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Characterization of *Escherichia coli* from Stool Samples in Children with Diarrhea and their Antibiotics Resistance Pattern

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A B S T R A C T

Diarrheal diseases are a major cause of morbidity and mortality in developing countries. In the past decades several new enteric pathogens, including bacterial, viral, and parasitic agents have been described. *Escherichia coli (Esch. coli)* is one of the leading cause of acute diarrhea in developing countries in children under 5 years old, with significant morbidity and mortality. The present study was carried out in the Department of Microbiology Mayo Institute of Medical Sciences and Research Lucknow. The study group included 81 children suffering from diarrhoea attending the inpatient or outpatient sections of the Department of Paediatrics. Among the bacterial pathogens detected, *Esch. coli* was the commonest. In the present study major symptoms reported were diarrhea, fever and vomiting. Examination of stool samples showed that 32 (39.5%) children harbored one or more of the *Escherichia coli* pathotypes. Out of those 32 who were subjected to serotyping 9 (28.12%) were untypable and 5 (15.62%) were rough strains. Of the typable strains, Enteropathogenic E.coli (EPEC) accounted for 3.12%, Enterotoxicogenic *E.coli* (ETEC) accounted for 12.5%, Enterohaemorragic (EHEC) accounted for 3.12 %. Of the ETEC strains, serogroups O173 (1), O11 (1), O15 (1) and O8 (1) were found. The EPEC strain found was O86 and EHEC was serogroup O172. Among the other serogroups which comprised of 37.5% strains, O161 (3) was predominant. Antibiotic resistance was documented for Ampicillin (100%).

Introduction

Diarrheal diseases are a major cause of morbidity and mortality in developing countries (Kosek *et al*., 2003). Globally 1.3 billion cases of acute diarrhea occur in children below 5 years annually. 80% of these deaths are in children below 2 years of age (Samal *et al*., 2008). In the past decades several new enteric pathogens, including bacterial, viral, and parasitic agents have been described (Presterl *et al*., 2003; Aslani *et al*., 2009; Alikhani *et al*., 2011). The

bacterial pathogens associated with diarrhea include species of *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia enterocolitica*. In developing countries in children under 5 years old *E.coli* is one of the leading causes of acute diarrhea, with significant morbidity and mortality. Each type of *E.coli* diarrhea is associated with a different pathotype of *E. coli*, and each pathotype has characteristic virulence determinants that contribute to its pathogenic mechanisms (Donnenberg *et al*., 2010)**.** Pathotypes of diarrheagenic *E.coli included* Enterotoxigenic *E.coli* (ETEC) Shiga toxin–producing *E. coli* (STEC), Enteropathogenic *E.coli* (EPEC), Entero-invasive *E. coli* (EIEC), and Entero-aggregative *E. coli* (EAEC) (Oberhelman *et al*., 1998). ETEC is a common cause of traveler's diarrhea which is caused by the production of heat-labile (LT) and/or heat-stable (ST) enterotoxins. EIEC is similar to *Shigella* species by means of genetic traits of virulence. EAEC has been associated with acute and persistent diarrhea in children and adults in developed as well as developing areas (Oberhelman *et al*., 2011; Presterl *et al*., 1999).

Materials and Methods

The present study was carried out in the Department of Microbiology Mayo Institute of Medical Sciences and Research Lucknow. A total of 81 children up to the age of 5 years suffering from diarrhoea form the study population were selected. Patients were given clean, autoclaved, wide-necked container having a tight-fitting, leak-proof lid for the collection of stool samples. The specimens collected were taken to the laboratory soon after collection and processed as soon as possible but no longer than 2 hours after collection. If there was delay, a portion of stool was transferred to Cary-Blair's transport medium with the help of a sterile swab. Macroscopic examination of stool samples was performed for consistency, colour, odour and presence of mucus and/or blood. Presence of any proglottids, larvae, or adult worms was also noted.

Specimens were plated immediately on McConkey agar, Xylose-lysine-deoxycholate (XLD) agar or Deoxycholate-citrate agar (DCA). Plates were incubated at 35-37˚C for 18-24 hrs. Gram staining was done on isolates to observe Gram negative bacilli. Indole, Methyl Red, Voges-Proskauer and Citrate (IMViC) tests were used to identify *E. coli*. *E.coli* isolates were randomly selected for serotyping and sent to Central Research Institute, Kasauli, Himachal Pradesh.

The antimicrobial sensitivity testing of the pathogens was performed by Kirby-Bauer method using commercially prepared antibiotic discs of Hi-media laboratories (India) and was interpreted as sensitive, intermediate sensitive and resistant as per CLSI guidelines (CLSI -2014).

Results and Discussion

The present study comprised of 81 children suffering from diarrhoea attending the inpatient or outpatient sections of the Department of Paediatrics, Mayo institute of medical sciences and research lucknow. The results obtained are tabulated as in table no.1.

Among the bacterial pathogens detected, *E.coli* was the commonest (39.5%) followed by *Vibrio cholerae* (4.59%), *Shigella* spp. (1.66%) and *Salmonella typhimurium* (0.42%). However, *Campylobacter* could not be isolated in any sample. In the present study we focus only on the prevalence of E.coli, pathogenicity, sensitivity and serotyping. The antimicrobial susceptibility profiles of *E. coli* are shown in Table no.3.

High rate of antibiotic resistance was documented for ampicillin (100%), followed by amoxicillin-clavulanic acid (59.3%), cefixime (59.2%), ampicillin-sulbactum (56.2) and tetracycline (56.2). However, *E. coli* were susceptible to amikacin (90.6%), piperacillin-tazobactum (71.8%), ticarcillin-clavulanic acid (71.8%).cefipime (68.7%) and meropenem (65.6%).

Table.1 Prevalence of *E.coli* in total sample

Organism	No. of samples	No. of Isolates
E.coli	81	32 (39.5%)

Table.2 Sex-wise prevalence of *E.coli*

Males in this study constituted 11 (34.3 %) and females were 21 (65.6%).

No. of Isolates	Male	Female
32 (100%)	11(34.3%)	21(65.6%)

Table.3 Age Wise Prevalence of *E.coli*

Age	No. of samples	*E.coli* isolated No. (%)
0-1	18	8(44.4)
1-2	9	2(22.2)
2-3	17	8(44.4)
3-4	16	10(62.5)
4-5	21	4(19.0)

Table 4 Antimicrobial susceptibility of *E.coli*

NAME OF ANTIBIOTICS	*E.COLI* (32)		
	S	I	R
AMPICILLIN	0	0	32 (100%)
AMPICILLIN-SULBACTUM	10(31.25%)	4(12.5%)	18(56.25%)
AMOXICILLIN-CLAVULANIC ACID	13(40.6%)	0	19(59.3%)
PIPERACILLIN-TAZOBACTUM	23(71.8%)	0	9(28.1%)
TICARCILLIN-CLAVULANIC ACID	23(71.8%)	0	9(28.1%)
CEFOTAXIME	14(43.7%)	4(12.5%)	14(43.7%)
CEFTAZIDIME	12(37.5%)	7(21.8%)	13(40.6%)
CEFIXIME	13(40.6%)	0	19(59.2%)
CEFEPIME	22(68.7%)	3(9.3%)	7(21.8%)
CEFTAZIDIME/CLAVULANIC ACID	19(59.3%)	3(9.3%)	10(31.2%)
TETRACYCLINE	9(28.1%)	6(18.7%)	18(56.2%)
AMIKACIN	29(90.6%)	0	3(9.3%)
GENTAMYCIN	18(56.25%)	8(23%)	6(18.7%)
TOBRAMYCIN	12(37.5%)	8(25%)	12(37.5%)
CIPROFLOXACIN	18(56.25%)	2(6.25%)	12(37.5%)
OFLOXACIN	19(59.2%)	0	13(40.6%)
IMIPENEM-CILASTATIN	12(37.5%)	11(34.3%)	9(28.1%)
MEROPENEM	21(65.6%)	0	11(34.3%)
DORIPENEM	19(59.3%)	0	13(40.6%)

Fig.1 Prevalence of *E.coli* in total sample

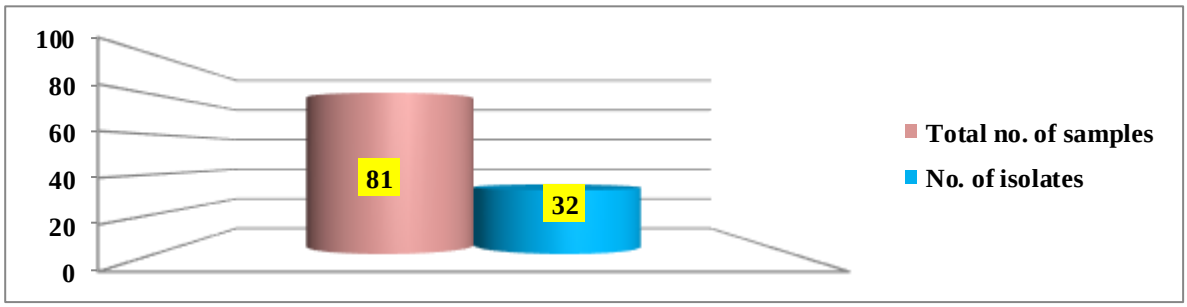


Fig.2 Sex-wise prevalence of *E.coli*

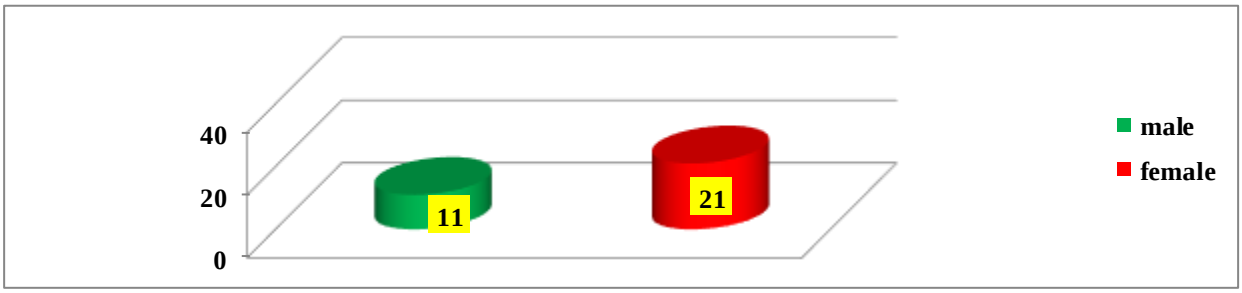
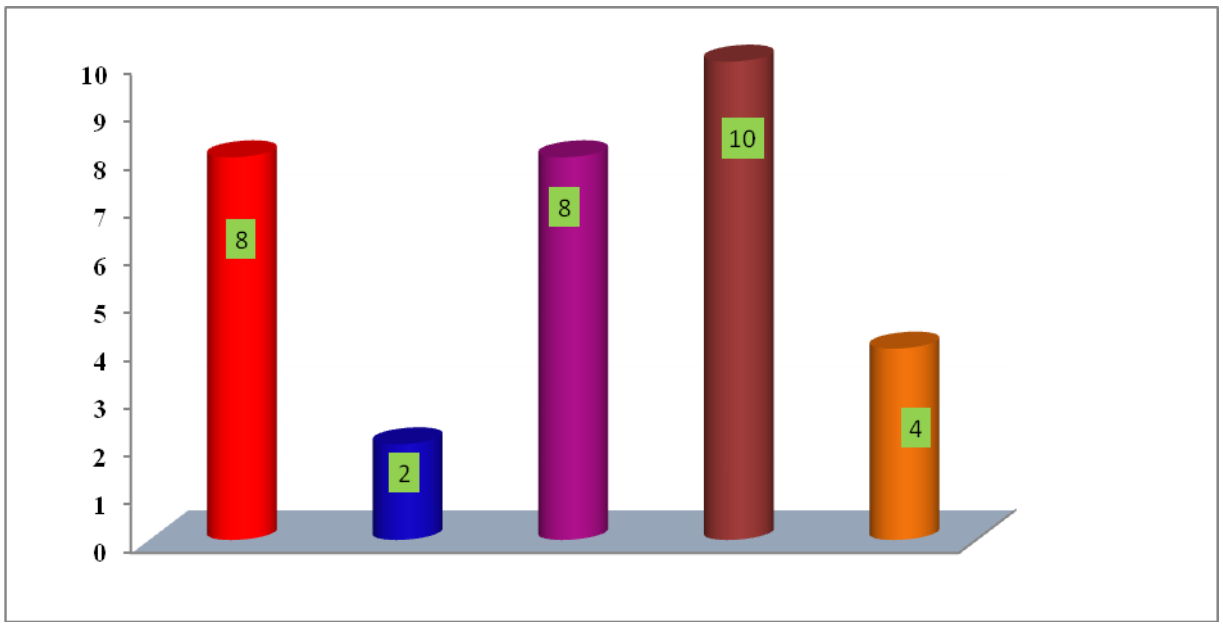


Fig.3



List of *E.coli* serotypes (n=32)

Enteropathogenic *E.coli* (EPEC) n=1 (3.12%)	O group Number	86 1				
Enterotoxigenic *E.coli* (ETEC) n=4 (12.5%)	O group Number	173 1	11 1	15 1	8 1	
Enterohaemorrhagic *E.coli* (EHEC) n=1 (3.12%)	O group Number	172 1				
Enteroinvasive *E.coli* (EIEC) n=0	O group Number					
Other O serogroups n=12 (37.5%)	O group Number	153 1	87 1	161 3	12 1	21 1
	O group Number	132 1	99 1	60 1	80 1	154 1
Rough Strains N=5 (15.62%)			Untypable Strains n=9 (28.12%)			

This table shows the list of various serotypes of *E.coli*. 32 *E.coli* isolates were subjected to serotyping. Out of those 32, 9 (28.12%) were untypable and 5 (15.62%) were rough strains. Of the typable strains, ETEC was predominant (12.5%) and EPEC and EHEC each comprised 3.12% of the total strains. Of the ETEC strains, serogroups O173 (1), O11 (1), O15 (1) and O8 (1) were found. The EPEC strain found was O86 and EHEC was serogroup O172. Among the other serogroups which comprised of 37.5% strains, O161 (3) was predominant.

A total of 81 stool samples from under five year age were collected during the study period. Out of all the samples examined *E.coli* was isolated from 32(39.5%) children. In the present study 62.5% *E.coli* isolated from the age group of 3-4 years, 3-4 year children are probably more mobile and playful than younger children. They have a higher chance than children below 1 year to get diarrhea from hand-contamination, especially while playing in the ground, playing with their toys or other objects, and unknowingly putting their dirty fingers into their mouth. In addition, the risk of ingesting contaminated materials is high, especially in unhygienic environments.

44.4% *E.coli* isolated from the age group of 0-1 year this could be related to the beginning of environmental exposure and increased introduction of solid foods to children whose immune system is still developing. Whereas study done by Sudershan *et al.,* (2014) shows higher prevalence 54% in children below one year of age and only 3% belonged to more than 4 years of age.

In the present study major symptoms reported were diarrhea, fever and vomiting similar to study done by Sudershan *et al.,* (2014).

In present study females have higher prevalence of infection 65.6% compare to men 34.3 % similar to the study done by Kandakai-Olukemi *et al.,* (2009).

Examination of stool samples showed that 32 (39.5%) children harbored one or more of the *E. coli* pathotypes. Out of those 32, 9 (28.12%) were untypable and 5 (15.62%) were rough strains. Of the typable strains, EPEC accounted for 3.12%, ETEC accounted for 12.5%, EHEC accounted for 3.12 %. Of the ETEC strains, serogroups O173 (1), O11 (1), O15 (1) and O8 (1) were found. The EPEC strain found was O86 and EHEC was serogroup O172.

Among the other serogroups which comprised of 37.5% strains, O161 (3) was predominant. The present study revealed that the prevalence of ETEC (12.5%) in diarrheal stool samples higher than the other serotypes of *E. coli*, similar to the study done by Samal *et al*., whereas Sudershan *et al.*, (2014) and Jafari *et al*., (2008) reported prevalence of EPEC 35% and 12.4% respectively in diarrheal stool samples higher than the other serotypes of *E. coli*. Pie *et al*., (1997) reported that EAEC was responsible for 55% of the diarrhea cases in a diarrheal epidemic in South India.

In the present study higher rate of antibiotic resistance was documented for ampicillin (100%), followed by amoxicillin-clavulanic acid (59.3%), cefixime (59.2%), ampicillin-sulbactum (56.2) and tetracycline (56.2).

Because they are inexpensive and can be obtained easily without a doctor's prescription. Resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid mediated antibiotic resistance found to be common in Escherichia coli.(10) However, *E. coli* were susceptible to amikacin (90.6%), piperacillin-tazobactum (71.8%), ticarcillin-clavulanic acid (71.8%).cefipime (68.7%) and meropenem (65.6%).

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