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Etiological Agents of Diarrhea in Hospitalized Pediatric Patients with Special Emphasis on Diarrhea Genic Escherichia Coli

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ABSTRACT

One of the "three giant killers" of newborns and children is diarrheal illness. 525,000 children under the age of five die from pediatric diarrheal illness each year, out of an estimated 1.7 billion cases worldwide. To look into the causes of diarrhea in children who are hospitalized, specifically focusing on Escherichia coli that causes diarrhea (E. coli). Along with finding additional contributory pathogens, the study aims to ascertain the frequency, pathotypes and clinical consequences of E. coli infections in pediatric diarrhea. The present study was a observational, descriptive study. This Study was conducted from One year. Total 100 patients were included in this study. In our study, 66 (66.0%) patients were Male and 34 (34.0%) patients were Female. The value of z is 4.5255. The value of p is <.00001. The result is significant at p<.05. In our study, 43 (43.0%) patients were <1 years of age, 29 (29.0%) patients were 1-3 years of age, 17 (17.0%) patients were 3-5 years of age and 11 (11.0%) patients were >5 years of age. The value of z is 5.0967. The value of p is <.00001. We came to the conclusion that E. coli continues to be a significant pathogen in the etiology of diarrhea in pediatric patients admitted to hospitals. Through more precise diagnosis, prompt treatment and the application of preventive measures like better sanitation and immunization programs, a thorough understanding of the various diarrhea genic E. coli strains and the use of suitable laboratory diagnostics will help to improve patient outcomes.

INTRODUCTION

One of the "three giant killers" of newborns and children is diarrheal illness. 525,000 children under the age of five die from pediatric diarrheal illness each year, out of an estimated 1.7 billion cases worldwide[1]. Morbidity, death and other serious consequences result from about half of these diarrheal cases, which are reported from South Asia and Africa. An estimated 300,000 (13%) of all diarrhea-related fatalities in children under five occur in India alone each year. In India, diarrheal illnesses account for almost one-third of hospital admissions and 17% of all hospitalized patient fatalities[2]. In poorer nations, the problem of controlling diarrhea is exacerbated by socioeconomic issues, inadequate sanitation, poor hygiene habits, low-quality drinking water and a lack of education. Infectious etiological agents such Rotavirus, Shigella sp., Salmonella sp., Entamoeba histolytica and diarrheagenic Escherichia coli (DEC) are responsible for the bulk of diarrheal illnesses that affect children in underdeveloped nations. Enteropathogenic E. Coli (EPEC), Enterohemorrhagic E. Coli (EHEC), Enterotoxigenic E. Coli (ETEC), Enteroaggregative E. Coli (EAEC) and Enteroinvasive E. Coli (EIEC) are the five types of DEC that are typically seen[3]. Most diarrheal infections in children in developing countries are caused by infectious etiological agents like Rotavirus, Shigella sp., Salmonella sp., Entamoeba histolytica and diarrheagenic Escherichia coli (DEC). The five kinds of DEC that are commonly observed are Enteropathogenic E. Coli (EPEC), Enterohemorrhagic E. Coli (EHEC), Enterotoxigenic E. Coli (ETEC), Enteroaggregative E. Coli (EAEC) and Enteroinvasive E. Coli (EIEC)[4]. For appropriate treatment to lower morbidity and mortality, early detection of the infectious agent causing diarrhea is crucial, particularly in young children. At the municipal and national levels, policymaking decisions require the identification of pathogens that cause diarrhea, such as bacteria, viruses and parasites. According to a number of studies, molecular techniques and enzyme immunoassay may improve the detection rate when compared to traditional techniques. There is little data on DEC, especially E. coli O157: H7, as the cause of diarrhea in children in India. Pal[5] India was the first country to report EHEC from nondiarrheagenic animal sources. Subsequent research on humans, food and animals has indicated that this enteropathogen could pose a threat to human health in the future. To provide more insights into the etiology of acute diarrhea with special emphasis on DEC in North India, this study was conducted in children below 12 years hospitalized due to diarrhea. to look into the causes of diarrhea in children who are hospitalized, specifically focusing on Escherichia coli that causes diarrhea (E. coli). Along with finding additional contributory pathogens, the study aims to ascertain the prevalence, pathotypes and clinical implications of E. coli infections in pediatric diarrhea.

MATERIALS AND METHODS

Study Type and Design: This was an observational, descriptive study.

Study Duration: One year.

Sample Size: 100.

Inclusion Criteria:

- **Age Group:** Pediatric patients aged 0-18 years (or a specific age range based on the study's focus, e.g., infants, children, or adolescents).
- **Hospitalized Patients:** Children who are admitted to the hospital for treatment of diarrhea.
- **Diarrhea Diagnosis:** Patients presenting with acute diarrhea, which is defined as having three or more loose stools per day or a change in stool consistency lasting for at least 24 hours.
- **Parental/Guardian Consent:** Informed consent obtained from the parents/guardians of the child (or assent from the child if age-appropriate), allowing participation in the study.
- **No Previous Antimicrobial Treatment (Optional):** Inclusion of patients who have not received antibiotics or antimicrobial treatment prior to admission, as prior treatments might interfere with pathogen detection or complicate diagnosis.
- **Availability of Stool Samples:** The availability of stool samples for microbiological analysis, including identification of diarrheagenic E. coli strains (e.g., ETEC, EPEC, EHEC).

Exclusion Criteria:

- **Non-Pediatric Patients:** Patients older than 18 years or younger than 1 month (or any age range outside the study's specific focus).
- **Chronic Diarrhea:** Children with a history of chronic diarrhea or underlying conditions such as inflammatory bowel disease, malabsorption syndromes, or chronic gastrointestinal conditions that could lead to persistent diarrhea.
- **Underlying Serious Medical Conditions:** Children with serious comorbidities, such as immuno compromised states (e.g., those undergoing chemotherapy or with HIV), severe malnutrition, or congenital gastrointestinal disorders that could confound the results.
- **Diarrhea from Non-Infectious Causes:** Patients whose diarrhea is believed to be due to non-infectious causes, such as medication side effects, food allergies, or metabolic disorders.
- **Pre-Existing Gastrointestinal Surgery:** Children who have undergone gastrointestinal surgery (e.g., bowel resection or corrective surgery for

malrotation), as this may alter the natural course of diarrhea or its microbiological findings.

Statistical Analysis: For statistical analysis, data were initially entered into a Microsoft Excel spreadsheet and then analyzed using SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism (version 5). Numerical variables were summarized using means and standard deviations, while categorical variables were described with counts and percentages. Two-sample t-tests, which compare the means of independent or unpaired samples, were used to assess differences between groups. Paired t-tests, which account for the correlation between paired observations, offer greater power than unpaired tests. Chi-square tests (χ^2 tests) were employed to evaluate hypotheses where the sampling distribution of the test statistic follows a chi-squared distribution under the null hypothesis., Pearson's chi-squared test is often referred to simply as the chi-squared test. For comparisons of unpaired proportions, either the chi-square test or Fisher's exact test was used, depending on the context. To perform t-tests, the relevant formulae for test statistics, which either exactly follow or closely approximate a t-distribution under the null hypothesis, were applied, with specific degrees of freedom indicated for each test. P-values were determined from Student's t-distribution tables. A p-value ≤ 0.05 was considered statistically significant, leading to the rejection of the null hypothesis in favour of the alternative hypothesis.

RESULTS AND DISCUSSIONS

Table 1: Basic information and Clinical Symptoms of the Study Population (n=100)

	Characteristics	Total (n)	Number of cases (%)
Sex	Male	100	66 (66)
	Female	100	34 (34)
Age (years)	<1	100	43 (43)
	1-3	100	29 (29)
	3-5	100	17 (17)
	>5	100	11 (11)
Clinical symptoms	Fever	100	82 (82)
	Vomiting	100	69 (69)
	Abdominal pain	23	17 (73.9)
	Tenesmus	23	6 (26.1)
	Headache	23	6 (26.1)
	Myalgia	23	6 (26.1)
	Low urine output	100	36 (36)
	Altered sensorium	100	16 (16)
Dehydration	Nil	100	3 (3)
	Mild	100	16 (16)
	Moderate	100	49 (49)
	Severe	100	32 (32)

Table 2: Pattern of Enteropathogens in Stool in the Study Population (n=100)

Etiological agents	N(%)
Diarrheagenic Escherichia coli	29 (29)
EPEC	18 (18)
EAEC	5 (5)
ETEC	2 (2)
EIEC	3 (3)
EHEC	1 (1)
Shigella flexneri	3 (3)
Vibrio cholerae serogroup O1	4 (4)
Aeromonas spp.	1 (1)
Entamoeba histolytica	1 (1)
Giardia lamblia	4 (4)
Ascaris lumbricoides	1 (1)
Enterobius vermicularis	1 (1)
Rotavirus	21/40(52.5)

Table 3: Bacterial Enteropathogens in Stool and their Resistance Pattern in Percentage

Antibiotic	Escherichia coli (n=92)	Shigella flexneri (n=3)	Vibrio cholerae (n=4)	Aeromonas spp. (n=1)
Ampicillin	97.3	100	50	100
Cefotaxime	95.95	66.67	50	100
Ceftriaxone	95.95	66.67	50	100
Cefixime	95.95	66.67	50	100
Cotrimoxazole	91.89	100	100	100
Tetracycline	97.3	100	50	100
Doxycycline	78.2	66.67	50	100
Azithromycin	74.32	66.67	50	100
Chloramphenicol	58.11	66.67	50	100
Ciprofloxacin	93.24	100	50	0
Nalidixic acid	100	100	0	0
Norfloxacin	91.89	100	50	100
Ofloxacin	66.79	0	0	0
Gentamicin	58.11	0	0	0
Amikacin	41.89	100	50	100
Cefuroxime	97.3	66.67	50	100
Cefepime	93.24	66.67	50	100
Ceftazidime	90.54	66.67	50	100
Cefoperazone/sulbactam	74.32	0	0	100
Piperacillin/tazobactam	94.59	100	50	100
Imipenem	50	66.67	50	100

In our study, 66 (66.0%) patients were Male and 34 (34.0%) patients were Female. The value of z is 4.5255. The value of p is <.00001. The result is significant at p <.05. In our study, 43 (43.0%) patients were <1 years of age, 29 (29.0%) patients were 1-3 years of age, 17 (17.0%) patients were 3-5 years of age and 11 (11.0%) patients were >5 years of age. The value of z is 5.0967. The value of p is <.00001. The result is significant at p < .05. In our study, 82 (82.0%) patients had Fever, 69 (69.0%) patients had Vomiting, 17 (73.9%) patients had Abdominal pain, 6 (26.1%) patients had Tenesmus, 6 (26.1%) patients had Headache, 6 (26.1%) patients had Myalgia 36 (36.0%) patients had Low urine output and 16 (16.0%) patients had Altered sensorium. The value of z is 10.8263. The value of p is <.00001. The result is significant at p<.05. In our study, 16 (16.0%) patients had Mild Dehydration, 49 (49.0%) patients had Moderate Dehydration and 32 (32.0%) patients had Severe Dehydration. The value of z is 7.4155. The value of p is <.00001. The result is significant at p<.05. In our study, 29 (29.0%) patients had Diarrheagenic escherichia coli, 18 (18.0%) patients had EPEC Etiological agents, 5 (5.0%) patients had EAEC Etiological agents, 3 (3.0%) patients had EIEC and Shigella flexneri and 21 (52.5%) patients had Rotavirus Etiological agents. In Escherichia coli, 97.3% patients had Ampicillin, 95.95% patients had Cefotaxime, 95.95% patients had Ceftriaxone, 95.95 patients had Cefixime, 91.89% patients had Cotrimoxazole, 97.3 % patients had Tetracycline, 78.2% patients had Doxycycline, 74.32% patients had Azithromycin, 58.11% patients had Chloramphenicol, 93.24% patients had Ciprofloxacin, 100% patients had Nalidixic acid, 91.89% patients had Norfloxacin, 66.79 % patients had Ofloxacin, 58.11% patients had Gentamicin, 41.89% patients had Amikacin, 97.3% patients had Cefuroxime, 93.24% patients had Cefepime, 90.54 patients had Ceftazidime, 74.32%

patients had Cefoperazone/sulbactam, 94.59% patients had Piperacillin/tazobactam and 50% patients had Imipenem. In Shigella flexneri coli, 100% patients had Ampicillin, 66.67% patients had Cefotaxime, 66.67% patients had Ceftriaxone, 66.67 patients had Cefixime, 100.0% patients had Cotrimoxazole, 100.0 % patients had Tetracycline, 66.67% patients had Doxycycline5 66.67% patients had Azithromycin, 66.67% patients had Chloramphenicol, 100.0% patients had Ciprofloxacin, 100% patients had Nalidixic acid, 100.0% patients had Norfloxacin, 100.0% patients had Amikacin, 66.67% patients had Cefuroxime, 66.67% patients had Cefepime, 66.67 patients had Ceftazidime, 100.0% patients had Piperacillin/ tazobactam and 66.67% patients had Imipenem. In Vibrio cholerae, 50.0% patients had Ampicillin, 50.0% patients had Cefotaxime, 50.0% patients had Ceftriaxone, 50.0 patients had Cefixime, 100.0% patients had Cotrimoxazole, 50.0 % patients had Tetracycline, 50.0% patients had Doxycycline, 50.0% patients had Azithromycin, 50.0% patients had Chloramphenicol, 50.0% patients had Ciprofloxacin, 50.0% patients had Norfloxacin, 50.0% patients had Amikacin, 50.0% patients had Cefuroxime, 50.0% patients had Cefepime, 50.0 patients had Ceftazidime, 50.0 % patients had Piperacillin/tazobactam and 50.0% patients had Imipenem. In Aeromonas spp., 100.0% patients had Ampicillin, 100.0% patients had Cefotaxime, 100.0% patients had Ceftriaxone, 100.0 patients had Cefixime, 100.0% patients had Cotrimoxazole, 100.0 % patients had Tetracycline, 100.0% patients had Doxycycline, 100.0% patients had Azithromycin, 100.0% patients had Chloramphenicol, 100.0% patients had Norfloxacin, 100.0% patients had Amikacin, 100.0% patients had Cefuroxime, 100.0% patients had Cefepime, 100.0 patients had Ceftazidime, 100.0% patients had Cefoperazone/ sulbactam, 100.0% patients had Piperacillin/ tazobactam and 100.0% patients had Imipenem.

In developing nations, diarrhea is the leading cause of morbidity and mortality among young children. One hundred children with diarrhea were included in the current study and it was discovered that 43% of the patients were younger than one year old, with a male majority. These results are consistent with those of prior studies[6]. 52.5% of children under the age of five had rotavirus overall in this study, which is much higher than in a comparable study[7]. which reported 35% stool positivity for rotavirus antigen by ELISA. Data from 22 Indian cities were analyzed., a total of 15,476 samples were tested by various tests and rates of rotavirus positivity ranged from 6-45%. The DEC overall prevalence was 29%. The most frequent pathotypes was EPEC 19 (65.5%), followed by EAEC 5 (17.2%), ETEC 2 (6%), EIEC 3 (10.3%) and no STEC was found. In another study conducted in 200 children with diarrhea, DEC infections were found in 26%., EAEC was the most common DEC identified by multiplex PCR followed by EPEC in 16% cases, ETEC in 3.5% and EIEC in 1.5% of the diarrheal cases. Other similar studies have reported the incidence of E. coli to be 27.6%, 43%, and 61.76%, respectively. The multiplex PCR assays are highly sensitive and useful for identification of DEC[8]. In this study, resistance rates of DEC to first-line therapeutic drugs were high, for example, 97.3% to ampicillin and 95.95% to co-trimoxazole higher than rates reported before but appears similar to the study by Karmali in which only 9.30% E. coli strains were susceptible. In this study, 89% of E. coli isolates were multidrug resistant, which is much higher compared to other studies which reported 66.6% and 70.2%. The resistance was 100% for amoxicillin clavulanate and nalidixic acid, 97.3% for tetracycline, 93.24% for ciprofloxacin and 97.3% for ampicillin. The E. coli isolates were susceptible to chloramphenicol (58.11%), gentamicin (48.19%), amikacin (58.11%) and imipenem (50%), respectively. The findings are similar to another study in hospitalized Indian children with diarrhea in which nalidixic acid was found to be 100% resistant and fluoroquinolone susceptibility was 13.95% among the DEC strains and in vitro sensitivity to amikacin was 83.72%. Uma[9] found that 90% of E. coli strains were resistant to most of the antimicrobial agents tested., all the isolates were resistant to ampicillin, imipenem and cotrimoxazole but were sensitive to amikacin. Approximately 66.67% of the Shigella sp. isolates in our investigation were resistant to the first-line medications ceftriaxone, cefixime and azithromycin. The strains were responsive to gentamicin and ofloxacin but resistant to ciprofloxacin, ampicillin, nalidixic acid, cotrimoxazole and furazolidone. In a related investigation, Shigella showed overall resistance to nalidixic acid, cotrimoxazole, ciprofloxacin and furazolidone of 63.6%, 58.1%, 25.92% and 16.3%, respectively. Although it is rare in Shigella, reports of ciprofloxacin resistance have also come from other regions of India[10]. V. cholerae O1 subtype Ogawa accounted for 4% of the isolates in this investigation. Subtype Ogawa infection has also been observed to be more common in India in other investigations. Isolates of V. cholerae demonstrated resistance to the first-line medications ciprofloxacin, azithromycin and doxycycline. Although the strains were resistant to furazolidone, cotrimoxazole and nalidixic acid, they were vulnerable to gentamicin. Tetracycline, ampicillin and amoxicillin clavulanate were all effective against two isolates. Numerous investigations have documented a rise in resistance to ampicillin, cotrimoxazole, furazolidone and nalidixic acid., isolates of V. cholerae have total resistance to furazolidone but are susceptible to gentamicin and tetracycline[11]. All nonbloody stools submitted for the examination of bacterial enteric pathogens should be cultured for E. coli O157:H7[12]. The agar medium most commonly used for the isolation of E. coli O157:H7 is SMAC which is 100% sensitive, 85% specific and 86% accurate for

detecting E. coli O157:H7. In this study, no E. coli O157:H7 was isolated by culture on SMAC agar. Our findings are also similar to many previous studies in India which found nil to rare isolates of E. coli O157. In the present study, an overall detection rate by ELISA was 1/100 (1%) from the study participants. Earlier studies reported cross-reaction of E. coli O157 lipopolysaccharide with antibodies to many other pathogens. E. coli O157:H7 in the present study was not detected by culture and PCR. Four (4%) patients had G. lamblia, and one (1%) had E. histolytica, both of which are known to cause diarrhea. According to studies, between 2.6% and 12% of children in India have giardiasis. The existence of Enterobius vermicularis and Ascaris lumbricoides in a single sample each seems to be of questionable importance. In the current investigation, Cryptosporidium sp. was not found. According to current reports, the prevalence rate of Cryptosporidium in India ranges from 1-16.7%. In 63% of the patients in this investigation, the pathogenic etiological agents were identified. Pathogens were found in 10-70% of patients in different studies[13]. Various studies have not found pathogen in up to 40%-50% of children with presumed infectious diarrhea.

CONCLUSION

We concluded that E. coli remains a crucial pathogen in the etiology of diarrhea in hospitalized pediatric patients. A comprehensive understanding of the different diarrheagenic E. coli strains, along with appropriate laboratory diagnostics, will aid in improving patient outcomes through more accurate diagnosis, timely treatment and implementation of preventive measures, such as improved sanitation and vaccination strategies. Further research is needed to explore emerging strains and antimicrobial resistance patterns to stay ahead in managing pediatric diarrheal diseases effectively.

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