ORIGINAL ARTICLE

The Epidemiological Characteristics of Diarrheagenic Escherichia Coli among Children with Acute Diarrhea

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ABSTRACT

Background: Diarrhea is known as one of the highly infectious diseases affecting the world population around the globe especially the children community. It is associated with the highest mortality and morbidity rates in children.

Objective: The study was carried out to find the characteristic features of diarrheagenic Escherichia coli among children with acute diarrhea under the age of 5 years.

Study design: It is a prospective based study conducted for the duration of the one year from September 2021 to August 2022. **Material and Methods:** There were 130 patients that participated in this study. The samples were collected from each patient. The clinical symptoms and demographic features of each patient were recorded. The SPSS was used for statistical analysis.

Results: Some of the features that were commonly observed among children were nausea (n=23), vomiting (n=32), abdominal pain (n=60) and fever (n=12). There were 27% patients that reported diarrhea in spring, 26% in autumn, 33% and 12% in summer and winter respectively. It was found that the isolate rate was decreasing in the order from EPEC, EAEC, ETEC, STEC and EIEC in age group 0-11.

Conclusion: This study reported that EPEC is an important phenotype present among children under the age of 5 who are suffering from DEC infection. The drug resistance cases are increasing in DEC strains with time. The antibiotic resistance was shown for ampicillin in both DEC strains and the aEPEC subtype. The data can be used to study the antimicrobial investigation against these strains.

Keywords: Escherichia coli.

INTRODUCTION

Diarrhea is known as one of the highly infectious diseases affecting the world's population around the globe, especially the children's community. It is associated with the highest mortality and morbidity rates in children¹⁻². The major cause of childhood diarrhea is Diarrheagenic Escherichia coli. For epidemiological investigation, there is a need to have sufficient knowledge about the characteristic features and other parameters. It is the major cause of pediatric morbidity and mortality. It is most commonly observed in the people of sub-Saharan Africa and south Asia. There are different pathotypes of DEC known to date. The most common phenotype of the DEC is EPEC. EAEC is highly known as the primary pathotype. Approximately 770 million episodes of pediatric diarrhea per annum are reported in the nations like China³⁻⁴. It is one of the 15 nations with a higher incidence ratio of diarrhea. According to the different report the rotavirus was reported as the primary cause of the acute diarrhea in children. The incidence and pathology of the microorganism vary with the changing geographical trends. The primary cause of pediatric diarrhea in underdeveloped countries is diarrheagenic Escherichia coli (DEC).⁵ DEC was observed to be the most prevalent bacterial infection among the pediatric under the age of five in a 5-year surveillance in China. There are six major categories of the DEC.6-7 Because of the lack of routinely screening practices in the countries like Pakistan the spare and insufficient information is available about the DEC and its variants.⁸⁻¹⁰ The drug resistance has complicated the treatment process of such infectious disease. Even the infectious agents of Enterobacteriaceae family have emerged the drug resistance against the antimicrobial drugs that were used as the first line treatment agents for such infections. This study was conducted to investigate the epidemiological features and characteristics of diarrheagenic Escherichia coli among children with acute diarrhea under the age of 5 years. DEC prevalence and resistance was also analyzed.11

MATERIAL AND METHODS

It is a prospective based study conducted for the duration of the one year from September 2021 to August 2022. The patients who

attended the pathology department of our institute tertiary care center were selected for the study. The ethical and review board committee of the hospital approved the study. The three abnormal appearance stool within the duration of 24 hours were characterized as diarrhea. There were 130 patients that participated in this study. The parents of the children were aware of the data collection and all basic guidelines were followed throughout the study.

Among 130 children, there were 63% males and 36% females. The acute diarrhea was characterized as the type of diarrhea which last more than 14 days. The diarrhea which lasted for less than 10 days was characterized as persistent diarrhea. The children diagnosed with persistent diarrhea were excluded from the study. The samples from each patients were collected. The clinical symptoms and demographic features of each patient was recorded. The microbiological tests were performed. The PCR was performed for the characterization of the DEC. The slide agglutination test was performed for determining the susceptibilities of the antimicrobial. The data collected was stratified by the statistical software SPSS. The results were recorded in the form of tables.

RESULTS

Study was carried out to find the characteristic features of diarrheagenic Escherichia coli among children with acute diarrhea under the age of 5 years. There were 130 patients that participated in this study. The parents of the children were aware of the data collection and all basic guidelines were followed throughout the study. Among 130 children, there were 63% males and 36% females. Basic features of the children are listed in table no.1. Some of the features that were commonly present among children were nausea (n=23), vomiting (n=32), abdominal pain=60) and fever n=12). There were 27% patients that reported diarrhea in spring, 26% in autumn, 33% and 12% in summer and winter respectively.

Table 1: Basic features of children suffering from acute diarrhea

Features	No. of patients
Age months	
0-11	35(26%)
12-23	41(31%)
24-35	24(18%)
36-47	15(11%)
48-59	15(11%)
Sex	
Male	82(63%)
Female	48(36%)
Season	
Spring	36(27%)
Autumn	35(26%)
Summer	43(33%)
Winter	16(12%)
Clinical symptoms	
Nausea	23
Vomiting	32
Abdominal pain	60
Fever	12
Other symptoms	3

Table 2 shows age distribution of DEC strains among patients suffering from acute diarrhea. It was found that the isolate rate was decreasing in the order from EPEC, EAEC, ETEC, STEC and EIEC in age group 0-11. Similar trend was found in all other age groups as well. EPEC was also found to be present commonly in all patients.

Table 2: Age distribution of DEC strains among children with acute diarrhea

Different age groups	DEC strains	Isolate rate (%)
0-11	EPEC	10
	EAEC	15
	ETEC	21
	STEC	24
	EIEC	26
12-23	EPEC	9
	EAEC	12
	ETEC	13
	STEC	14
	EIEC	-
24-35	EPEC	5
	EAEC	6
	ETEC	-
	STEC	-
	EIEC	-
36-47	EPEC	5
	EAEC	6
	ETEC	7
	STEC	-
	EIEC	-
48-59	EPEC	4
	EAEC	5
	ETEC	-
	STEC	-
	EIEC	-

The season wise distribution of DEC strain was also studied and it was analyzed that the isolate rate was maximum in case of EIEC in spring, STEC in summer and autumn and winter. EIEC strain was not as such found in all seasons except spring.

Table 3: Season wise distribution of DEC strains among children suffering from acute diarrhea

Season	DEC Strain	Isolate rate
Spring	EPEC	2
	EAEC	4
	ETEC	7
	STEC	-
	EIEC	8
Summer	EPEC	12
	EAEC	15
	ETEC	-

	STEC	16
	EIEC	-
Autumn	EPEC	10
	EAEC	15
	ETEC	-
	STEC	17
	EIEC	-
Winter	EPEC	3
	EAEC	4
	ETEC	5
	STEC	7
	EIEC	-

Drug resistance was measured in case of DEC strain and eEPEC subtype. It was found that the maximum percentage of drug resistant isolate in DEC strain was for ampicillin. Similar data was found in EPEC subtype as well.

Table 4: Drug resistance	of DEC strains and eE	PEC subtypes

Antibiotic drugs	Percentage of resistant isolate in DEC strains (%)	eEPEC subtype(%)
Meropenem	9.6	14.9
Imipenem	9.4	14.1
Tazobactum	17.8	21.4
Co-trimoxazole	66.8	67.0
Amikacin	8	4.3
Gentamicin	49	47.9
Levofloxacin	41.9	37.9
Ciprofloxacin	51.9	42.3
Cefoxitin	32	10
Aztreonam	41.2	34.8
Cefapime	37.9	38.2
Ampicillin	78.2	82

DISCUSSION

DEC is a major health concern in children mostly in developing countries¹³. In this study the epidemiological features and characteristics of the DEC, its prevalence and resistance was analyzed. The study was carried out on 130 patients, among these there were 82 males and 48 females. Some of the DEC strains were revealed in previous studies therefore their data was analyzed in our patients¹⁴. EPEC was revealed by China in 1995, and this is the most commonly found phenotype in DEC patients as per studies¹⁵⁻¹⁶. However, our study showed its rate a little bit less than the other strains like EAEC, ETEC, STEC and EIEC. As per studies, EAEC is considered as traveler's diarrhea in developed as well as developing countries. According to some studies the strain EAEC was found to be present as primary pathotype¹⁷. But in case of our study the strain was second most prevalent strain in these patients. This difference can be due to different geographical distribution of strains. The strain STEC was linked with severe form of diarrhea. In our study this strain was prominently found among 0-11 and 12-23 months' patients. The strain STEC is prevailing at a rapid pace and its monitoring is a must need to decrease its infection rate.

With the increase in age the infection rate was also decreasing as per our studies. Similar reports are obtained from other studies as well where age stratification decreased the infection rate of DEC¹⁸⁻¹⁹. With the passage of time child gain age related immunity and it can cope with the DEC strains that's why the infection rate was reduced as the age of child increased. Infection rate was quite high up till 23 months of age. In children of age greater than 23 months the infection rate of almost all strains was reduced. The seasonal variation showed that infection rate was maximum in summer and autumn. And it was much reduced in winter and spring. Such seasonal variations were reported by other studies as well²⁰. This data supports the point that may be environmental factors, temperature, and humidity plays role in increasing the infection rate in case of diarrhea patients. There was resistance found for some of the first line therapeutic drugs used such as ampicillin (78%) and co-trimoxazole (66%) in our

study. This percentage was lower than that found in the literature where antibiotic resistant rate was quite high for ampicillin and cotrimoxazole. As per literature aEPEC is one of the most important subtype of EPEC as it found in 42% of the DEC strains. However, the association of this subtype with diarrhea is still under study. Recent studies reported that the incidence of this subtype is increasing in both developed as well as under developed countries. And 78% of the EPEC cases were linked to this subtype in a study²¹. As per another study the other strains of this class tEPEC has been absent from most of the studies therefore a proper contrast between the two cannot be made. This study linked the aEPEC strain with diarrhea and this is supported by some of the other reports as well. As per studies 80% of the aEPEC strain was not belonging to the classical type and it was from O non-typeable. In our study it was found that only 33% of the aEPEC strains belonged to classical subtype, may be our serotyping was unable to diagnose the classical aEPEC subtype. Serotyping is a popular procedure used in many countries including China22. aEPEC strains that were identified in Mexico reported high antibiotic resistance. Similarly, another study showed high multidrug resistance in case of aEPEC strain. In our study it was found that aEPEC strain showed resistance to many broad spectrum antibiotics including cephalosporin. In our study it was found that highest resistance was shown against ampicillin in both DEC strains and aEPEC subtype.

This study also has some limitations some of the DEC pathotypes could not be linked to age and seasons as they were quite few, there is need for large sample size, coverage of more cases can help study the matter more easily. So that many other important factors can be discussed. There is need for longer monitoring duration to get clearer picture of DEC prevalence.

CONCLUSION

The knowledge about the characteristic features and other parameters is vital for the epidemiological investigation. This study reported that EPEC is an important phenotype present among children under the age of 5 who are suffering from DEC infection. aEPEC has become a crucial subtype of EPEC. The drug resistance cases are increasing in DEC strains with the passage of time. The antibiotic resistance was shown for ampicillin in both DEC strains and aEPEC subtype. The data can be used to study the antimicrobial investigation against these strains.

REFERENCES

- Nguyen TV, Le Van P, Le Huy C, Weintraub A. Diarrhea caused by rotavirus in children less than 5 years of age in Hanoi, Vietnam. Journal of Clinical Microbiology. 2004 Dec;42(12):5745-50.
- Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic Escherichia coli isolated from adolescents and adults in Hamedan, Western Iran. Iranian journal of microbiology. 2013 Mar;5(1):42.
- Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F. Diarrheagenic Escherichia coli and Shigella with high rate of extended-spectrum Beta-lactamase production: two predominant etiological agents of acute diarrhea in Shiraz, Iran. Microbial Drug Resistance. 2017 Dec 1;23(8):1037-44.
- Zhang SX, Zhou YM, Tian LG, Chen JX, Tinoco-Torres R, Serrano E, Li SZ, Chen SH, Ai L, Chen JH, Xia S. Antibiotic resistance and molecular characterization of diarrheagenic Escherichia coli and nontyphoidal Salmonella strains isolated from infections in Southwest China. Infectious diseases of poverty. 2018 Jun 1;7(03):24-34.
- Nweze EI. Virulence properties of diarrheagenic E. coli and etiology of diarrhea in infants, young children and other age groups in southeast, Nigeria. American-Eurasian J Sci Res. 2009;4(3):173-9.

- Chao AW, Bhatti M, DuPont HL, Nataro JP, Carlin LG, Okhuysen PC. Clinical features and molecular epidemiology of diarrheagenic Escherichia coli pathotypes identified by fecal gastrointestinal multiplex nucleic acid amplification in patients with cancer and diarrhea. Diagnostic Microbiology and Infectious Disease. 2017 Nov 1;89(3):235-40.
- Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic Escherichia coli by multiplex PCR. Indian Journal of Medical Microbiology. 2012 Jul 1;30(3):279-84.
- Acosta GJ, Vigo NI, Durand D, Riveros M, Arango S, Zambruni M, Ochoa TJ. Diarrheagenic Escherichia coli: prevalence and pathotype distribution in children from Peruvian rural communities. The American journal of tropical medicine and hygiene. 2016 Sep 9:95(3):574.
- Jafari F, Hamidian M, Rezadehbashi M, Doyle M, Salmanzadeh-Ahrabi S, Derakhshan F, Reza Zali M. Prevalence and antimicrobial resistance of diarrheagenic Escherichia coli and Shigella species associated with acute diarrhea in Tehran, Iran. Canadian journal of infectious diseases and medical microbiology. 2009 Oct;20(3):e56-62.
- Nataro JP, Kaper JB. Diarrheagenic escherichia coli. Clinical microbiology reviews. 1998 Jan 1;11(1):142-201.
- Tobias J, Vutukuru SR. Simple and rapid multiplex PCR for identification of the main human diarrheagenic Escherichia coli. Microbiological research. 2012 Oct 12;167(9):564-70.
- Lozer DM, Souza TB, Monfardini MV, Vicentini F, Kitagawa SS, Scaletsky IC, Spano LC. Genotypic and phenotypic analysis of diarrheagenic Escherichia coli strains isolated from Brazilian children living in low socioeconomic level communities. BMC infectious diseases. 2013 Dec;13(1):1-6.
- Zhou Y, Zhu X, Hou H, Lu Y, Yu J, Mao L, Mao L, Sun Z. Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: a hospital based study. BMC infectious diseases. 2018 Dec;18(1):1-0.
- Qu M, Lv B, Zhang X, Yan H, Huang Y, Qian H, Pang B, Jia L, Kan B, Wang Q. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in Beijing, China (2010–2014). Gut pathogens. 2016 Dec;8(1):1-9.
- Tian L, Zhu X, Chen Z, Liu W, Li S, Yu W, Zhang W, Xiang X, Sun Z. Characteristics of bacterial pathogens associated with acute diarrhea in children under 5 years of age: a hospital-based cross-sectional study. BMC infectious diseases. 2016 Dec;16(1):1-8.
- Chen Y, Chen X, Zheng S, Yu F, Kong H, Yang Q, Cui D, Chen N, Lou B, Li X, Tian L. Serotypes, genotypes and antimicrobial resistance patterns of human diarrhoeagenic Escherichia coli isolates circulating in southeastern China. Clinical microbiology and infection. 2014 Jan;20(1):52-8.
- Hien BT, Scheutz F, Cam PD, Serichantalergs O, Huong TT, Thu TM, Dalsgaard A. Diarrheagenic Escherichia coli and Shigella strains isolated from children in a hospital case-control study in Hanoi, Vietnam. Journal of clinical microbiology. 2008 Mar;46(3):996-1004.
- Moyo SJ, Maselle SY, Matee MI, Langeland N, Mylvaganam H. Identification of diarrheagenic Escherichia coli isolated from infants and children in Dar es Salaam, Tanzania. BMC infectious diseases. 2007 Dec;7(1):1-7.
- Shine S, Muhamud S, Adanew S, Demelash A, Abate M. Prevalence and associated factors of diarrhea among under-five children in Debre Berhan town, Ethiopia 2018: a cross sectional study. BMC infectious diseases. 2020 Dec;20(1):1-6.
- Workie GY, Akalu TY, Baraki AG. Environmental factors affecting childhood diarrheal disease among under-five children in Jamma district, South Wello zone, Northeast Ethiopia. BMC infectious diseases. 2019 Dec;19(1):1-7.
- Dagnew AB, Tewabe T, Miskir Y, Eshetu T, Kefelegn W, Zerihun K, Urgessa M, Teka T. Prevalence of diarrhea and associated factors among under-five children in Bahir Dar city, Northwest Ethiopia, 2016: a cross-sectional study. BMC infectious Diseases. 2019 Dec;19(1):1-7.
- Qiu FZ, Shen XX, Li GX, Zhao L, Chen C, Duan SX, Guo JY, Zhao MC, Yan TF, Qi JJ, Wang L. Adenovirus associated with acute diarrhea: a case-control study. BMC infectious diseases. 2018 Dec;18(1):1-7.