich, USA). Gels were run at 120V for 1 hour and visualized with UVP Gel documentation system (Benchtop Trans-illuminator System BioDoc-it, UK). PCRs included negative controls (*E. coli* ATCC25922 & sterile nuclease free water) and positive controls (PDAS 101, ATCC 35401, 29930, 933W, 35150 & E 2348/69 from the Kenya Medical Research Institute).

Statistical analysis was performed with SPSS v23 and comparisons performed with the Chi square test to assess associations between variables, for which p-values less than 0.05 were considered statistically significant. Outcome variables were presence or absence of DEC pathotypes; predictor variables were age, sex, prior use of antibiotics and HAART, temperature and HIV-serostatus.

List of Abbreviations

DEC: Diarrhoeagenic *Escherichia coli*

EAEC: Enteroaggregative *Escherichia coli*

EPEC: Enteropathogenic *Escherichia coli*

ETEC: Enterotoxigenic *Escherichia coli*

EIEC: Enteroinvasive *Escherichia coli*

EHEC: Enterohemorrhagic *Escherichia coli*

CDEC: Cell-detaching *Escherichia coli*

Declarations

Ethics approval and consent to participate

Scientific and ethical approval was obtained from the School of Biomedical Sciences higher degree research and ethics committee (SBS-HDREC), Makerere University. Written informed consent was obtained from the caretakers of these children and confidentiality of the information was emphasized through coding of participants' details and restricting accessibility of compiled data under a computer password as well as key and lock for hard copies. Consent to participate was obtained from the parent or legal guardian of the children, and the consent was written. Participation was voluntary and any participant was free to withdraw from the study without any due cost.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

David Kateete is an editorial board member for BMC Infectious Diseases

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Authors' contributions

FM, FCN, DPK and HK conceptualized, designed and supervised the study. FM performed the experiements and data analysis (under supervision of FCN & HK) in partial fulfilment of the requirements for the award of the degree of Master of Science in Immunology & Clinical Microbiology of Makerere University. EK helped with the design of molecular assays. FM, EK and DPK wrote the manuscript. All authors read and approved the final manuscript.

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