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Study of *Escherichia coli* as a Cause of Diarrhoea in the Ashanti Region of Ghana

Frank Awuah, MSc
Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
Abetifi Presbyterian College of Education, Abetifi Kwahu
Mathew Glover Addo, PhD
Linda Aurelia Ofori, PhD
Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

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Abstract

Even though diarrhoeagenic *Escherichia coli* (*E. coli*) is accepted worldwide as essential diarrhoea-causing agent, especially among children, routinely they are not sought as a stool sample pathogen in many microbiological laboratories. The conventional biochemical means are not able to differentiate *E. coli* which is a normal flora in the gastrointestinal tract from the pathogenic strains. This research work sought to detect and identify diarrhoeagenic *E. coli* in the Ashanti region of Ghana by using conventional multiplex PCR. A convenient sampling method was employed to select a total of 502 patients from Ashanti Regional and St. Michael's Hospitals for the study. In all, five pathotypes of diarrhoeagenic *E. coli* were detected with enterotoxigenic *E. coli* (ETEC) as the most frequent. The target genes considered for each group of diarrhoeagenic *E. coli* were eaeA (*E. coli* attaching- effacing) and bfpA (bundle forming pilus A) for enteropathogenic *E. coli* (EPEC), elt and Stla for enterotoxigenic *E. coli* (ETEC), ial for enteroinvasive *E. coli* (EIEC), CVD432 for enteroaggregative *E. coli* (EAEC), and hlyA for enterohaemorrhagic *E. coli* (EHEC). Generally, some of the participants (31.08% = n/N= 69/222) exhibited symptoms of diarrhoea while

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others did not (68.92% = n/N =153/222), although they were positive for the pathotypes. The virulence factors considered were seen as contributory factors to the symptomatic situations. Participants in the ≤5 and ≥ 42-year groups were seen to be more vulnerable to diarrhoea. Additionally, it was discovered that men were more susceptible to diarrhoea than women. The study therefore recommends more studies into the ≥ 42-year group not neglecting that of children. In addition, this current study suggests the routine utilization of molecular methods such as conventional multiplex PCR to identify and detect the pathotypes of *E. coli* causing diarrhoea.

Keywords: *E. coli*, diarrhoeagenic *E. coli*, diarrhoea, virulence factors, pathotypes

Introduction

Diarrhoea is a pathogenic disease that can affect individuals of all ages (Schiller, 2019). It is the second leading killer of children, and nearly one in every five children under the age of five dies as a result of dehydration, weakened immunity, or malnutrition associated with diarrhoea (UNICEF/WHO, 2012). Ahs et al. (2010) observed that diarrhoea due to infection is a major disease. Although, studies have led to a lot of progress in producing drugs against infectious agents, bacteria, viruses, fungi, and parasites, Florez et al. (2018) opine that infectious diseases cause by these organisms are still a major cause of socio-economic disturbances, disability, and death for millions around the world. In lower middle-income countries, of all medical conditions, diarrhoea is the second leading cause of healthy time lost to illness (72. 8 million Disability Adjusted Life Years (DALYs)), and dehydration as a result of diarrhoea in 1.8 million death every year (Ahs et al., 2010; WHO, 2008). Even currently, the top 10 disorders that cause DALYs worldwide include diarrhoea (Behera and Mishra, 2022) and 1.8 million individuals in developing nations pass away annually from diarrhoeal diseases (Demissie et al., 2021).

Diarrhoeagenic *E. coli* is the leading cause of bacterial pediatric diarrhoea in developing regions (Canizalez-Roman, 2016; Msolo et al., 2020). Predominantly, *E. coli* is a normal flora in the gastrointestinal tract but in a situation of an immunocompromised and immunosuppressed human host or when the mucosal barrier between the gut and other normally sterile sites of the body is violated, even the normal nonpathogenic *E. coli* tend to be opportunistic (Yang et al., 2019). In such a situation, the patient is not able to limit this avirulence *E. coli* in their natural habitat.

Even so, certain *E. coli* strains have evolved adaption to cause a broad spectrum of human diseases in a more robust human host (Pakbin et al., 2021). Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or

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can disseminate throughout the body. Urinary tract infections, sepsis or meningitis, and enteritis or diarrhoea are the three broad clinical syndromes due to infection as a result of strains of inherently pathogenic *E. coli* (Soltani et al., 2018; Zeljković, 2018).

Possession of fimbriae which has the property to adhere to surfaces is a characteristic feature of almost all *E. coli* strains (Schiller et al., 2020; Esteban-López, 2020). This means that even the nonpathogenic *E. coli* is no exception in this case. Even so, the *E. coli* strains that cause diarrhoea elaborate certain specific fimbriae antigens. This promotes their colonization and adherence to sites that are mostly not colonized like the small intestinal mucosa (Damalanka et al., 2021; Milton et al., 2021).

The ability of *E. coli* strain to cause disease varies with their specific virulence factors which include adhesins, invasins, toxins, and capsules (Croxen et al., 2010;Sarowska et al., 2019). Genes responsible for the virulence are either on the chromosome (e.g.pathogenicity islands), on large plasmids, or phages, and these can be transmitted horizontally between strains (Leimbach et al., 2013).

In underdeveloped countries, *E. coli* which has acquired the ability to cause diarrhoea is the most common bacterial cause of juvenile diarrhoea (Canizalez-Roman, 2016; Msolo et al., 2020). Diarrhoeagenic strains can be divided into at least five different categories with corresponding distinct pathogenic schemes dictated by their respective virulence genes (Bugarel et al., 2011; Prah et al., 2021). Therefore, it is possible to distinguish and identify the different pathotypes of diarrhoeagenic *E. coli* by using these virulence genes by genotyping. Taken together, these organisms probably represent the most common cause of diarrhoea worldwide among all ages (Jesser and Levy 2020). These strains of diarrhoeagenic *E. coli* can be distinguished by their respective pathogenesis which is encoded by their respective virulence genes. These among others are mainly enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC).

Tetteh et al. (2018) reported from the UNICEF Ghana document published in 2016 that the diarrhoea disease burden in Ghana is overwhelming and estimated that over 300,000 children under-five died from diarrhoea. Peprah et al. (2016) observed that, in 2011, Ghana recorded average annual diarrhoeal cases of 2,218 per 100,000 populations for children under-five with the Ashanti region recording the third highest. Prah et al. (2021), have reported on the virulence profile of diarrhoeagenic *E. coli* in the Western Region of Ghana but this study attests to the fact that there is variation in the distribution of the pathotypes of diarrhoeagenic *E. coli* even when addressing the same country. Despite this, not much has been reported on the pathotypes of diarrhoeagenic *E. coli* in the Ashanti Region of Ghana. This paper, therefore,

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seeks to detect and identify diarrhoeagenic *E. coli* in the said Region of the Republic of Ghana.

2. Materials and Methods

2.1 Sampling Procedure

The binomial model, No = [Z2 (P) (1-P)] /(d)2 was applied to predict the minimum sample size of 379 for this study where N = sample size, P = prevalence, q = 1 – p and d (error) is the precision level (0.05). Z is the critical value of the binomial distribution at the 5% level (1.96). Between July 2020 and July 2021, a total of 502 stool specimens were collected from the patients who attended Ashanti Regional and St. Michael's Hospitals in the Ashanti Region of Ghana. The age range of patients was from ≤5 to ≥42 years. Diarrhoea was defined as a history of more than one liquid stool per day or three or more stools of loose consistency during the previous 24 hours (WHO, 2017).

2.2 Research Design

A cross-sectional study was conducted to assess the diarrhoeagenic *E. coli* in patients who reported at Ashanti Regional and St. Michael's Hospitals in the Ashanti Region of Ghana. Structured questionnaires were used to obtain information to assess the attitudes, opinions, and practices of the participants concerning the diarrhoeagenic *E. coli* in the St. Michael's and Ashanti Regional Hospitals. In this research, the questionnaires were administered to the selected patients and relatives (in the case of children and critically ill patients). To ascertain how the questionnaires would work and also to find out whether changes were needed, they were pre-tested before the commencement of the actual survey. Based on the findings from the pretest results, these questionnaires were then subjected to revision and finalization.

2.3 Isolation of *E. coli* from the Faecal samples

The stool samples of the Participants were streaked on MacConkay agar. Colonies showing bright pinky-red after overnight culture were presumptive for organisms capable of lactose fermentation such as *E. coli*. One isolated colony each was subcultured on Cystine- Lactose electrolyte deficiency agar (CLED) and incubated at 37℃ for 24 hrs. Yellow colonies were taken as possible *E. coli*. Isolates from the CLED were inoculated into a 5ml test tube of tryptophan broth and incubated at 44℃ for 24 hours. A drop of Kovacs' reagent was then added to the positive tubes of tryptophan broth. After gentle agitation, all tubes that developed red ring color indicated the presence of indole and were identified as possible thermotolerant coliforms (*E. coli*). When an isolate from the indole test was streaked on Eosin Methylene Blue (EMB) agar as confirmation, the colonies with a green

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metallic sheen were identified as *E. coli*. The isolates were then stored in plane tubes containing Mueller-Hinton agar awaiting DNA extraction.

2.4 DNA Extraction and Pathotype Identification

Using the boiling process, genomic DNA was extracted from the isolated bacterial cells, and the template was then exposed to multiplex PCR using specific primers, as described by Hegde et al. (2012). In all, the study made use of two multiplex PCRs for the detection of the genes of interest which were the virulence markers.

2.4.1 Multiplex Polymerase Chain Reaction (PCR) Assay 1

The interest of the PCR 1 was to detect EHEC, EAEC, and ETEC. The optimized protocol was done by using 25µl Ben Taq mixture (Beneficial Bio, UK),12µl of nuclease-free water, 5 µl of the DNA template, 1 µl each of the forward and the reverse of the primers (hlyA for EHEC, CVD432 for EAEC, elt, and Stla for ETEC isolates). In each of the reactions, DNA samples with the relevant virulence gene or genes served as positive controls. However, sterile distilled water was used as a negative control.
Each of the assays was performed by adhering to the optimal cycling condition as illustrated below: 95°C for 1 min for one cycle followed by 35 cycles of 94°C for 1 min, 55°C for I min, 72°C for 1min and72°C for 5 min(Hegde et al. 2012).

2.4.2 Multiplex Polymerase Chain Reaction (PCR) 2

Although the protocol for PCR 2 was just as that of the PCR 1, the primers used in the PCR 2 were eaeA and bfpA for isolates of EPEC and ial for isolates of EIC. Also, 14 µl of nuclease-free water was used in PCR 2 instead of the 12 µl in PCR 1.

In both cases (PCR 1 and PCR 2), the products of the PCR reactions were subjected to gel electrophoresis with 1.5% (W/V) agarose gel in 120mil of buffer solution. The gel was stained with ethidium bromide which is an intercalating dye and therefore made visualization of the DNA bands possible when photographed under UV light.

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Table 1. Primers sequences and their corresponding bands used for the detection of virulence genes of diarrhoeagenic *E. coli*.

Reference strain	Target gene	Primer name	Primers (5′ to 3′)	Product size (bp)	Reference
EPEC	eaeA	eaeA-F	TGATAAGCTGCAGTCGAATCC	229	Hegde, et al., 2012: Detection of diarrhoeagenic *Escherichia coli*
		eaeA-R	CTGAACCAGATCGTAACGGC		
	bfpA	bfpA-F	CACCGTTACCGCAGGTGTGA	450	
		bfpA-R	GTTGCCGCTTCAGCAGGAGT		
ETEC	elt	elt-F	CTCTATGTGCACACGGAGC	322	
		elt-R	CCATACTGATTGCCGCAAT		
ETEC	Stla	Stla-F	TCTTTCCCCTCTTTTAGTCAGTC	170	
		Stla-R	CCGCACAGGCAGGATTAC		
EIEC	ial	ial-F	CTGGTAGGTATGGTGAGG	320	
		ial-R	CCAGGCCAACAATTATTTCC		
EAEC	CVD432	CVD432-F	CTGGCGAAAGACTGTATCAT	630	
		CVD432-R	CAATGTATAGAAATCCGCTGTT		
EHEC	hlyA	hlyA-F	GCATCATCAAGCGTACGTTCC	534	
		hlyA-R	AATGAGCCAAGCTGGTTAAGCT		

Source: Hegde et al. (2012)

3.0 Results

3.1 Frequency and prevalence of diarrhoeagenic *E. coli*

The study recovered 312 (62.15%, n/N =312/502) *E. coli* isolates from the 502 stool samples collected. The frequency of diarrhoeagenic *E. coli* (DEC) was 71.15% (n/N = 222/312) among the *E. coli* isolates and a prevalence of 44.22% (n/N = 222/502) among the study population. Ninety (28.85%, n/N = 90/312) of the *E. coli* isolates tested negative for any of the target genes.

Participants from Ashanti Regional Hospital had DEC prevalence of 44.78% (n/N = 90/201) while that of St. Michael was 43.85% (n/N = 132/301). Statistically, the DEC prevalence in the two hospitals was not significantly different (P = 0.8373).

Generally, 69(31.08%, n/N=69/222) of the participants who tested positive for DEC exhibited symptoms of diarrhoea. This means that 153(68.92%, n/N=153/222) of the DEC isolates came from those without diarrhoea. This is statistically different from those with diarrhoea who were infected with DEC (P < 0.0001).

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Out of the the 90 samples from Ashanti Regional Hospital which tested positive for DEC, 33(36.67%, n/N =33/90) of them were diarrheic. Additionally, 27.27% (n/N = 36/132) of the samples from St. Michael's Hospital which tested positive for DEC were diarrheic. Therefore, there were individuals who tested positive for DEC who were diarrheic, and some individuals who tested positive for DEC but did not exhibit any symptoms of diarrhoea (Table 2). However, there was no statistical difference between the proportion of participants from the Ashanti Regional Hospital (36.67%, n/N =33/90) who suffered from diarrhoea compared with their counterpart from the St. Michael's Hospital (27.27%, n/N =36/132) (P=0.1382). while Table 2 shows the frequency and Prevalence of diarrhoeagenic *E. coli*, Fig. 1 shows an image of PCR results in this study.

Table 2. Frequency and prevalence of diarrhoeagenic *E. coli*

Hospital	Samples collected	EC	DEC	Frequency of DEC (%)	Prevalence of DEC (%)	DEC symp. (%)	DEC not symp.
Ashanti Regional Hospital	201	122	90	73.77 N= 122	44.78 N=201	33(36.67) N= 90	57(63.33) N=90
St. Michael's Hospital	301	190	132	69.47 N= 190	43.85 N= 301	36(27.27) N= 132	96(72.73) N=132
Total	502	312	222	71.15 N= 312	44.22 N= 502	69(31.08) N= 222	153(68.92)

Symp. = symptomatic, DEC= diarrhoeagenic *E. coli*, EC = *E. coli*

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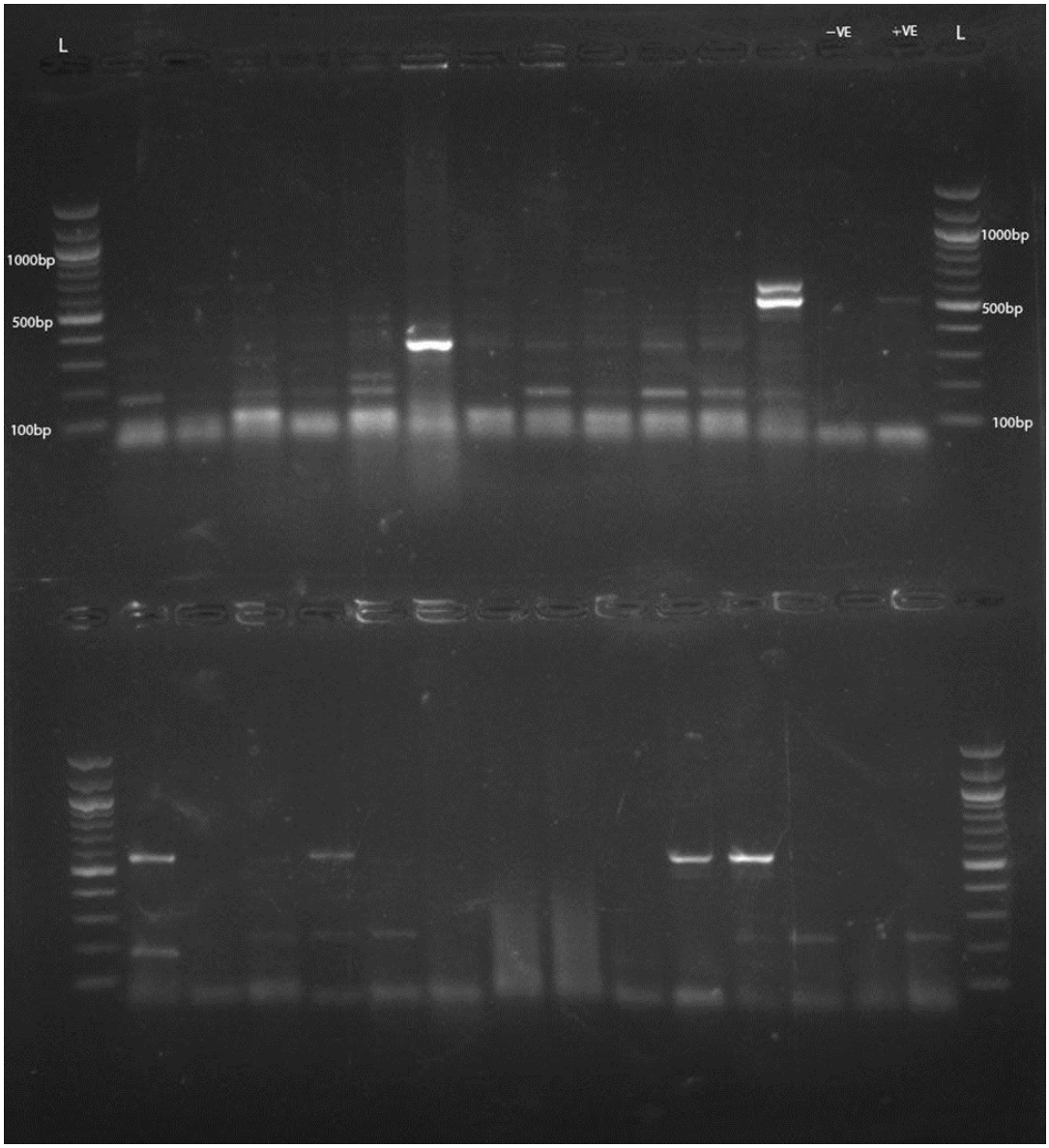

L = DNA molecular size marker((100bp ladder), -VE = negative control,
+VE = positive control.
Fig. 1. Image showing multiplex PCR Products.

3.2 The target genes, eaeA, bfpA, elt, Stla and ial, CVD432,

The five main pathotypes of diarrhoeagenic *E. coli* were detected in this area under study. The target genes that were employed for each group of diarrhoeagenic *E. coli* were eaeA (*E. coli* attaching- effacing) and bfpA (bundle forming pilus A) for enteropathogenic *E. coli* (EPEC), elt and Stla for enterotoxigenic *E. coli* (ETEC), ial for enteroinvasive *E. coli* (EIEC),

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CVD432 for enteroaggregative *E. coli* (EAEC), and hlyA for enterohaemorrhagic *E. coli* (EHEC). The pathotype with the highest frequency is ETEC, followed by atypical EPEC (aEPEC), which has a frequency of 16.03% (n/N =50/312). The pathotype with the lowest frequency is typical EPEC, EPEC with both eaeA and bfpA (8.33%, 26/312) as shown in Table 3. The pathotype of highest prevalence is ETEC with that of the Stla and elt being 25.23% (n/N = 127/502) and 21.31% (n/N = 107/502) respectively. EPEC with both eaeA and bfpA recorded the least prevalence 5.18%(n/N = 26/502) followed by EIEC (7.17, n/N = 36/502). The study observed that some of the study participants, although they were positive for the pathotypes, did not exhibit symptoms of diarrhoea(Table3).

Table 3. Prevalence and the Frequency of the Pathotypes of the Diarrhoeagenic *E. coli*

Target gene	Pathotype	Number of isolates	Frequency (%)	Prevalence(%)	Symptomatic (%)
eaeA		86	27.56	17.13	23(26.74)
bfpA and eaeA	EPEC	26	8.33	5.18	8(30.77)
bfPA		50	16.03	9.96	30.00%
elt		107	34.29	21.31	26(24.30)
Stla	ETET	127	40.71	25.23	40(31.50)
elt and Stla		59	18.91	11.75	18(30.51)
ial	EIEC	36	11.54	7.17	13(36.11)
CVD432	EAEC	51	16.35	10.16	15(29.41)
hlA	EHEC	58	18.59	11.55	18(31.03)

EPEC = enteropathogenic *E. coli*, ETEC = enterotoxigenic *E. coli*, EIEC= enteroinvasive *E. coli*, EAEC = enteroaggregative *E. coli*, EHEC = enterohaemorrhagic *E. coli*.

3.3 Prevalence of diarrhoea in relation to sex and age of participants

Out of the 502 samples collected, 63.94% (n/N = 321/502) were collected from females (67.91%, n/N= 218/321 being pregnant women) and 36.06% (n/N = 181/502) males. It was observed that 31.67(n/N=159/502) of the entire study participants were diarrheic. More males (38.67%, n/N = 70/181) had diarrhoea compared to females (27.73%, n/N = 89/321) (P = 0.0115).

Additionally, when the total participants of members of each age category in this study are taken into consideration, the results indicate that the highest prevalence of diarrhoea was recorded in the ≤5(44.00%, n/N=40/91). There was no statistically significant variation in the prevalence of diarrhoea between the ≤5 and ≥42 age groups. However, there was a statistically significant difference in the prevalence of diarrhoea between the ≤5 and the

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18–23 age groups with the ≤5year group having the greatest prevalence (P = 0.0271). Also, the statistical disparity between the ≤5 and 24-29-year age groups was significant (P = 0.0008). The disparity in diarrhoea prevalence between the ≤5year group and the 30-35year group is statistically significant (P = 0.0118).

The results from the two hospitals show that participants from Ashanti Regional Hospital (43.78%, n/N =88/201) have a higher prevalence of diarrhoea than those from St. Michael's (23.59%, n/N =71/301). Statistically, the difference between the two prevalences was significant(P<0.0001). Table 4 displays the prevalence of diarrhoea in connection to participant demographics of the two hospitals and the total prevalence.

Table 4. Prevalence of diarrhoea

Variable	Samples (N)	Prevalence of diarrhoea %(n/N)		
		Ashanti Regional Hospital	St. Michael Hospital	Total prevalence
SEX				
Female	321	38.52(47/122)	21.11(42/199)	27.73(89/321)
Male	181	51.90(41/79)	28.43(29/102)	38.67(70/181)
AGE (yrs.)				
≤5	91	59.46(22/37)	33.33(18/54)	44.00(40/91)
6-11	26	50.00(7/14)	16.67(2/12)	34.62(9/26)
12-17	28	37.50(3/8)	25.00(5/20)	28.57(8/28)
18-23	64	37.5(8/22)	21.43(9/42)	26.56(17/64)
24-29	92	23.40(11/47)	17.78(8/45)	20.65(19/92)
30-35	49	33.33(4/12)	18.92(7/37)	22.45(11/49)
36-41	38	50.00(5/10)	21.43(6/28)	28.95(11/38)
>42	114	54.90(28/51)	25.40(16/63)	38.60 (44/114)
Total	502	43.78(88/201)	23.59(71/301)	31.67(159/502)

3.4 Diarrhoeagenic *E. coli* pathotypes (virulence markers) in the various age groups

All the virulence markers, eaeA, bfp, elt, Stla, elt, ial, CVD432 and hly which served as the target genes for the molecular identification of the pathotypes of the DEC considered were detected in this study. Among these markers of the DEC type, stla was the highest in all the age categories except eaeA which was the highest in the 24-29 age group.

The distribution of the DEC virulence markers(pathotype) regarding the various age groups in this study is higher with respect to the age group 24-29, followed by the age group ≥42 and the age group ≤5. The least distribution was seen in the 12-17 year category. The distribution of the virulence markers is presented in Table 5.

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Statistically, the difference in the distribution of the virulence factors (the target genes) among the ages, 24-29 and >42 is not significant (P = 0.9475). Also, there was no statistical difference in the distribution of these target genes between the 24-29 and the ≤5 age groups (P = 0.3800). This is in spite of the fact that higher cases of diarrhoea was recorded among the ≤5 and the ≥42 years compared to the 24-29 age groups (Table 4).

Even though males had a greater prevalence of diarrhoea than females (Table 4), the difference in the distribution of the virulence markers by sex was significant, with females having the highest (p = 0.0001).

Table 5. The distribution of diarrhoeagenic *E. coli* virulence factors(pathotypes) among the various age groups

Variable	eaeA (%)	eaeA and bfpA (%)	bfpA (%)	elt (%)	Stla (%)	elt and Stla (%)	Ial (%)	CVD432(%)	hlA (%)	Total (%)
Age (yrs.)										
0-5	23(26.74)	7(26.92)	11(22.00)	20(18.69)	24(18.900)	10(16.95)	9(25.00)	9(17.65)	10(17.24)	123(20.50)
6-11	2(2.33)	0(0.00)	4(8.00)	6(5.61)	5(3.94)	3(5.08)	3(8.33)	5(9.80)	2(3.44)	30(5.00)
12-17	3(3.49)	2(15.38)	3(6.00)	3(2.80	8(6.30)	2(3.39)	2(5.56)	1(1.96)	0(0.00)	24(4.00)
18-23	5(5.81)	1(8.85)	3(6.00)	10(9.35)	17(13.39)	6(10.17)	3(8.33)	4(7.84)	7(12.07)	56(9.33)
24-29	28(32.56)	9(34.62)	14(28.00)	28(26.17)	23(18.11)	11(18.64)	8(22.22)	16(31.37)	13(22.3 1)	150(25.00)
30-35	4(4.65)	1(8.85)	3(6.00)	7(6.54)	11(8.66)	6(10.17)	0(0.00)	1(1.96)	6(10.34)	39(6.50)
36-41	2(2.33)	0(0.00)	1(2.00)	6(5.61)	7(5.51)	5(8.47)	2(5,56)	2(3.92)	5(8.62)	30(5.00)
>42	19(22.09)	6(23.08)	11(22.00)	27(25.23)	32(25.20)	16(27.12)	9(25.00)	13(25.49)	15(25.86)	148(24.67)
Total	86	26	50	107	127	59	36	51	58	600
Sex										
Male	37(43.02)	14(53.85)	25(50.00)	37(34.58)	44(34.65)	20(33.90)	16(44.44)	25(49.02)	16(27.59)	234(39.00)
Female	49(56.98)	12(46.15)	25(50.00)	70(65.42)	83(65.35)	39(66.10	20(55.56)	26(50.98)	42(72.41)	366(61.00)
Total	86	26	50	107	127	59	36	51	58	600

Discussion

Considering the total sample size of 502, 159 of them were symptomatic of diarrhoea and hence a prevalence of 31.67% (159/502). This prevalence is higher than the 27.22%, 22.1% and 17.0% prevalence of diarrhoea observed by Tetteh et al. (2018) in the Volta region of Ghana, Getachew et al.(2018) in the North Gondar zone of Ethiopia and Apanga and Kumbeni (2021) cross-sectional survey of Ghana respectively. These disparities in prevalence may be brought on by variations in the local environmental conditions in the research areas such as contaminated water, inadequate sanitation, and unsanitary settings, in addition to malnutrition and subpar food hygiene practices. This prediction by this study is necessitated by the fact that according to estimates, the environment is responsible for ninety-four percent of the burden of diarrhoeal disease and is linked to risk factors such as unclean drinking water, low socioeconomic level, inadequate sanitationand and poor hygiene (Prüss-Üstün et al., 2007; Pruss-Ustun et al., 2006). The high prevalence of diarrhoea in low socioeconomic areas is the result of exposure to environmental factors that cause diarrhoea such as contaminated water, poor sanitation, and unhygienic conditions in addition to malnutrition and poor food-hygiene practices (Agustina et al., 2013).

One sample could yield both lactose-fermenting *E. coli* and non-lactose-fermenting *E. coli* giving rise to more than one isolate. This observation is supported by Yaratha et al. (2017) who stated that 10% of *E. coli* isolates have historically been reported to be sluggish or non-lactose fermenting, despite the fact that *E. coli* are facultatively anaerobic, Gram-negative bacteria that will ferment lactose to make hydrogen sulfide.

Out of the 502 samples, 312 tested positives for *E. coli*. From the PCR results, the frequency of DEC in the total isolate is 71.15% (n/N= 222/312) with a prevalence of 44.22%. (222/502). Ninety (90) of the *E. coli* isolates tested negative for any of the target genes. This can be explained by the fact that in the digestive system, *E. coli* is primarily part of the natural flora, and hence the fact that the biochemical test detected 312 in the samples collected does not mean that they were necessarily pathogenic or diarrhoeagenic (Basu et al., 2020). *E. coli* is typically a normal component of the gut flora, but it can become opportunistic when the immune system of the human host is compromised, the immune system is suppressed, or the mucosal barrier between the gut and other normally sterile sites of the body is breached (Fossen, 2019).

All five main pathotypes of diarrhoeagenic *E. coli* were detected in the area under study. This is in support of the assertion that there are five main pathotypes of diarrhoeagenic *E. coli* (Tareen et al., 2022; Redha et al., 2022). The study observed that some of the study participants exhibited symptoms of diarrhoea while others did not show any signs of diarrhoea although they were

positive for the pathotypes proved by the presence of the virulence factors (Tables 2 and 3). This finding of the present study agrees with the assertion that an individual can habour DEC without exhibiting any signs of being infected with the pathogen (Gautam 2021; Hatyoka et al., 2022). Host immunity might be attributed to the situation in which an individual is infected with the pathogen but does not experience the disease caused by the pathogen (Subekti et al.,2003; Donnenberg et al.,2013; Rainard et al.,2020). This finding of the research suggests that the diarrhoeagenic *E. coli* may have coexisted with the people in this area of the current research for a very long time, and as a result, immunity has evolved as a means of adapting to survive the presence of this pathogen.

The rate of recovery of the DEC was higher in females than in males (Table 5) with the difference being statistically significant ($P<0.05$). This can be attributed to the fact that normally females interact more with children, food, and meat during food preparation(Pelto and Armar-Klemesu, 2011; Abokyi et al.,2023). Most of the females in this present study were pregnant women who attended hospitals for medical review. The would-be babies of these pregnant women are potentially at risk of being infected with DEC hence the tendency of suffering from diarrhoea.

Considering the frequency of the virulence markers (DEC pathotypes), it is observed that, 127(40.71%) of the 312 *E. coli* isolates tested positive for Stla, 107(34.29%) were elt, 86(27.56%) were eaeA, and 58(18.59) were hlA. 50(16. 03) were bfpA, 51(16.35%) were CVD432, and 36(11.54) were ial. 26(8.33%) were positive for bfpA and eaeA combined. Table 3 illustrates that both Stla and elt were target genes for ETEC. This means that the most highly detected diarrhoeagenic *E. coli* prototype as discovered by this research is ETEC. In the work done by Prah et al. (2021) in the Western Region of Ghana and Yadav et al. (2020), among the pathotypes of diarrhoeagenic *E. coli* studied, ETEC was the one with the highest number of occurrences.

This study reveals that although Stla and elt were target genes for ETEC, there are instances that some isolates were detected with Stla but not elt and vice versa (Table 3). LT operons (elt or etx) are located on the plasmid which may also contain genes encoding ST operon, stla (Kim, 2020 and van, 2017). It can therefore be deduced from this study that ETEC elaborates Stla or elt and in some situations, both. ETEC is defined as containing the *E. coli* strains that cause diarrhoea through the activity of Heat-labile toxin (LT) and or Heat-stable toxin (ST) producing virulence genes. This pathotype (virotype) of *E. coli* may elaborate only an LT, and ST only, or both (Fleckenstein et al., 2010; Fleckenstein, 2013).

Also, eaeA and bfpA were target genes for EPEC(Table 3). Some isolates revealed the presence of eaeA (aEPEC) with the absence of bfpA and some possessed both eaeA and bfpA (tEPEC). The study, therefore, reveals

that there are strains of EPEC containing either eaeA only or some with both eaeA and bfpA. This is in concordance with the finding that EPEC is subdivided into typical EPEC (tEPEC), which carries both the intimin gene (eae) and the bundle forming pili (bfp) genes, and atypical EPEC (aEPEC) which carries eae gene but lacks the bfp gene (Khairy et al., 2020). Again, 86 isolates tested positive for eaeaA and 26 isolates revealed the presence of both eaeA and bfpA. This means that there were more atypical EPEC (aEPEC) than typical EPEC (tEPEC) circulating in the area under study. Devi et al.(2018) observed that, of the EPECs identified according to the specific genotypes, atypical EPECs which have only eaeA were more than typical EPECs which have both eae and bfpA genes.

It must also be pointed out that this present study detected strains of EPEC with only bfpA without eaeA. This is in agreement with Prah (2021) who has revealed that there are strains of EPEC with only bfpA and not eaeA in the Western Region of Ghana. This study, therefore, recommends that EPEC with only bfpA should be included in the classification.

Additionally, the results indicate that more samples (44.00%, n/N=40/91) and 38.60% (44/114) respectively, came from people in the ≤5 and ≥42 age groups who have diarrhoea more frequently than those in the younger adult range of 18–23, 24-29 and 30-35 with the prevalence being 26.56% (n/N = 17/64), 20.65%(n/N = 19/92), 22.45%(n/N = 11/49) respectively. There was no statistically significant variation in the prevalence of diarrhoea between the ≤5 and ≥42year age groups($P>0.05$). There was however, a statistically significant variation in the prevalence of diarrhoea between the ≤5 and the 18–23, 24-29, and 30-35 age groups ($P < 0.05$) with the ≤5 age category having the highest prevalence.

The association between age group and diarrhoea is therefore a zig-zag type. This can be explained by the fact that the proportion of individuals suffering from diarrhoea among the various age groups is higher among the children category especially those within the ≤5 years but decreases among the young adult category (18-23-, 24-29- and 30–35-year groups) and increases again as the age increases (≥42 age group) as illustrated in Table 4. This means that comparatively, greater percentages of the participants in the age groups ≤5 and ≥ 42 respectively exhibited symptoms of diarrhoea more than those within the umbrella of 18-23-, 24-29- and 30–35 (young adult category) age groups. Generally, the distribution of the DEC is highest in the 24-29, ≤5, and ≥42 age groups (Table 5). According to statistics, there is no change in the distribution of the virulence factors (the target genes) between individuals aged 24-29, ≤5, and ≥42 ($p >0.05$). This situation is dangerous as all things being equal, children are normally the ones with weaker immunity compared to the young adult year groups (Ciarambino et al., 2021; Galletti and 2021; Brink et al., 2021). This might explain the reason for the highest

number of participants in the ≤5-year group exhibiting symptoms of diarrhoea. A higher percentage of the ≥ 42-year group exhibits symptoms of diarrhoea is so because the immune system is suppressed as one grows older (King and Londrigan 2021; Ciarambino et al., 2021). This study therefore supports the assertion that diarrhoeagenic *E. coli* is an important cause of diarrhoea in children (Angulo-Zamudio et a., 2021; Zhou et al., 2021; Emami et al., 2021; Manhique-Coutinho et al., 2022;) but also reveals an important finding that the ≥42year group is also very vulnerable to diarrhoea when infected with DEC, although many studies on diarrhoea have shifted more emphasis on children (Manhique-Coutinho et al., 2021; Tareen et al., 2022).

Although there was a large distribution of DEC pathotypes in the 24-29 age group, similar to the ≤5 and ≥42 age groups, there were statistically fewer cases of diarrhoea in this 24-29 age group ($p>0.05$). This can be explained by the possibility that someone between the ages of 24-29 (young age group) has a better immune system than those in the ≤5 and over-42 age groups. The young are more able to produce B and T cells in their bone marrow and thymus glands respectively, and also have effective lymphocyte activity in their secondary lymphoid tissue. Hence, they react to immune challenges more robustly than the elderly (Montecino-Rodriguez et al., 2013). People in the 24–29 year age group are, on average, younger than those in the ≥ 42-year group; as a result, the ≥42 year group may need fewer pathogens, such as diarrhoeagenic *E. coli*, to cause an infection like diarrhoea considered in this study. Therefore, even though the pathogen (diarrhoeagenic *E. coli*) is more widely distributed in the younger age than in older adults, as this research found, it makes sense in a situation where the prevalence of diarrhoea becomes higher in an older adult (≥42 years) than among the younger adults (24-29). Therefore, this recent research suggests that age might be a risk factor for diarrhoea. This assertion is in agreement with several other findings that diarrhoea risk is affected by age (Gupta et al., 2015; Genser et al., 2006; Gebbia et al., 2023).

Conclusion

The results of this study show that all five pathotypes of diarrhoeagenic *E. coli* are in circulation in this region of Ghana and provide light on the significant virulence features that may raise public health concerns. The study observed that some of the study participants exhibited symptoms of diarrhoea while others did not show any signs of diarrhoea, although they were positive for the pathotypes proved by the presence of the virulence factors.

The virulence factors considered in this study might be a contributory factor to the diarrhoea episode observed. The children in the ≤5 and ≥ 42-year groups are more vulnerable to diarrhoea when infected with DEC. There are strains of EPEC with only bfPA without eaeA.

The study, therefore, recommends more studies on diarrhoea in the ≥ 42-year group not neglecting that of the children (≤5 year group) category. There is a need for routine utilization of molecular methods such as the conventional multiplex PCR to identify and detect the pathotypes of *E. coli* causing diarrhoea. Also, there should be an inclusion of EPEC with only bfpA and not eaeA in the classification of EPEC.

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Ethical Approval

This study has been approved by committee on human research publication and ethics of Kwame Nkrumah University of Science and Technology and the principles of the Helsinki Declaration were followed.

Conflicts of Interest: The authors declare that they have no conflict of interest in the publication.

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