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DIARRHEAGENIC *ESCHERICHIA COLI* AMONG STUDENTS IN THE ASHANTI REGION OF GHANA

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Abstract – *Objective:* Diarrhea-causing *Escherichia coli* (*E. coli*) is a significant etiological agent that is always evolving due to its genomic plasticity, necessitating its routine surveillance. However, diarrheagenic *E. coli* (DEC) among Ghanaian students remains uncharacterized. This current study focused on characterizing the DEC pathotypes recovered from the stool samples of students with and without diarrhea seeking health care from the Ashanti region of Ghana.

Patients and Methods: There were 119 students who participated in this research. A structured questionnaire was utilized to gather the data. *E. coli* was isolated from participant stool samples using a biochemical method. DEC was distinguished from the *E. coli* isolates using a two-multiplex Polymerase Chain Reaction (PCR).

Results: The study detected all the five main pathotypes of DEC with the enterotoxigenic *E. coli* (ETEC), which elaborate only heat-labile enterotoxin gene, elt, or heat-stable enterotoxin gene, *stla* being most frequent. When comparing individuals with diarrhea to those without it, it was shown that ETEC with simply *elt* or *stla* was statistically significantly lower in cases of diarrhea. In general, there was no statistically significant difference in the likelihood of any pathotype producing diarrhea between students who experienced diarrhea and those who did not.

Conclusions: The study has provided insight into the five main pathotypes of diarrheagenic *E. coli* among students in the Ashanti region of Ghana, which calls for monitoring by public health authorities. This current study suggests that more research be done in this field on the host immunological response to DEC infection.

Keywords: Students, Pathotypes, *E. coli*, Diarrheagenic *E. coli*, Diarrhea.

INTRODUCTION

One of the most prevalent health issues, particularly in the case of younger children, is diarrhea.

Although diarrhea is preventable, it is still the third most common cause of death among children aged 59 months and less, with approximately 443,832 children under the age of five and 50,851 children between the ages of five and nine dying from it each year[1].

Children under three years old in low-income nations often have three episodes of diarrhea per year[2]. This can potentially make the youngster lose the nourishment required for development with every episode. As a result, diarrhea is a possible contributor to malnutrition, and since undernourishment tends to impair immunity, undernourished children are more vulnerable to diarrheal illness.

One of the illnesses that rank among the top five in Ghana for morbidity and death in children under five is diarrhea[3]. In a study to determine the prevalence of diarrhea in the Ashanti region of Ghana[4], it was observed that 31.67% of the entire study participants were diarrheic. Even though most studies on diarrhea have limited themselves to children, especially those under five, it should be noted that diarrhea affects people of all ages[5]. The likelihood of contracting diarrheal sickness can be greatly influenced by the environment to which an individual is exposed[6-9]. Students are among the groups of individuals who are more likely to experience diarrhea, according to a previous study[10]. This threat will probably cost these students some valuable time, which will interfere with their ability to complete their academic work.

Addressing the root cause of the high prevalence of diarrhea among these students is necessary to resolve the diarrhea issue. It is a well-known fact that diarrhea is caused by infectious organisms, such as bacteria, among other etiological agents, with the majority of these organisms transmitted from one person to another by the fecal-oral route[11]. Although several studies conducted globally have found that rotavirus is the main cause of acute pediatric diarrhea, the role of bacteria in diarrhea seems to differ depending on the location[12-14]. Alhaji et al[15] asserted that the most common cause of disorders associated with diarrhea is *E. coli*, among other things. Despite the reality that *E. coli* makes up most of the facultative flora in the human gut that is non-pathogenic[16], certain *E. coli* strains have developed adaptations to infect and harm the urinary tract, central nervous system, or gastrointestinal tract, even in the most resilient human hosts. There are at least five main categories of *E. coli* strains that cause diarrhea, and each has its own distinct pathogenic scheme[17]. According to some research[18,19], there may still be more types of diarrheagenic *E. coli*. Since *E. coli* is a potential fecal indicator, its presence may also indicate the presence of other etiological agents. Nevertheless, not many papers have been published on diarrheagenic *E. coli,* which has the potential to cause a high frequency of diarrhea in the students under investigation. Hence, the purpose of this study is to look into diarrheagenic *E. coli* that causes diarrhea in students in this research region.

MATERIALS AND METHODS

Identification of Bacterial Strains

This study used *E. coli* isolates stored at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR). These samples were collected between July 2020 and July 2021 from both symptomatic and asymptomatic patients with diarrhea who consented to this study. These patients sought health care at the Ashanti Regional and St. Michael's Hospitals of the Republic of Ghana. Samples were taken from patients who had not initiated any antibiotic treatment for the past month at the time of sampling. Suspected *E. coli* isolates stored in sample tubes containing Mueller-Hinton agar were subcultured on 5% sheep blood agar and incubated in 5% CO_2 at 35-37^{o}C for 18-24 h.

Preparation of Template DNA and Identification of Pathotypes of Diarrheagenic *E. coli*

Genomic DNA was extracted by employing the boiling method[20]. Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enterohaemorrhagic *E. coli* (EHEC) were detected by multiplex polymerase chain reaction (PCR). Target genes: *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) were taken into consideration. Primers and procedures as described by[21] were utilized to analyze these target genes.

Statistical Analysis

The findings were summarized using descriptive statistics. When suitable, tables were utilized to display frequencies.

TABLE 1. DISTRIBUTION OF DIARRHEAGENIC *E. COLI* AMONG STUDY PARTICIPANTS.

Hospital	Sample	EC	DEC	Prevalence of DEC/%	DEC symptomatic	DEC not symptomatic	No DEC symptomatic
Ashanti Regional Hospital	64	42	35	54.67	13	22	15
St. Michael's Hospital	55	34	24	43.64	6	18	9
Total	119	76	59	49.58	19	40	24

EC = *E. coli*, DEC = diarrheagenic *E. coli*.

The Chi-square test was used to determine statistical differences between categorical variables and to determine their association[22,23]. *p*-values were used to evaluate the results and determine the significance level statistically. Significant *p*-values were defined as those with a 95% confidence level of less than 0.05. Fisher's Exact Probability statistics were used to get the odds ratio.

RESULTS

Prevalence of Diarrheagenic *E. coli*

A total of 76 *E. coli* isolates were recovered from 119 students seeking health care at the Ashanti Regional and St. Michael's Hospitals. The prevalence of DEC among these participants was 49.58%. It was observed that 32.20% (n/N=19/59) of those who tested positive for diarrheagenic *E. coli* suffered from diarrhea. This current study found that 77.63% (n/N=59/76) of the *E. coli* isolates were diarrheagenic. Also, while testing negative for diarrheagenic *E. coli*, 20.17% (n/N=24/119) of the study subjects had symptoms of diarrhea. Table 1 shows the distribution of diarrheagenic *E. coli* among the study participants, while the result of agarose gel electrophoresis following PCR amplification is shown in Figure 1.

Pathotypes of Diarrheagenic *E. coli*

All five main pathotypes of DEC (EPEC, ETEC, EIEC, EAEC, and EHEC) were detected in this study. ETEC with only *elt* or *stla* was mostly detected among the study participants with a prev-

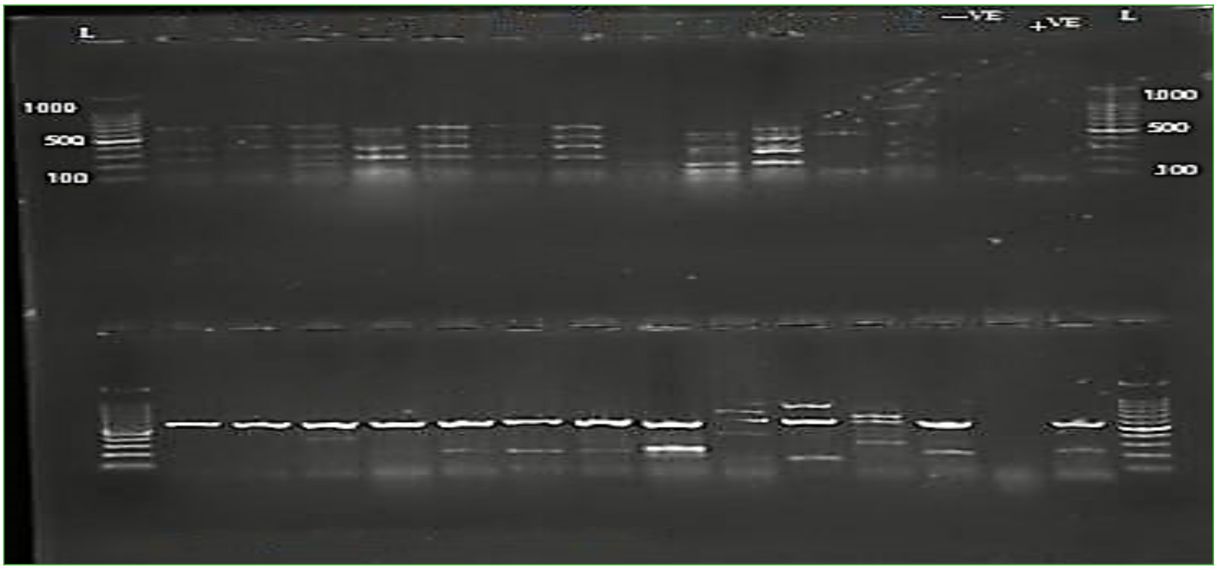

Figure 1. Agarose gel electrophoresis after PCR amplification of target genes of DEC pathotypes. +VE stands for positive control, -VE for negative control, and L for ladder (a 100 bp molecular marker).

alence of 26.89% (32/119) and 24.37% (29/119), respectively. EPEC with both *eaeA* and *bfpA,* ETEC with both elt and *stla,* EIEC and EHEC were the least detected pathotypes of DEC in this study. In general, there is no virulence gene (pathotype) that has a significantly higher risk of infecting a host with diarrhea than it does with those without diarrhea (p>0.05). On the other hand, compared to those without diarrhea, those who had diarrhea had a decreased likelihood of harboring ETEC with just *elt* (OR=0.28, CI=0.10, 0.81, p= 0.0184) or only *stla* (OR= 0.27, CI= 0.10, 0.72, p= 0.0088) (Table 2).

DISCUSSION

According to Saka et al[24], DEC is responsible for 30-40% of acute diarrhea cases in children under the age of five in underdeveloped nations. However, because most districts' clinical laboratories lack the tools necessary to identify it, the significance of this essential pathogen is underestimated[17]. Even though diarrhea caused by this pathogen has the potential to waste the instructional contact hours of students, even the few studies in this regard have been limited to children under the age of five. The scarcity of DEC data and related virulence factors in Ghana among students has been exacerbated by this circumstance. The present study examined DEC among students in the Ashanti region of Ghana.

The prevalence of DEC among the students in this study was 49.57%, and for the first time, this study detected all five main pathotypes of DEC among students in Ghana. This prevalence is lower than the 66.70%, as observed by Prah et al[17]. The discrepancy may stem from the fact that all study participants in the previous study were diarrheic, whereas both diarrheic and non-diarrheic volunteers were selected for the current study. This current study found that 77.63% (n/N = 59/76) of the *E. coli* isolates were diarrheagenic. This frequency of DEC is higher than 35.10%, which was previously noted by previous scholars[25]. The difference might result from the two studies' different sample sizes. This current study used 119 stool samples as the sample size, whereas the previous study used 230 stool samples. Given that these DEC coexist with the typical flora of *E. coli* in the gastrointestinal tract, there is a risk that the high frequency of DEC seen in this research region will rise. The pathogenic *E. coli* found in this study may be causing the typical commensal *E. coli* to become more harmful. This study's prediction supports the idea that, typically, benign commensals can transform into highly adapted pathogens by absorbing mobile genetic elements from pathogenic ones[26,27,19]. These pathogens can cause a range of diseases, including extraintestinal infections of the urinary tract, bloodstream, and central nervous system, as well as gastroenteritis[28]. These diseases have the potential to endanger the lives of the students who are being studied.

It was shown in this study that diarrhea affected 32.20% (n/N = 19/59) of the individuals who tested positive for *E. coli,* which causes diarrhea (Table 1). This means that, despite the possibility that DEC may have contributed to the instances of diarrhea among the students in the current research, a sizable portion of them had DEC infections while not exhibiting any sign of the disease. Given that the people in the research region may have been exposed to these DEC for a very long period, it is conceivable that they have evolved particular adaptations to help them withstand this infection. This finding agrees with the observation made by similar studies which found that while some research participants displayed diarrheal symptoms, others did not exhibit any symptoms while testing positive for the pathotypes of DEC indicated by the presence of virulence factors[4,29]. The risk that this study anticipates is that the asymptomatic DEC-positive individuals may potentially spread the infection of the DEC virulence factors of the pathotypes to other non-diarrheic students and possibly to those they come into contact with since they may not even be aware that they are carriers of the pathogen.

This is because all the five main pathotypes of DEC (EPEC, ETEC, EIEC, EAEC, and EHEC) were detected among the students in this study. The isolation of these subtypes from these students in this study area suggests possible circulation of these pathotypes and may have contributed to the burden of diarrhea among the local population. ETEC with only *elt* or *stla* was mostly detected among the study participants with a prevalence of 26.89% (32/119) and 24.37% (29/119), respectively. Several other studies have observed that among the five main pathotypes of DEC, ETEC is the most common[30,31,17]. In comparison to those who were not infected, the current investigation found that ETEC, which were defined by the produc-

TABLE 2. DISTRIBUTION OF PATHOTYPE OF DIARRHEAGENIC *E. COLI*.

Target genes	Pathotypes	Isolates	Prev./%	+ve symp.	+ve not symp.	-ve symp.	-ve not symp.	OR	*p*-value
eaeA	EPEC	27	22.69	10	17	16	33	1.21 (0.45, 3.24)	0.7000
bfpA		20	16.81	8	12	18	38	1.41 (0.49, 4.05	0.525
eaeA and *bfpA*		11	9.24	4	7	12	28	1.33 (0.33, 5.42)	0.6876
elt	ETEC	32	26.89	6	26	20	24	0.28(0.10, 0.81)	0.0184
stla		29	24.37	10	19	31	16	0.27 (0.10, 0.72)	0.0088
elt and *stla*		13	10.92	4	9	14	14	0.44 (0.1, 1.79)	0.2533
ial	EIEC	11	9.24	4	7	22	43	1.12 (0.29, 4.23)	0.8707
CVD432	EAEC	21	17.65	6	15	20	35	0.70 (0.23, 2.09)	0.5231
hlA	EHEC	13	10.92	6	7	16	29	1.55 (0.45, 5.42)	0.4896

Prev. = prevalence, Symp. = symptoms.

tion of *elt* (OR=0.28, CI=0.10, 0.81, p=0.0184) and *stla* (OR=0.27, CI=0.10, 0.72, p=0.0088), had considerably reduced odds of causing diarrhea. This suggests that ETEC is becoming protective rather than causing diarrhea. However, the earlier assertion made by similar other studies indicated that ETEC is the principal cause of acute diarrhea, which affects children less than five years old and travellers[32,33]. The variations in the inclusion criteria may be the cause of the discrepancies in the findings between the current and the earlier investigations. Although the majority of participants in this study had remained in the study region for over five years, the previous research focused on travelers and children under the age of five. Time exposure-induced mucosal immunity to ETEC infections may account for the current study's findings.

In developing nations, infantile diarrhea is mostly caused by the pathotypes of EPEC. EPEC infections can range from asymptomatic to deadly diseases[34]. EPEC are classified into typical EPEC (tEPEC), characterized by the presence of both eaeA and bfpA genes, which are carried on the EPEC adherence factor plasmid (pEAF), and atypical EPEC (aEPEC), which possess only the eaeA gene encoding the outer membrane protein intimin, but lack the pEAF plasmid[17]. This investigation detected the presence of tEPEC and aEPEC and another strain of EPEC, which only harbors *bfpA.* Similarly, a previous study[4] also identified EPEC with only *bfpA.*

Another pathotype, EHEC, whose infection lasts for a very long time and has the capacity to damage the gastrointestinal mucosal barrier and is known to cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS)[35-38], was also detected among the students in this study. The identification of these isolates highlights the necessity of EHEC surveillance among these students as well as the whole Ghanaian population.

Additionally, 20.17% (n/N =24/119) of the study participants experienced diarrheal symptoms, although they tested negative for diarrheagenic *E. coli*. This suggests that other etiological factors for diarrhea might exist in this area that is under investigation. Even though research has made significant strides to combat diarrhea, bacteria, viruses, fungi, and parasites remain infectious agents of the disease[39-41].

Generally speaking, no virulence gene (pathotype) has a notably higher chance of spreading an infection to a host that has diarrhea than it does to hosts that do not (p>0.05). This supports the earlier assertion by this study that although DEC has potentially contributed to the diarrhea episodes observed by this study, there is an equal number of participants who have developed adaptation to withstand the infection of the virulence factors of DEC due to long-term exposure to this diarrhea pathogen. This bolsters the claim that DEC pathotypes may be isolated from hosts who have diarrhea and non-diarrheic hosts[42].

CONCLUSIONS

The findings of this study offer preliminary evidence of the circulation of the five main pathotypes of DEC among students in this area and shed light on the significant strains that require serious and immediate concern to public health. While ETEC with only *elt* or *stla* is more common compared with the other pathotypes, the odds of carrying these strains of ETEC were statistically less among those with diarrhea than those without diarrhea. Generally, none of the pathotypes had significant odds of causing diarrhea among the diarrheic students than it does in non-diarrheic students. The study, therefore, recommends further studies into host immune response to infection of DEC in this study area. It is also recommended that future research be conducted in other parts of the country on the circulation of DEC pathotypes. It is suggested that the Public Health Division (PHD) of the Ghana Health Service of the Republic of Ghana monitor the emergence of the five principal DEC pathotypes, especially EHEC, which have been circulating in this study region. In this field of study, more investigation is also necessary into other etiological agents of diarrhea.

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Ethical Approval

The Kwame Nkrumah University of Science and Technology's School of Medical Sciences (SMS) Committee on Human Research, Publications, and Ethics (CHRPE) provided ethical permission, while Komfo Anokye Teaching Hospital (KATH) provided management approval. Additionally, authorization was received from Ashanti Regional and St. Michael's Hospitals. The study has been performed according to the Helsinki Declaration.

Conflict of Interest

The author declares no conflict of interest.

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AI Statement

AI technology was not employed in this study.

Informed Consent

Participants provided their informed consent to take part in this study.

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REFERENCES

1. WHO. Diarrheal disease: WHO fact sheet on diarrheal disease, 2024. Available at: https://www.who.int/news-room/fact-sheets/detail/diarrheal-disease.
2. Larbi RT, Atiglo DY, Peterson MB, Biney AA, Dodoo ND, Dodoo FN. Household food sources and diarrhea incidence in poor urban communities, Accra Ghana. PLoS One 2021; 16: e0245466.
3. Afrifa-Anane GF, Kyei-Arthur F, Agyekum MW, Afrifa-Anane EK. Factors associated with comorbidity of diarrhea and acute respiratory infections among children under five years in Ghana. Plos One 2022; 17: e0271685.
4. Awuah F, Addo MG, Ofori LA. Study of *Escherichia coli* as a Cause of Diarrhea in the Ashanti Region of Ghana. European Scientific Journal 2023; 19: 321-325.
5. Schiller LR. Chronic diarrhea evaluation in the elderly: IBS or something else?. Current Gastroenterology Reports 2019; 21: 1-7.
6. Robert E, Grippa M, Nikiema DE, Kergoat L, Koudougou H, Auda Y, Rochelle-Newall E. Environmental determinants of *E. coli*, link with the diarrheal diseases, and indication of vulnerability criteria in tropical West Africa (Kapore, Burkina Faso). PLoS Neglected Tropical Diseases 2021; 15: e0009634.
7. Mahmud MH, Isa ZM. Environmental risk factors of diarrhea among vulnerable population: a narrative review. Malaysian Journal of Public Health Medicine 2022; 22: 165-75.
8. Anita AR, Windusari Y, Sunarsih E, Fajar NA. Association between the incidence of diarrheal diseases and environmental risk factors: A systematic review: a systematic review. Jambi Medical Journal: Jurnal Kedokteran dan Kesehatan 2024; 12: 24-32.
9. Yeboah SI, Antwi-Agyei P, Kabo-Bah AT, Ackerson NO. Water, environment, and health nexus: understanding the risk factors for waterborne diseases in communities along the Tano River Basin, Ghana. Journal of Water and Health 2024; 22: 1556-1577.
10. Awuah F. Diarrheagenic *E. coli* among patients seeking health care at the Ashanti Regional and St. Michael's Hospitals in the Ashanti Region of Ghana (Doctoral dissertation, Kwame Nkrumah University of Science and Technology).
11. Bashar MA, Soundappan K. Outbreak investigation of acute watery diarrhea in a village of North India: timely action saved lives. The Journal of Infection in Developing Countries 202; 16: 843-849.
12. Arakaki L, Tollefson D, Kharono B, Drain PK. Prevalence of rotavirus among older children and adults with diarrhea: A systematic review and meta-analysis. Vaccine 2021; 39: 4577-4590.
13. Chang H, Guo J, Wei Z, Huang Z, Wang C, Qiu Y, Xu X, Zeng M. Aetiology of acute diarrhea in children in Shanghai, 2015–2018. PLoS One 2021; 16: e0249888.
14. Kumar CG, Giri S, Chawla-Sarkar M, Gopalkrishna V, Chitambar SD, Ray P, Venkatasubramanian S, Borkakoty B, Roy S, Bhat J, Dwibedi B. Epidemiology of rotavirus diarrhea among children less than 5 years hospitalized with acute gastroenteritis prior to rotavirus vaccine introduction in India. Vaccine 2020; 38: 8154-8160.

15. Alhaji AI, Mulade GL, Ajibade GA, Benjamin OI. Isolation, characterization and antibiotic susceptibility pattern of diarrheagenic *Escherichia coli* (DEC) among children attending some selected hospitals within Kaduna metropolis. Fudma Journal of Sciences 2022; 6: 169-1674.
16. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 1998; 11: 142-201.
17. Prah I, Ayibieke A, Nguyen TT, Iguchi A, Mahazu S, Sato W, Hayashi T, Yamaoka S, Suzuki T, Iwanaga S, Ablordey A. Virulence profiles of diarrheagenic *Escherichia coli* isolated from the western region of Ghana. Japanese Journal of Infectious Diseases 2021; 74: 115-121.
18. Pokharel P, Dhakal S, Dozois CM. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorganisms 2023; 11: 344.
19. Santos AC, Santos FF, Silva RM, Gomes TA. Diversity of hybrid-and hetero-pathogenic *Escherichia coli* and their potential implication in more severe diseases. Frontiers in Cellular and Infection Microbiology 2020; 10: 339.
20. Ahmed OB, Dablool AS. Quality improvement of the DNA extracted by boiling method in gram negative bacteria. International Journal of Bioassays 2017; 6: 5347-5349.
21. Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. Indian Journal of Medical Microbiology 2012; 30: 279-284.
22. Richardson JT. The analysis of 2× 2 contingency tables—Yet again. Statistics in Medicine 2011; 30: 890- 892.
23. Campbell I. Chi-squared and Fisher–Irwin tests of two-by-two tables with small sample recommendations. Statistics in medicine 2007; 26: 3661-3675.
24. Saka HK, Dabo NT, Muhammad B, García-Soto S, Ugarte-Ruiz M, Alvarez J. Diarrheagenic *Escherichia coli* pathotypes from children younger than 5 years in Kano State, Nigeria. Frontiers in Public Health 2019; 7: 348.
25. Abbasi E, Mondanizadeh M, van Belkum A, Ghaznavi-Rad E. Multi-drug-resistant diarrheagenic *Escherichia coli* pathotypes in pediatric patients with gastroenteritis from central Iran. Infection and Drug Resistance 2020; 13: 1387-1396.
26. Pokharel P, Dhakal S, Dozois CM. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorganisms 2023; 11: 344.
27. Baumgart LA, Lee JE, Salamov A, Dilworth DJ, Na H, Mingay M, Blow MJ, Zhang Y, Yoshinaga Y, Daum CG, O'Malley RC. Persistence and plasticity in bacterial gene regulation. Nature Methods 2021; 18: 1499-1505.
28. Peng Z, Wang X, Huang J, Li B. Pathogenic *Escherichia coli*. In Molecular Medical Microbiology 2024; 1065-1096.
29. Gautam K. Prevalence of diarrheagenic *E. coli* isolated from diarrheal stool from Children under 5 years of age at Kanti Children's Hospital. Journal of Ayurveda Campus 2021; 2: 41-45.
30. Belete MA, Demlie TB, Chekole WS, Sisay Tessema T. Molecular identification of diarrheagenic *Escherichia coli* pathotypes and their antibiotic resistance patterns among diarrheic children and in contact calves in Bahir Dar city, Northwest Ethiopia. Plos One 2022; 17: e0275229.
31. Yadav M, Sujatha R, Kumar A. Characterization of Enterotoxigenic and Enterohemorrhagic *Escherichia coli* in Paediatric Patients. J. Pure Appl. Microbiol 2020; 14: 375-81.
32. Khalil I, Walker R, Porter CK, Muhib F, Chilengi R, Cravioto A, Guerrant R, Svennerholm AM, Qadri F, Baqar S, Kosek M. Enterotoxigenic *Escherichia coli* (ETEC) vaccines: Priority activities to enable product development, licensure, and global access. Vaccine 2021; 39: 4266-4277.
33. Hosangadi D, Smith PG, Kaslow DC, Giersing BK. WHO consultation on ETEC and Shigella burden of disease, Geneva, 6–7th April 2017: Meeting report. Vaccine 2019; 37: 7381-90.
34. Hassan AO, Ojo BO, Abdulrahman AO. *Escherichia coli* as a global pathogen. Funksec here 2021; 3(1): 239-260.
35. Lang C, Fruth A, Holland G, Laue M, Mühlen S, Dersch P, Flieger A. Novel type of pilus associated with a Shiga-toxigenic *E. coli* hybrid pathovar conveys aggregative adherence and bacterial virulence. Emerging Microbes & Infections 2018; 7: 1-6.
36. Krause M, Barth H, Schmidt H. Toxins of locus of enterocyte effacement-negative Shiga toxin-producing *Escherichia coli*. Toxins 2018; 10: 241.
37. Chiang CJ, Huang PH. Metabolic engineering of probiotic *Escherichia coli* for cytolytic therapy of tumors. Scientific Reports 202; 11: 5853.
38. Gillespie M. Escape the EM boards: interactive virtual escape room for GI board review. Journal of Education & Teaching in Emergency Medicine 2021; 6: SG8.
39. Al-Sulivany BS, Mohammad P, Ahmed D, Omer R, Omer E. Transmission of zoonotic infections (bacteria, parasites, viruses, and fungi) from aquaculture to humans and molecular methods for organism identification. Journal of Zoonotic Diseases 2024; 8: 580-591.
40. Paul J. Introduction to Infectious Diseases. Disease Causing Microbes 2024; 1-63.
41. Florez ID, Veroniki AA, Al Khalifah R, Yepes-Nunez JJ, Sierra JM, Vernooij RW, Acosta-Reyes J, Granados CM, Perez-Gaxiola G, Cuello-Garcia C, Zea AM. Comparative effectiveness and safety of interventions for acute diarrhea and gastroenteritis in children: a systematic review and network meta-analysis. PloS One 2018; 13: e0207701.
42. Dias RC, Dos Santos BC, Dos Santos LF, Vieira MA, Yamatogi RS, Mondelli AL, Sadatsune T, Sforcin JM, Gomes TA, Hernandes RT. Diarrheagenic *Escherichia coli* pathotypes investigation revealed atypical enteropathogenic *E. coli* as putative emerging diarrheal agents in children living in B otucatu, S ão P aulo S tate, B razil. Apmis 2016; 124: 299-308.