Subjects' Sociodemographics Influence the Transmission Patterns of Diarrheagenic *Escherichia coli* Pathotypes among Children under 5 Years in Nakuru County

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Abstract

Background: Diarrheagenic *Escherichia coli* (DEC) infections constitute the leading causes of morbidity and mortality among children in Sub-Saharan Africa. However, little has so far been done to properly reveal the pathogenic endowments of DEC in these populations. **Aims and Objectives:** We evaluated 4 DEC strains among children under 5 years. **Materials and Methods:** A cross-sectional study design was employed among 384 positive cases. **Results:** There was a significant decline in infections associated with DEC as the children grew older (χ^2[12] = 87.366: P = [0.000]. A total of 56 (14.6%) cases were 0–12 months, 168 (43.8%) were 13–24 months, 88 (22.9%) were 25–36 months, 40 (10.4%) were 37–48 months, and 32 (8.3%) were 49–60 months. A total of 248 (64.6%) male subjects exhibited more susceptibility to DEC infections than their female counterparts (n = 136 [35.4%]) (χ^2[3] =13.313: P = [0.004]. Subjects from urban areas (n = 248 [64.6%]), significantly bored the brunt of infections than those from rural areas (n = 136 [35.4%]) (χ^2[3] = 35.147: P = [0.000]. The prevalence of DEC appeared significantly higher during rainy seasons (n = 269 [70.1%]). **Conclusion:** Young age, male gender, crowding, and rainy season play a central role in the transmission of DEC pathotypes.

Keywords: Children, diarrheagenic *Escherichia coli*, Nakuru County, pathotypes, prevalence

Résumé

Contexte: Les infections à Escherichia coli entéropathogène (DEC) constituent les principales causes de morbidité et de mortalité chez les enfants en Afrique subsaharienne. Cependant, jusqu'à présent, peu de choses ont été faites pour révéler correctement les caractéristiques pathogènes de DEC dans ces populations. **Objectifs:** Nous avons évalué 4 souches de DEC chez les enfants de moins de 5 ans. **Matériel et méthodes:** Un plan d'étude transversal a été utilisé parmi 384 cas positifs. **Résultats:** Il y a eu une diminution significative des infections associées à DEC à mesure que les enfants grandissaient (χ2 [12] = 87,366 : P = [0,000]). Un total de 56 (14,6 %) cas avaient entre 0 et 12 mois, 168 (43,8 %) avaient entre 13 et 24 mois, 88 (22,9 %) avaient entre 25 et 36 mois, 40 (10,4 %) avaient entre 37 et 48 mois, et 32 (8,3 %) avaient entre 49 et 60 mois. Un total de 248 (64,6 %) sujets masculins ont montré une plus grande susceptibilité aux infections à DEC que leurs homologues féminins (n = 136 [35,4 %]) (χ2 [3] = 13,313 : P = [0,004]). Les sujets des zones urbaines (n = 248 [64,6 %]) ont significativement supporté le fardeau des infections par rapport à ceux des zones rurales (n = 136 [35,4 %]) (χ2 [3] = 35,147 : P = [0,000]). La prévalence de DEC semblait significativement plus élevée pendant la saison des pluies (n = 269 [70,1 %]). **Conclusion:** L'âge jeune, le sexe masculin, la surpopulation et la saison des pluies jouent un rôle central dans la transmission des pathotypes de DEC.

Mots-clés: Enfants, Escherichia coli diarrhéagéniques, Comté de Nakuru, pathotypes, prévalence

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Introduction

According to the WHO,[1] there is a staggering 1.7 billion cases of pediatric diarrheal disease worldwide with an estimated 525,000 fatalities of children under the age of 5 years annually. Diarrhea epidemic patterns among children under 5 years of age are on the rise, and diarrheagenic *Escherichia coli* (DEC) has been implicated in this trend. DEC strains distinctly exist in six different pathotypes (pathogenic types): Enterohemorrhagic *E. coli*, Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli*.[2] Both EPEC and EAEC have been linked to chronic and severe cases of diarrhea among both economically thriving and underprivileged countries in the world. These two pathotypes are not only predominant but also ubiquitous.[3,4] In most of the Sub-Saharan African countries, several studies have been done to characterize different pathotypes of DEC both in stool samples and drinking water. For instance, Bonkoungou[5] characterized DEC isolated from drinking water and fecal matter of patients among them children under 5 years of age presenting symptoms of diarrheagenic disease in Ouagadougou, Burkina Faso. Interestingly, the most common *E. coli* strain found was EAEC, followed by EPEC, EIEC, and Shiga toxin-producing *E. coli*. This trend was largely attributed to the difference in demographic factors among the children. However, coinfections with EAEC rather than isolated cases of Enterotoxigenic *E. coli* (ETEC) were found. Other studies done in Africa have presented a consistent pattern of prevalence with this study.[6-8] In Kenya, there is a notable paucity of information regarding patterns of DEC pathotypes. *E. coli* with multidrug resistance has been found in asymptomatic school children in the Kenyan slums with the highest prevalence in occurring in Kibra.[9] However, cases of DEC have recently been reported among children under 5 years of age in a cosmopolitan Kenyan setting.[10]

Despite these studies' emphasis on characterizing the various DEC pathotypes in Kenya, there are little data available on the prevalence of the most frequently isolated DEC pathotypes from stool samples of hospitalized children under 5 years of age at Provincial General Hospital (PGH) in Nakuru County. Therefore, the purpose of this study was to describe the DEC pathotype isolates from children aged below 5 years attending PGH.

Materials and Methods

Ethics statement

The research was reviewed and approved by the Kenyatta National Hospital–University of Nairobi Ethics and Research Committee (KNH-UoN ERC), reference number KNH-ERC/A/216. Permission to conduct the study was sought through the County Medical Director and Public Health and the Hospital Medical Superintendent of Nakuru Provincial General Hospital (Ref No.RII/Vol 1/08). Research authorization was further sought from National Commission for Science, Technology, and Innovation (NACOSTI), reference number NACOSTI/P/22/20744. Each caregiver provided assent to allow their children to be subjects in the current study through the signing of a consent form.

Study design and population

Between July 2017 and July 2018, approximately 1124 children (0–60 months) who reported to Nakuru Provincial General Hospital pediatric unit experiencing diarrhea (July 2017– July 2018) were sampled as part of a cross-sectional study using random sampling.

Inclusion and exclusion criteria

All children under the age of five with diarrhea episodes were included. The selection of the cohort was based on definitive laboratory diagnosis of DEC. A total of 384 cases were found after meeting the selection criteria. The subjects with prior antibiotic consumption (within 7 days preceding sample collection) were excluded from the study. Diarrhea was defined by the occurrence of more than three loose stools, the liquid of watery or bowel movements in any 24 h.

Sample collection, isolation, identification, and characterization

Anal swabs were obtained aseptically when the child is in a supine position qualified laboratory personnel. Each swab was labeled with a unique participant number before specimen collection. A sterile cotton-tipped swab was shallowly inserted three times into the anal canal (maximum of an inch) before withdrawal. The anal swab was then returned to its plastic casing with the Amies transport media (Oxoid, Basingstoke, UK). This is meant to sustain microorganisms in a viable but slow state of growth during transportation to the laboratory. Portions of swabs were then placed in a special container with Cary-Blair transport medium (ThermoFisher Scientific), kept at 4°C using a cold box, and then transferred to the laboratory for analysis.

The swabs were then transferred into sterile tubes containing buffered peptone water, a nonselective enrichment media. The swabs were rolled severally onto the sides of the tube to wash off all possible bacteria on in the tube containing buffered peptone rinse water. The inoculated buffered peptone rinse solution was incubated at 37°C for 3–4 h. Subsequently, 200 μL of peptone water cultures were streaked on Eosin Methylene Blue (EMB) agar (Oxoid, Basingstoke, UK) and incubated overnight at 37°C for 24 h. The plates were removed from the incubator after 24 h and visually examined to identify *E. coli* colonies presumptively (deep purple colonies with a green metallic sheen formed.). Gram staining was performed to determine the morphology of the growing colonies, and a pure culture was obtained by sub-cultivating a single *E. coli* colony on another EMB agar plate. The plate was then incubated at 37°C for 24 h. A single colony was then selected for confirmative identification of *E. coli* through a series of biochemical tests. IMViC biochemical tests were performed as described by Cheesbrough.[11] Isolates that were positive to Indole and Methyl Red tests but negative for Voges-Proskauer, Citrate, and Urease tests were identified as *E. coli*. *E. coli* ATCC 25922 and *Klebsiella pneumonia* were used as negative and positive controls, respectively. Multiplex polymerase chain reaction was used to characterize the categories of DEC as described by Ohmura-Hoshino *et al*.[12]

Statistical analysis

Data were analyzed using SPSS (IBM Inc., Chicago, IL, USA, version 25). The Chi-square test was used to determine the association between the DEC distribution and various demographic factors where $P \leq 0.05$ was considered significant. Furthermore, frequencies and percentages of DEC pathotypes based on these factors were obtained.

Results

This study evaluated subjects' sociodemographic characteristics and how they influence the transmission of selected DEC pathotypes among under 5 children in Nakuru County, Kenya.

Subjects' socio demographic characteristics

Results in Table 1 show that 28 (14.6%) of the cases sampled were aged between 0 and 12 months, 84 (43.8%) aged 13–24 months, 44 (22.9%) aged 25–36 months, 20 (10.4%) aged between 37 and 48 months, while 16 (8.3%) were aged between 49 and 60 months. On the other hand, 28 (14.6%) of the sampled controls were aged between 0 and 12 months, 84 (43.8%) aged 13–24 months, 44 (22.9%) aged 25–36 months, 20 (10.4%) aged 37–48 months while 16 (8.3%) were aged between 49 and 60 months. Table 1 results also show that 124 (64.6%) of the sampled cases were male, while 68 (35.4%) were female. Furthermore, 124 (64.6%) of the cases were from rural setups, while 68 (35.4%) were from urban areas. The same pattern was true for controls. About 20 (10.4%) of the cases had no formal education at all, 32 (16.7%) had primary school level, 68 (35.4%) had secondary school level, while; 72 (37.5%) had attained tertiary level. Interestingly, 20 (10.4%) and 72 (37.5%) of the controls had no formal education and tertiary level respectively. Importantly, 124 (64.6%) of the cases had acute diarrhea at the time of admission to the study, while 68 (35.4%) had had chronic diarrhea. The same pattern was true for controls.

Distribution of diarrheagenic *Escherichia coli* pathotypes based on subject sociodemographics

It was noted that there was statistical significance in the prevalence of DEC pathotypes with regards to all the demographic factors investigated. Based on sex, there is a variation in the prevalence of DEC pathotypes among the sexes. Of the total ($n = 384$) cases reported for both sexes. The total number of females was $n = 136$ (35.4%) while the number of males was $n = 248$ (64.6%). Regarding females, the sequence of dominance within the group was: EAEC ($n = 58$ [42.6%])

Table 1: Sociodemographic characteristics of children under 5 years

Sociodemographic characteristics	Cases, *n* (%)	Controls, *n* (%)	Total
Age (months)			
0–12	28 (14.6)	28 (14.6)	56 (14.6)
13–24	84 (43.8)	84 (43.8)	168 (43.8)
25–36	44 (22.9)	44 (22.9)	88 (22.9)
37–48	20 (10.4)	20 (10.4)	40 (10.4)
49–60	16 (8.3)	16 (8.3)	32 (8.3)
Gender			
Male	124 (64.6)	124 (64.6)	248 (64.5)
Female	68 (35.4)	68 (35.4)	136 (35.4)
Place of residence			
Urban	124 (64.6)	124 (64.6)	248 (64.5)
Rural	68 (35.4)	68 (35.8)	136 (35.4)
Guardians/parent level of education			
None	20 (10.4)	20 (10.4)	40 (10.4)
Primary school	32 (16.7)	28 (14.6)	60 (15.6)
Secondary school	68 (35.4)	72 (37.5)	140 (36.5)
Tertiary school	72 (37.5)	72 (37.5)	144 (37.5)
Income			
Wage-earner	40 (20.8)	44 (22.9)	84 (21.9)
Self-employed	88 (45.8)	92 (47.9)	180 (46.9)
Unemployed	64 (33.3)	56 (29.2)	120 (31.3)
Season			
Dry	56 (29.2)	59 (30.7)	115 (29.9)
Rainy	136 (70.8)	133 (69.3)	269 (70.1)
Clinical symptoms			
Acute diarrhea	124 (64.6)	124 (64.6)	248 (64.6)
Chronic diarrhea	68 (35.4)	68 (35.4)	136 (35.4)

>EPEC (n = 46 [33.8%]) > EIEC (n = 16 [11.8%]) = ETEC (n = 16 [11.8%]) [Table 1]. Conversely, the trend for the males within the group was: EAEC (n = 118 [47.6%]) >ETEC (n = 52 [21.0%]) >EPEC (n = 46 [18.5%]) > EIEC (n = 32 [12.9%]). A significant difference was noted in the proportion of all pathotypes among males and females (χ^2[3] = 13.313: P = [0.004].

Ages were grouped at an interval of 12 months with the least being 0 months and the highest being 60 months. Of the total (n = 384) cases reported across all the different age groups, a total of n = 56 (14.6%) cases were 0–12 months, n = 168 (43.8%) were 13–24 months, n = 88 (22.9%) were 25–36 months, n = 40 (10.4%) were 37–48 months and n = 32 (8.3%) were 49–60 months. Notably, there were variations in the trend of the DEC pathotypes across all the age brackets. For the age 0–12 months, the sequence of prevalence within the group was: EAEC (n = 48 [85.7%]) >EPEC (n = 8 [14.3%]) >EIEC (n = 0 [0.0%]) = ETEC (n = 0 [0.0%]). For the age 13–24 months, the sequence of prevalence within the group was: EAEC (n = 68 [40.5%]) >EPEC (n = 48 [28.6%]) >ETEC (n = 36 [21.4%]) >EIEC (n = 16 [9.5%]). Reportedly for the age 25–36 months, the order of prevalence within the group was: EAEC (n = 36 [40.9%]) >EIEC (n = 24 [27.3%]) >EPEC (n = 20 [22.7%]) >ETEC (n = 8 [9.1%]). Interestingly, for the age 37–48 months, there was an equal prevalence of three pathotypes, these are; EAEC, EIEC, and EPEC, each at n = 8 (20.0%). ETEC was the most predominant pathotype within this age group (n = 8 [9.1%]). Lastly, for the age 49–60 months, the order of prevalence was: EAEC (n = 16 [50.0%]). Both EPEC and ETEC showed equal prevalence (n = 8 [25.0%]) with no reported prevalence for EIEC (n = 0 [0.0%]). A significant difference in proportions across the different age groups was reported(χ^2[12] = 87.366: P = [0.000].

Pathotypes distribution according to the place of residence also varied significantly across all the pathotypes(χ^2[3] = 35.147: P = [0.000]. The total number of cases reported for the population living outside the Nakuru municipality (rural) was n = 136 (35.4%) while those from the population within Nakuru municipality (urban) were n = 248 (64.6%). The prevalence of pathotypes for the population living outside the Nakuru municipality (rural) within this group was: EAEC (n = 52 [38.2%]) >EPEC (n = 40 [29.4%]) >EIEC (n = 32 [23.5%]) >ETEC (n = 12 [8.8%]). On the other hand, the prevalence of pathotypes for the population living within the Nakuru municipality (urban) within this group was: EAEC (n = 124 [50.0%]) >ETEC (n = 56 [22.6%]) >EPEC (n = 52 [21.0%]) >EIEC (n = 16 [6.5%]).

Moreover, pathotype distribution according to the occupation of the parents/guardians varied significantly across all the pathotypes(χ^2[6] = 15.644: P = [0.016]. Occupation categories consisted of self-employed (n = 180 [46.9%]), unemployed (n = 120 [31.3%]) and wage-earners (n = 84 [21.9%]) of the total n = 384. The order of prevalence within the self-employed group was: EAEC (n = 76 [42.2%]) >EPEC (n = 40 [22.2%]) >ETEC (n = 36 [20.0%]) >EIEC (n = 28 [15.6%]). On the other hand, the unemployed group was characterized by the following prevalence: EAEC (n = 48 [40.0%]) >EPEC (n = 36 [30.0%]) >ETEC (n = 24 [20.0%]) >EIEC (n = 12 [10.0%]). Finally, the Wage-earner group was characterized by the following prevalence: EAEC (n = 52 [61.9%]) >EPEC (n = 16 [19.0%]) while both the ETEC and EIEC had an equal prevalence (each n = 8 [9.5%]). There existed a similar trend in the order of prevalence of pathotypes between the two groups – self-employed and unemployed. That is, EAEC > EPEC > ETEC > EIEC.

Another important factor that affected the distribution of DEC pathotypes was the level of education of the mothers/guardians. There was a statistically significant difference among the different levels of education and across all the pathotypes(χ^2[9] = 64.607: P = [0.000]. Of the 384 cases, those with no education were n = 40 (10.4%), those with primary education were n = 60 (15.6%), those with secondary education were n = 140 (36.5%) and those with tertiary education were n = 144 (37.5%). In the distribution of the pathotypes within the children of parents/guardians without education, the following sequence was observed: Both EAEC and ETEC were equally predominant (each n = 16 [40.0%]) followed by EIEC (n = 8 [20.0%]). No case was reported for the EPEC (n = 0 [0.0%]). Just like those without education, the primary level also showed equal prevalence for EAEC and ETEC (each n = 16 [27.7%]) followed by EPEC (n = 28 [46.7%]). However, there was no reported case of EIEC (n = 0 [0.0%]) within the primary level. The secondary level characterized by the following order: EAEC (n = 64 [45.7%]) >EPEC (n = 28 [20.0%]) while both EIEC were equally prevalent ETEC (each n = 24 [17.1%]). Lastly, the order of prevalence for the tertiary level was as follows: EAEC (n = 80 [55.6%]) >EPEC (n = 36 [25.0%]) >EIEC (n = 16 [11.1%]) >ETEC >(n = 12 [8.3%]).

The number of days with diarrhea was observed to have been a factor in segregating the DEC pathotypes. These days were grouped into two: diarrhea lasting <14 days which comprised n = 248 (64.6%) cases and diarrhea lasting more than 14 days accounting for n = 136 (35.4%). Just like the other categories previously mentioned, there were statistically significant differences between the two groups across all the pathotypes(χ^2[3] = 11.390: P = [0.010]. For the cases within the group of <14 days of diarrhea, the following prevalence of the pathotypes was observed: EAEC (n = 110 [44.4%]) >EPEC (n = 65 [26.2%]) >EIEC (n = 38 [15.3%]) >ETEC (n = 35 [14.1%]). On the other hand, the order of prevalence for cases within the group of more than 14 days of diarrhea was: EAEC (n = 66 [48.5%]) >ETEC (n = 33 [24.3%]) >EPEC (n = 27 [19.9%]) >EIEC (n = 10 [7.4%]).

Finally, the season of the year was also observed to play a critical role in the distribution of the pathotypes. The seasons were either categorized as rainy or dry. Notably of the total $n = 384$ cases, $n = 269$ (70.1%) were found during the rainy season while the rest $n = 115$ (29.9%) were from the dry season. Significant differences among these seasons were found to be significant across all the pathotypes($\chi^2[3] = 51.155$: $P = [0.000]$. The distribution of pathotypes during the rainy season was: EAEC ($n = 124$ [46.1%]) >EPEC ($n = 84$ [31.2%]) >ETEC ($n = 45$ [16.7%]) >EIEC ($n = 16$ [5.9%]). On the other hand, the dry season presented the following order: EAEC ($n = 52$ [45.2%]) >EIEC ($n = 32$ [27.8%]) >ETEC ($n = 23$ [20.0%]) >EPEC ($n = 8$ [7.0%]).

In general, regardless of the different categories of sociodemographic characteristics, the distribution of pathotypes significantly varies from one group to another. Cumulatively, however, the prevalence of the pathotypes from the total $n = 384$ is as follows: EAEC ($n = 176$ [45.8%]) >EPEC ($n = 92$ [24.0%]) >ETEC ($n = 68$ [17.7%]) >EIEC ($n = 48$ [12.5%]) [Table 2].

Discussion

Contingent on gender, males were more susceptible to the DEC than females. This could be explained by the inherent capabilities of the females to resist infectious pathogens.[13] According to Zhou *et al*.,[13] females also showed a predominance of DEC infections (61.6%). However, unlike the current study where EAEC was the most dominant pathotype, Zhou *et al*.[13] noted the predominance of EPEC pathotype across the gender. The prevalence in males could be attributed to the aggressive nature of the males at a younger age.[14] Males could have higher chances of interaction with the DEC strains during their playful moments. Other studies have also reported significant differences in the prevalence of DEC between males and females.[15,16] So far, there is little or no data indicating a high prevalence of DEC among females. Females have been observed to have a preponderance in any given population with the interplay of various immune factors such as the existence of a higher number of CD4+ T cells and higher CD4/CD8 T cell ratios at the age of 0–60 months which seemingly plays a significant role in pathogen-host immune interactions.[17] Thus, the observed predominance of DEC infections among the males.

Regardless of age, the EAEC pathotype is predominant among the sampled population. This pathotype has always been observed to occur ubiquitously across the populations of

Table 2: Diarrheagenic *Escherichia coli* pathotype distribution based on subjects' sociodemographic characteristics

Sociodemographic characteristics	What type of pathotype from Stool sample				Total	Value	df	*P*
	EAEC	EIEC	EPEC	ETEC				
Sex								
Female	58 (42.6)	16 (11.8)	46 (33.8)	16 (11.8)	136 (100.0)	13.313[a]	3	0.004
Male	118 (47.6)	32 (12.9)	46 (18.5)	52 (21.0)	248 (100.0)			
Ages (months)								
0–12	48 (85.7)	0	8 (14.3)	0	56 (100.0)	87.366[a]	12	0.000
13–24	68 (40.5)	16 (9.5)	48 (28.6)	36 (21.4)	168 (100.0)			
25–36	36 (40.9)	24 (27.3)	20 (22.7)	8 (9.1)	88 (100.0)			
37–48	8 (20.0)	8 (20.0)	8 (20.0)	16 (40.0)	40 (100.0)			
49–60	16 (50.0)	0	8 (25.0)	8 (25.0)	32 (100.0)			
Present place of residence								
Rural (outside Nakuru municipality)	52 (38.2)	32 (23.5)	40 (29.4)	12 (8.8)	136 (100.0)	35.147[a]	3	0.000
Urban (within Nakuru municipality)	124 (50.0)	16 (6.5)	52 (21.0)	56 (22.6)	248 (100.0)			
Parent/guardians occupation								
Self-employed	76 (42.2)	28 (15.6)	40 (22.2)	36 (20.0)	180 (100.0)	15.644[a]	6	0.016
Unemployed	48 (40.0)	12 (10.0)	36 (30.0)	24 (20.0)	120 (100.0)			
Wage-earner	52 (61.9)	8 (9.5)	16 (19.0)	8 (9.5)	84 (100.0)			
Education level of mothers/guardian								
None	16 (40.0)	8 (20.0)	0	16 (40.0)	40 (100.0)	64.607[a]	9	0.000
Primary school	16 (26.7)	0	28 (46.7)	16 (26.7)	60 (100.0)			
Secondary school	64 (45.7)	24 (17.1)	28 (20.0)	24 (17.1)	140 (100.0)			
Tertiary school	80 (55.6)	16 (11.1)	36 (25.0)	12 (8.3)	144 (100.0)			
Number of days with diarrhea								
<14	110 (44.4)	38 (15.3)	65 (26.2)	35 (14.1)	248 (100.0)	11.390[a]	3	0.010
>14	66 (48.5)	10 (7.4)	27 (19.9)	33 (24.3)	136 (100.0)			
Month/season								
Rainy season	124 (46.1)	16 (5.9)	84 (31.2)	45 (16.7)	269 (100.0)	51.155[a]	3	0.000
Dry season	52 (45.2)	32 (27.8)	8 (7.0)	23 (20.0)	115 (100.0)			
Total	176 (45.8)	48 (12.5)	92 (24.0)	68 (17.7)	384 (100.0)			

E. coli=*Escherichia coli*, EAEC=Enteroaggregative *E. coli*, EIEC=Enteroinvasive *E. coli*, EPEC=Enteropathogenic *E. coli*, ETEC=Enterotoxigenic *E. coli* children under 5 years of age across the population. There is a declining number of infections detected in the stool samples as the age of the children advances. This trend in older children is arguably associated with age-related immunity. This has been observed in several studies.[18,19] For instance, Zhou *et al*.[20] characterized DEC among children under 5 years of age with acute diarrhea. The study established that the rate of infection with DEC decreases as age advances. However, these studies found EPEC to be the most predominant pathotype, indicating the existence of variations in pathotype prevalence in different settings and populations. The variation in the predominance of Dec pathotypes thus could be influenced by demographic factors including geographical locations.

The reduction in the overall prevalence of pathotypes at the age of 0–12 months is possibly due to the passive transfer of competent immunological correlates like immunoglobulin (Ig) M and IgA via maternal breast milk. This age is characterized by exclusive breastfeeding, which plays a significant role in conferring protection against infections. Mothers' milk has been established to strengthen immunity and is hence necessary for providing crucial protection against DEC infection. Further, this study presumes the possibility that children that are exclusively breastfed could have reduced risk of direct contamination from water used, utensils and food among other possible sources of infections. Several studies have established that the host's susceptibility to infection is dependent on the age of the child and other protective maternal factors, such as transplacental antibodies (secretory IgG) and human milk (lysozyme, oligosaccharides, and lactoferrin).[21,22] As soon as breastfeeding is withdrawn (13–24 months), the child becomes highly susceptible to DEC infections. This susceptibility does not last for long. As the immune factors become decreased (postbreastfeeding), and the adaptive immunity of the child begins to become more elaborate, the child's immune system progressively becomes more competent in fighting infections associated with DEC with age. Thus they observed a decline in the number of infections for age groups that is 37 to 48 and 49 to 60 months. This observation is, therefore, well demonstrated in the current study.

High prevalence of DEC strains among subjects from urban areas as compared to rural populations was observed. However, the EIEC pathotype shows a high prevalence in both urban and rural settings. In a recent study, Garrine *et al*.[23] demonstrated the predominance of EIEC among children under the age of 5 years in rural Mozambique. This proves the possibility of EIEC being influenced by several factors. For example, economic activities that include animal husbandry might play a central role in the transmission of DEC pathotypes in rural settings through the contamination of important water points. In urban settings where human interactions are notably high, the number of DEC infections is therefore on the rise. However, the rationale behind these assertions is yet to be well elucidated.

Furthermore, the high prevalence of DEC strains among the urban population could be attributed to several factors, including population pressure that leads to scarce sanitation services. Population pressure could play a significant role in infection progression.[24] Several studies have cited population pressure as one of the factors implicated in DEC infections. In a study by Taneja and Sharma,[25] poor water quality, lack of rigorous infection management practices, and inappropriate disposal of human waste are the leading cause of infectious pathogens. Similarly, both the rural and urban areas are characterized by a lack of sanitization and a safe water supply for the population thus introducing risk factors for the transmission of infections. Sambe-Ba *et al*.[26] also observed that enteric infections such as DEC are mostly reported in urban settings. They established, however, that seasonality characterized by rainfall patterns has a significant influence on the infection progression. Since the sampled area around the urban setting, Nakuru and its outskirts experience frequent episodes of convectional rainfall between 700 mm and 2000 mm annually because of its proximity to Lake Nakuru,[27] the infection patterns could be attributed to such occurrence of rainfall.

Conversely, the rural areas around the Nakuru area receive moderate rainfall because of their distance from Lake Nakuru, an excellent source of convection rainfall around the urban settings. The findings from this study are consistent with those from the studies mentioned above. Boru *et al*.[28] sampled an urban population of children between 2 and 5 years of age in one of the business hubs of Kenya's capital (Kenya) and found that ETEC accounts for the highest number of reported diarrheal cases in most of the urban settings. However, in the current study, EAEC continues to be the predominant isolate among urban settings. These findings are consistent with several studies.[29-31]

Corroborating these findings, seasonality also influences the transmission patterns of the DEC pathotypes. In general, the rainy season accounted for most of the DEC prevalence. In various places in Nakuru County, there are excessive surface runoffs due to the rugged terrains.[32] These surface runoffs contribute to possible contamination of water sources that potentiate the transmission of DEC during the rainy season. However, contrary to the findings of this study, Shah *et al*.[33] showed that the prevalence of bacterial pathogens in central Kenya (Kiambu County) was high during the dry season. Nonetheless, there is indisputable evidence that DEC infections peaked during the rainy season.[34] This study thus possibly rules out that most cases presenting diarrhea do not necessarily construe rotavirus infections, which are most prevalent during the age of 5 years and below.[35]

This study also investigated the role of socioeconomic factors such as income, occupation and the level of education of the parents. These factors play a pivotal role in the transmission of pathogens. Interestingly, as the level of education advances, there is a general increase in the number of DEC pathogens. EAEC still dominates the prevalence with regard to the level of education. The rest of the pathotypes show a general trend in the patterns of infections. As an individual becomes more literate, the quest to look for jobs in urban centres increases. As mentioned above, there is a high prevalence among individuals living in urban settings compared to those in rural settings thus increasing the likelihood of infections among the populations within such settings.[20]

Contrary to this observation, the caregiver's occupation shows a strikingly discordant trend in the transmission pattern of DEC pathotypes. Among all the pathotypes, wage-earners show the least transmission of pathotypes. This could be attributed to the quality of life and medical attention the children are given by parents with constant income. However, for the self-employed, the highest prevalence could be attributed to several factors, including long working hours and less attention from parents to the children. Self-employed solely rely on what they generate through business and other activities such as farming. These activities shift the attention of the parents to their children and thus making the children more vulnerable to infections. These findings are consistent with a study by.[34]

Finally, the clinical information on the duration of diarrhea shows a slight difference among children who had episodes that lasted <14 days and that that lasted more than 14 days. However, diarrhea lasting more than 14 days had shown a lower rate of DEC infections. This observation is consistent with the report by Omore R *et al.*[35] They reported the highest prevalence of diarrhea lasting <7 days. Nonetheless, it is imperative to always ascertain the pathogen type with persistent diarrhea every time there is a presentation of diarrhea among children under the age of 5 years to rule out the existence of any other type of diarrhea other than that caused by DEC.

In general, this study shows that the EAEC pathotype was found predominant across all the demographic and clinical factors presented by the study. These results are consistent with the recent study by Webale *et al*.[36] and Iijima *et al*.[10] who explored the epidemiological patterns of antimicrobial-resistant DEC isolates and other bacteria in Kenya. Another study by Nyanga *et al*.[37] showed a similar trend in pathotype distribution. Several other studies also support these findings.[18] However, the majority of the studies have shown the predominance of the EPEC pathotype. For instance, Ori *et al*.[38] observed that the frequencies of DEC pathotypes are EPEC > EAEC > ETEC > EIEC. Thus, there is an existence of variability in the patterns of prevalence among the DEC isolates depending on different socioeconomic and demographic factors. Hence, the occurrence of DEC strains within a specific region is possibly influenced by prevailing factors such as climate and anthropogenic factors. Most of the studies done in Kenya tend to have the same trend. Evidently, region seems to have a greater influence on the existence and prevalence of DEC strains. It is imperative to mention that the epidemiology of the DEC is largely dependent on the geographical locations, techniques used in the detection, patient's age, gender and socioeconomic status what are your findings on these issues,[39] therefore the existence of the observed inconsistency in the prevalence of the pathotypes.

Conclusions

The findings of this study established the highest prevalence of the EAEC pathotype as compared to the rest of the pathotypes. Also, EIEC showed the lowest prevalence. Thus, the existence of pathogenicity of DEC-associated diarrhea could greatly be influenced by the presence of EAEC. In addition, the study concludes that the distribution of DEC pathotypes is largely dependent on the region, occupation, employment status, seasons, gender, age, and place of residence, with age being the most associative factor.

Data availability statements

The data presented in this study are openly available in (Havard Dataverse) at (https://doi.org/10.7910/DVN/IIGIAH), (CCO 1.0).

Table 1 shows the distribution of DEC pathotypes based on various subjects' sociodemographic characteristics. Some of the characteristics assessed include sex, age, place of residence, and guardian's occupation. Young age, male sex, rainy season, and living in crowded areas have shown significant association with the transmission of DEC pathotypes.

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Conflicts of interest

There are no conflicts of interest.

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