

Diarrhoea with Special Reference to Enteropathogenic *E. coli* in Children below Five Years at a Tertiary Care Centre

Sweta Muni¹, Ajay Kumar¹, S K Shahi², Deepak Pankaj³, Aninda Sen⁴, Swati Salila⁵

ABSTRACT

Introduction: Diarrhoea is defined as passage of loose, liquid or watery stools. In the developing countries, diarrhoea is major cause of death in children below 5 years. Various organisms have been implicated as causes of bacterial diarrhoea. These include *Escherichia coli*, *Vibrio cholerae*, *Salmonella* and *Shigella* species etc. Enteropathogenic *Escherichia coli* (EPEC) causing diarrhoea in children less than five years was isolated and identified in this study.

Material and Methods: 230 cases of diarrhoea in children below 5 years were studied over a period of two years with emphasis on 106 samples of enteropathogenic *E. coli* and various strains of EPEC were identified by slide agglutination test and subjected to antimicrobial disc diffusion susceptibility tests.

Results: The sex incidence revealed a male to female ratio of 1.25:1. *E. Coli* being the most common pathogen with 92.17% in cases of diarrhoea. Majority of EPEC were found in age group 13-24 months and 25-36 months (30.2% each). Three types of serotypes O111, O26, O55 of *E. coli* were studied.

Maximum resistance was seen to Ampicillin (83.01%).

Conclusion: The present study revealed a large number of cases of diarrhoea due to EPEC strain of *E. coli*. Consideration and emphasis should be laid on this variety of *E. coli* for devising strategies for children in this part of the world.

Keywords: Antibiotics, *E. coli*, EPEC, Diarrhoea, Stool samples

INTRODUCTION

Diarrhoea is a well known cause of mortality and morbidity among children. As per UNICEF and WHO, respiratory tract infection especially pneumonia is the most common cause of death in children below the age of 5 years which is very closely followed by diarrhoea as the second most common cause of death. It was also observed by them that India accounts for more number of deaths than any other country of the world due to diarrhoeal cases in children.¹ In India, according to figures of the health ministry, more than one lakh children under the age of five fail to resist diarrhoea and die every year due to it. Diarrhoea has a varied etiology, it cannot be attributed to a specific etiology and so different diarrhoeal symptoms can be caused by bacteria, viruses and parasitosis of either single or multiple aetiology.² Considering diarrhoea caused by *E. Coli*, six groups responsible are known, which are enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC).³ Among the six types, Enteropathogenic *Escherichia coli*

(EPEC) is among the most important pathogens infecting children. This is because of their high and increasing prevalence not only in the community but also at hospital settings.⁴ These are one of the important causes of persistent diarrhoea in the childhood group⁵ and worsened by seasonal outbreaks, EPEC organisms also are a major cause of endemic infantile diarrhoea. EPEC has various serotypes, of which there are certain serotypes which are much more likely to be encountered in diarrhoea than the others.⁶ Various serotypes have been reported in different geographical areas which are significantly associated with diarrhea. These serotypes are Serotypes O55:H12/45, O86:H48, O127:H21, O142:H48, O126:H48, and O126:H19, O55:H6 and O111:H2.^{7,8} Rehydration therapy is the most effective form of treatment in EPEC diarrhoea and the use of antibiotics should be limited due to increasing number of antibiotic resistance.⁹ To understand the various parameters associated with a disease like its consequences, complications along with organisms involved, there has to be monitoring and documentation at serial intervals. Therefore this study was undertaken to find out various microbial organisms involved in childhood diarrhoea especially with reference to enteropathogenic serotype of *E. coli* which is long delayed in this region.

MATERIAL AND METHODS

The sample population of our study included both male and female patients below five years of age, attending the Out Patients Department of Pediatrics and other departments of Katihar Medical College, with complaints of diarrhoea and gastroenteritis. Patients in the study were included from paediatric ICU and ward, neonatal ICU, other wards with children less than five years with diarrhoea. Those children less than 5 years in the state of shock and children more than 5 years were excluded from the study. Collection of stool samples were done as per guidelines given by World Gastroenterology Organisation (WGO) from patients

¹Senior Resident, ²Professor and Head, ⁵Junior Resident, Department of Microbiology, ³Assistant Professor, Department of General Surgery, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, ⁴Professor, Department of Microbiology, Katihar Medical College, Katihar, India

Corresponding author: Dr. Sweta Muni, Shanti Niwas, Amba Vihar Colony, Ambedkar Path, Near Mahima Mandir, Rukanpura, Patna-800014, Bihar, India

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with passing of two or more unformed stool in the last 24 hours. It also included watery stool samples accompanied with fever, abdominal pain and vomiting. Stool samples collected were put in sterile containers with wide mouth and a lid and were further processed within three hours of sample collection in the department of microbiology. Stool samples were inoculated in the following media- (1) Mac Conkey agar (MCA) (2) Sorbitol Mac Conkey agar (SMCA) and (3) Deoxycholate Citrate agar (DCA). Those samples which were lactose fermenting non mucoid colonies were further processed as they were suspected to be *E. coli* and those colonies which were non lactose fermenting on DCA were processed to look for *Salmonella*, *Shigella* and other non lactose fermenting bacteria. Standard protocol were followed for biochemical tests where the purified isolated bacterial growth were identified. Disc diffusion techniques were done for antibiotic susceptibility testing (AST). *E. coli* serotyping of isolates was done with antisera which was obtained from Denka Seiken co. Ltd from Japan and manufacturers's instructions were followed to perform the test.

RESULTS

Two hundred and thirty (230) samples collected from children below five years of age presenting with diarrhoea and / or gastroenteritis showed the percentage of male infants and children with diarrhoea was 55.65% while it was 44.35% in female infants and children with diarrhoea with male to female ratio was 1.25:1. During the study it was found that only children 30% were exclusively breastfed whereas mixed feeding was seen in 70.0% children. Age distribution of infants and children with diarrhea showed that maximum number of cases were seen in age group 25-36 months (33.04%) followed by age group 49-60 months (28.69%). This was followed by age group 13 -24 months (19.13%) and age group 0-12 months (15.65%). Minimum number of cases were seen in age group 37-48 months. The distribution of different bacteria isolated in different age-groups showed majority of them to be *E. coli* (212/230) while few in numbers were *Klebsiella* (7/230), *Proteus* (7/230) and *Shigella* (4/230). Maximum number of *E. coli* was isolated from age group 25-36 months (31.3%), followed by age group 49-60 months (26.08%) followed by 0-12 months and 13-24 month age group (15.65% each). 3.47% of organisms were isolated from age group 37 to 48 months which was the least. (Table 1)

Among distribution of diarrheagenic strains of *E. coli*, maximum number of strains were found to be

Enteropathogenic *E. coli* EPEC (50%), and this was followed by Diffusely adherent *E. coli* DAEC (34.9%). Enteroaggregative *E. coli* EAEC and enterotoxigenic *E. coli* ETEC accounted for only 10.4% and 4.7% of strains respectively. (Figure 1) In this study, Enteropathogenic *E. coli* (EPEC) was isolated in various age groups of children with diarrhea. EPEC was isolated most commonly from age-group 13-24 and 25-36 months (30.2%). This was followed by age group 0-12 months (18.7%) and age group 49-60months (13.2%). Least number of EPEC strain was found in age group 37-48 months (7.5%).

Three types of serotypes of Enteropathogenic *E. coli* (EPEC) were isolated from various age groups which were serotypes O111, O26 and O55 respectively. Serotype O26 (39.6%) was the most common serotype of EPEC. This was followed by serotype O111 (37.7%) and serotype O55 (22.6%). Serotype O111 was most prevalent in age group 25 to 36 months (16/40) while both serotype O26 (16/42) and serotype O55 (11/24) were prevalent in age group 13 to 24 months. (Table 2)

Various parasites were found as co-infection with diarrhoeagenic *E. coli* on wet mount (microscopic) examination. These were *Trichuris trichiura* (3.8%), *Ascaris lumbricoides* (7.5%), *hymenolepis nana* (4.7%) and *Giardia lamblia* (3.8%). Antibiotic susceptibility pattern of various isolates were studied in 106 cases of Enteropathogenic *E. coli* (EPEC). Maximum resistance was found with ampicillin 88/106 (83.01%) followed by cefixime 42/106 (39.62%). The strains were maximally sensitive to amikacin 94/106 (88.67%) followed by gentamicin 79/106 (74.52%) and ofloxacin 61/106 (57.54%). (Table 3)

DISCUSSION

This study was based on 230 children below five years presenting with diarrhoea in various out and in patient department with special reference to enteropathogenic (EPEC) strain of *E. coli*. The overall male to female ratio in our study was found to be 1.25:1. In various studies for



■ EPEC(50%) ■ ETEC(4.7%) ■ EAEC(10.4) ■ DAEC(34.9)
Figure-1: Distribution of Diarrheagenic strains of *E. coli*

Age (in months)	<i>Escherichia coli</i> (%)	<i>Klebsiella pneumoniae</i> (%)	<i>Proteus vulgaris</i> (%)	<i>Shigella flexneri</i> (%)
0-12	36 (15.65)	-	-	-
13-24	36 (15.65)	4 (1.73)	-	4 (1.73)
25-36	72 (31.3)	-	4 (1.73)	-
37-48	8 (3.47)	-	-	-
49-60	60 (26.08)	3 (1.3)	3 (1.3)	-
Total	212 (92.17)	7 (3.04)	7 (3.04)	4 (1.73)

Table-1: Distribution of Different Bacteria Isolated from Different Age Group

Age-Group (in Months)	Serotypes of Enteropathogenic <i>E. coli</i> (EPEC)			Total
	O111 (%)*	O26 (%)*	O55 (%)*	
0-12	8 (40)	8 (40)	4 (20)	20
13-24	4 (12.5)	16 (50)	12 (37.5)	32
25-36	16 (50)	8 (25)	8 (25)	32
37-48	8 (100)	-	-	08
49-60	4 (28.6)	10 (71.4)	-	14
Total	40 (37.7)	42 (39.6)	24 (22.6)	106

*Percentages (%) calculated horizontally

Table-2: Serotypes of Enteropathogenic *E. coli* Isolated From Various Age Groups

Antibiotics	Sensitive (%)	Moderate Sensitive (%)	Resistant (%)
CIP (Ciprofloxacin)	38 (35.84)	30 (28.3)	38 (35.84)
GE (Gentamicin)	79 (74.52)	16 (15.09)	11 (10.37)
OF (Ofloxacin)	61 (57.54)	28 (26.41)	17 (16.03)
AK (Amikacin)	94 (88.67)	8 (7.54)	4 (3.77)
CXM (Cefixime)	12 (11.32)	52 (49.05)	42 (39.62)
NA (Nalidixic acid)	52 (49.05)	14 (13.20)	40 (37.73)
AMP (Ampicillin)	4 (3.77)	14 (13.20)	88 (83.01)

Percentages (%) calculated horizontally out of a total of 106

Table-3: Antibiotic Susceptibility Pattern of Isolates from EPEC infection

children done under 5 years with diarrhoea the number of male children was more as compared to female children. In a similar studies conducted by Behiry I K et al,¹¹ the ratio of male to female was 1.4:1 while in a study conducted by Dessalegn M et al,¹² the Male: Female ratio was 1.7:1. In this study, it was seen that diarrhoea was more common in children who were mixed fed in first 6 months of life than those who were exclusively breast fed in first 6 months of life. Whether it be developed or developing nation breastfeeding is very important as it decreases not only the risk of diarrhoea but also respiratory tract infection.¹³ It can be also emphasized that with increasing awareness and encouraging breastfeeding, death of an estimated 820,000 children under the age of five could be prevented globally every year.¹⁴ Maximum number of diarrhoeal cases were seen in age group 25-36 months while minimum number of cases were seen in age group 37-48 months. Among different types of bacteria isolated in different age group, *E. coli* was the most common pathogenic organisms (92.17%) followed by *Klebsiella pneumoniae* (3.04%). Also *Proteus Vulgaris* was present in 3.04% of cases and *Shigella* was responsible for 1.73% of cases. Various authors also found out *E. coli* to be most common agent for childhood diarrhoea which was similar to our study.^{11,15} *E. coli* was the most common pathogen (63.48%) isolated from 115 diarrhoeal stool samples in a recent study by Sang W K et al¹⁶ which was followed by *Salmonella* (20%), *Shigella* (13.04%) and *Vibrio Cholerae*. The findings of this study were more or less similar to that of the present study except for the fact that *Salmonella* and *Vibrio* were not isolated in the present

study. Amisano G et al¹⁷ have also reported that *E. coli* was the most common pathogen (46.88%) isolated by them. Serotypes of *E. coli* were studied. *Enteropathogenic E. coli* (EPEC) was found to be most common serotype among the *E. coli* serotypes (50%). Diffusely adherent *E. coli* (DAEC) accounted for 34.9% of cases while EAEC and ETEC accounted for 10.4% and 4.7% of strains respectively. Addy PAK et al¹⁸ also found EPEC to be the most common pathogen in diarrhoeal stool samples from infants in Ghana. Various authors also reported EPEC as the most common isolate in diarrhoeal stool samples. EPEC was the most common pathogen for diarrhoea in children as reported by Aggarwal et al who found them to be 55.1% in their study followed by ETEC 33.3%, EAEC 10.3% and EHEC 1.1%.¹⁵ Maximum percentage of EPEC (30.2%) was seen in the age group 13-24 months and 25-36 months (32/106) each. Next age group in which EPEC was most common was the age group up to 12 months (18.7%). The serotypes of enteropathogenic *E. coli* encountered in the study were O26, O111 and O55. O26 was present in maximum 39.6% of EPEC cases followed by O11 and O55 which accounted for 37.7% and 22.6% of EPEC respectively. A study from Vellore, India conducted by Maiya P P et al¹⁹ in infants and children with acute gastroenteritis showed isolation of serotypes O26, O126, O111 and O128. Addy PAK et al¹⁸ also reported serotype O11, O26 and O55, which were similar to the findings of the present study. The authors however, also reported isolation of additional serotypes viz. O125, O119, O168a, O86a, O169 and O15. Also a study done two decades back by Hill S M et al²⁰, they reported serotype O111 along with other serotypes. Bacterial pathogens mixed with parasitic infection was seen in (19/106) 17.9% of EPEC infection. These were *Trichuris trichiura* (3.8%), *Ascaris lumbricoides* (7.5%), *Hymenolepis nana* (4.7%) and *Giardia lamblia* (3.8%). The reason for finding the parasites in bacterial diarrhoea may be attributed to increased motility of gut during episodes of diarrhoea which helps them to flush out the parasites from the gastrointestinal tract. Majority of strains in the present study were resistant to ampicillin (88/106; 83.01%) followed by cefixime (42/106; 39.62%) and nalidixic acid (40/106; 37.73%). Among the sensitivity pattern seen, organisms were maximally sensitive to Amikacin 94/106 (88.67%). Gentamicin sensitivity was 79/106 (74.52%) while sensitivity to Oflocacin was 61/106 (57.54%). Resistance to Amoxicillin, Nalidixic acid and Ciprofloxacin was reported by Taneja N et al²¹ in 17/20 (85.0%), 14/20 (70.0%) and 10/20 (50.0%) cases respectively. Resistance to Amikacin was seen in 1/20 (5.0%) of cases. These findings are similar to that of our present study. Sang WK et al¹⁶ also reported maximum resistance (95%) to Ampicillin and Co-trimoxazole. Also recent study by Aggarwal et al¹⁵ showed maximum resistance to Ampicillin (90.8%), Nalidixic acid (100%), Ciprofloxacin (72.4%) while aminoglycosides like Amikacin and Gentamicin were highly effective against EPEC in antimicrobial sensitivity testing of children with diarrhoea which is quite similar to our study.

CONCLUSION

Since diarrhoeagenic *E.coli* results in mortality in children less than five years, studies based on characterization of these agents will be of great value in reducing the number of deaths in children. Strict measures need to be taken to reduce over the counter availability of drugs and imparting of health education at every level to prevent self medication. Prompt and proper treatment should be instituted to prevent development of resistant strains.

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