### A BACTERIOLOGICAL STUDY AMONG PATIENTS BELOW FIVE YEARS OF AGE SUFFERING FROM DIARRHOEA AND GASTROENTERITIS WITH SPECIAL REFERENCE TO THE RESPONSIBLE SEROTYPES OF ESCHERICHIA COLI AT A TERTIARY HEALTHCARE HOSPITAL IN EASTERN BIHAR

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ABSTRACT: INTRODUCTION: Diarrhoea is defined as passage of loose, liquid or watery stools. In the developing countries, diarrhea remains a wet season disease with bacteria playing a greater role. The agents responsible for infantile diarrhea may be bacteria, viruses, parasites, malnutrition and drugs. Infectious agents that cause diarrhoeal disease are usually spread by the fecal-oral route. Escherichia coli (E. coli) is a gram-negative bacillus that may be found in the normal intestinal flora of humans and animals, but can also be an important cause of enteric illness. Our present study is an attempt to fulfill this vacuum and investigate the most common pathogens causing these diseases amongst patients in and around a tertiary care hospital of eastern Bihar, with special reference to the serotypes of Escherichia coli. MATERIAL AND METHODS: Samples were collected from both male and female patients below five years of age, admitted to the Pediatrics Department, with complains of diarrhea and gastroenteritis. Samples were processed, identified and antibiotic sensitivity was done as per standard protocol. Serotyping of E. coli isolates was carried out using antisera as per manufacturer's instructions. RESULTS: Different diarrhoeagenic strains of E. coli showed that the maximum numbers of strains were EPEC, followed by DAEC. EAEC and ETEC accounted for only a small percentage of strains. Upon Serotyping the different E. coli isolates from patients with diarrhea, the results showed that the Serotype 026, 0111 and 055 were the predominant serotypes of EPEC. Among the ETEC strains only 2 serotypes were seen viz. 06 and 015. Serotype 044 and 0125 were the only two serotypes of EAEC seen. Among the DAEC, the only serotype was 01. The antibiotic susceptibility pattern of various isolates shows maximum resistance against ampicillin followed by cefixime. Strains were significantly sensitive to amikacin, gentamicin and ofloxacin. CONCLUSION: Our result shows high rate of diarrhoeagenic E. coli among Indian children presenting with diarrhea particularly in Katihar / Kosi region of Bihar. The finding of diverse E. coli subtypes even amongst the small number of E. coli isolates highlights the importance of pathogenic E. coli in cases of diarrhea and gastroenteritis and stresses upon the need for enhanced surveillance of children with diarrhea in these parts of the globe.

KEYWORDS: E. coli, EPEC, ETEC, DAEC, EAEC, Diarrhoea.

**INTRODUCTION:** Diarrhoea is defined as passage of loose, liquid or watery stools. These liquid stools are usually passed more than three times a day. However, it is recent change in consistency and character of stools rather than the number of stools that is more important.<sup>1</sup> In the developing countries, diarrhoea remains a wet season disease with bacteria playing a greater role.

The agents responsible for infantile diarrhoea may be bacteria, viruses, parasites, malnutrition and drugs. These organisms cause diarrhoea alone, or with others.<sup>2</sup> Infectious agents that cause diarrhoeal disease are usually spread by the fecal-oral route, specifically by ingestion of contaminated food / water, or, by contact with contaminated hands. The usual pathogenic mechanisms for infectious diarrhea include toxin production, tissue invasion or invasion of intestinal cells with consequent alteration of their function and reproduction.

Mixed infections with two or more enteropathogens occur in 15% to 20% of cases, but their clinical significance is difficult to interpret, and, may be caused by one or more of the organisms recovered.<sup>3</sup> Escherichia coli (E. coli) is a gram-negative bacillus that may not only be found in the normal intestinal flora of humans and animals, but can also be an important cause of enteric illness. Enteropathogenic strains of E. coli (EPEC) have an association with diarrhea in infants in developing, low-income nations and can cause chronic diarrhea.<sup>4</sup>

Similarly, Enterotoxigenic strains of E. coli (ETEC) are a common cause of traveler's diarrhea and a very important cause of diarrhea in infants and children.<sup>5</sup> Although studies on pathogens causing diarrhea and gastroenteritis have been carried out elsewhere, there are not many reports about pathogens involved in diarrhea and gastroenteritis in recent years, and none whatsoever from Katihar / Kosi region of Bihar in India. Clinical and bacteriological pattern vary not only from region to region but also in the same region from time to time.

A periodic assessment of the problem is vital to any region to understand the current bacteriological pattern and reduce its complication. Furthermore, no study on Escherichia coli serotypes in this region has ever been carried out, and such a study is long overdue. Our present study is an attempt to full fill this vacuum and investigate the most common pathogens causing these diseases amongst patients in and around a tertiary healthcare hospital of eastern Bihar, with special reference to the serotypes of Escherichia coli, which until now is the commonest known diarrhoeagenic, and gastroenteritis-causing bacteria in the under-five year age group.

MATERIALS AND METHODS: The sample population of our study included both male and female patients below five years of age, attending the Out Patients Department (OPD), or admitted to the Pediatrics Department of our College, with complains of diarrhea and gastroenteritis. Patients admitted to the Intensive Care Unit (ICU), Pediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU), and those patients who were in a state of shock were excluded from the study.

Before beginning the study the attendants of the patients were explained the condition of the patient and the purpose of our study. Written consent from the attendants was also obtained. After obtaining consent, detailed clinical history of each patient was taken in details. 120 Stool samples were collected from patients who presented with history of passing more than 2 unformed watery stools during the last 24 hours, or any voiding of watery stools if accompanied with fever, abdominal pain and/or vomiting as per WGO guidelines<sup>6</sup> for our study.

Samples were collected in wide mouthed sterile plastic containers with lid. All the above samples were taken to the Microbiology department for further processing. Processing was done within 3 hours of collection. The collected Stool samples were directly inoculated on Mac Conkey agar, Sorbitol Mac Conkey agar and Deoxycholate Citrate agar. Lactose fermenting non-mucoid colonies on Mac Conkey agar were suspected to be Escherichia coli and processed further.

NLF colonies on DCA were likewise processed to look for Shigella, Salmonella or any other non – lactose fermenting bacteria. The isolated bacterial growth were purified, and identified using standard biochemical tests. Antibiotic Susceptibility Testing (AST) was done on Mueller–Hinton agar (MHA) plates by modified Kirby–Bauer disc diffusion technique using commercially available antibiotic discs.

Interpretation of results was done as per CLSI recommended guidelines. All the media, stains and biochemicals used were obtained from HiMedia, Mumbai, and used as per their guidelines. Further, Serotyping of E. Coli isolates was carried out using antisera obtained from Denka Seiken Co. Ltd., Japan and tests were performed as per the manufacturer's instructions.

**RESULTS:** Out of the total of one hundred and twenty (120) samples collected from children below 5 years of age presenting to the Paediatric Department (both inpatient and outpatient) with diarrhoea and / or gastroenteritis, the percentage of male infants and children with diarrhoea was 55.0% while it was 45.0% in female infants and children with diarrhoea. The male to female ratio was 1.22:1. During the study it was found that only 36 children (30%) were exclusively breastfed whereas mixed feeding was seen in 84 (70.0%) children.

When the incidence of diarrhoea was broken up into seasons, maximum number of cases were found in summer (41.7%), followed by autumn (30%), while the least cases were seen in winter (10%) [Figure 1]. The distribution of different bacteria isolated in different age-groups showed that a majority of E. coli was isolated from age group 25-36 months (30.0%), followed by age group 49-60 months (28.3%) followed by 0-12 months and 13-24 month age group (15.0% each). The least number of organisms were isolated from age group 37-58 months (3.2%) [Table 1].

An analysis of the distribution of different diarrhoeagenic strains of E. coli showed that the maximum number of strains were EPEC (49.1%), followed by DAEC (34.5%). EAEC and ETEC accounted for only 10.9% and 5.5% of strains respectively [Figure 2]. Upon Serotyping the different E. coli isolates from patients with diarrhoea, the results showed that the Serotype 026 (40.7%), 0111 (37%) and 055 (22.2%) were the predominant serotypes of EPEC. Among the ETEC strains only 2 serotypes were seen viz. 06 and 015 (both 50%). Serotype 044 (66.7%) and 0125 (33.3%) were the only two serotypes of EAEC seen.

Among the DAEC, the only serotype isolated was O1 [Table 2]. Enteropathogenic E. Coli serotypes isolated from various age groups showed that Serotype O26 (40.7 %) was the most common serotype of EPEC. This was followed by O111 (37.03%) and O55 (22.2%) [Table 3]. ETEC isolated from age group 13-24, 25-36 and 49-60 months showed 2 (33.33%) strains being isolated from each of these group. Isolation of Enteroaggregative E. Coli (EAEC) in various age groups of children with diarrhoea showed maximum number of EAEC in the age group 49-60 months (83.3%) while two EAEC strains were detected in the 25-36 month age group (16.7%).

Diffusely Adherent E. coli (DAEC) isolated from children with diarrhoea was maximum in the age group 25-36 months (42.1%), followed by the age group 49-60 months (36.9%) and the age group of 0-12 months (21.0%) [Table 4]. The co-infection of diarrhoeagenic E. Coli with various parasites was also seen. Trichuris trichiura was seen in 2 (3.7%) cases of EPEC diarrhoea. Ascaris lumbricoides was seen in 4 (7.4%) and 5 (83.3%) cases of EPEC and ETEC diarrhoea respectively. Hymenolepis nana was seen in 3 (5.6%) cases of EPEC diarrhoea. Giardia lamblia was found in EPEC (3.7%), DAEC (5.3%) and ETEC (16.7%) diarrhoea cases [Table 5].

The antibiotic susceptibility pattern of various isolates shows maximum resistance against ampicillin 100/120 (83.3%) followed by cefixime 48/120 (40.0%). The strains were sensitive to amikacin 106/120 (88.3%) followed by gentamicin 90/120 (75.0%) and ofloxacin 70/120 (58.3%) [Table 6].

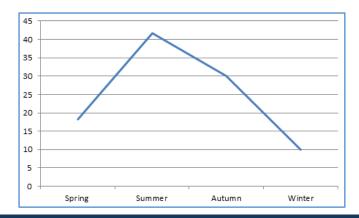


Fig. 1: Seasonal Distribution of Cases with Diarrhoea

Age (in months)	Escherichia coli (%)	Klebsiella pneumoniae (%)	Proteus Vulgaris (%)	Shigella flexneri (%)
0-12	18 (15.0)	-	-	-
13-24	18 (15.0)	2 (1.7)	-	2 (1.7)
25-36	36 (30.0)	-	2 (1.7)	-
37-48	4 (3.2)	-	-	-
49-60	34 (28.3)	2 (1.7)	2 (1.7)	-
Total	110 (91.5)	4 (3.4)	4 (3.4)	2 (1.7)

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

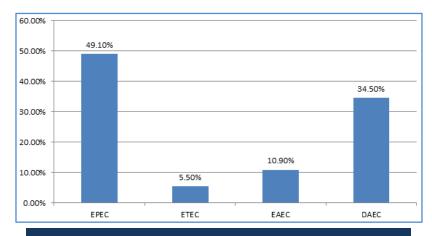


Fig. 2: Distribution of Diarrhoeagenic strains of E. Coli

Diarrhoeagenic		No. of	Percentage	
	E. coli	strains	rereentage	
1.	EPEC (n=54)			
	Serotype 026	22	40.7	
	Serotype 055	12	22.2	
	Serotype 0111	20	37.0	
2.	ETEC (n=6)			
	Serotype 06	03	50.0	
	Serotype 015	03	50.0	
3.	EAEC (n=12)			
	Serotype 044	08	66.7	
	Serotype 0125	04	33.3	
4.	DAEC (n=38)			
	Serotype 01	38	100.0	

Table 2: Isolation of Different Serotypes in E. coli in Patients with Diarrhoea

Age-Group	Serotypes of Enteropathogenic E. coli (EPEC)			
(in Months)	0111 (%)*	026 (%)*	055 (%)*	Total
0-12	4 (40)	4 (40)	2 (20)	10
13-24	2 (12.5)	8 (50)	6 (37.5)	16
25-36	8 (50)	4 (25)	4 (25)	16
37-48	4 (100)	-	-	04
49-60	2 (25)	6 (75)	-	08
Total	20 (37.03)	22 (40.7)	12 (22.22)	54

Table 3: Serotypes of Enteropathogenic E. coli Isolated From various Age Groups

<sup>\*</sup>Percentages (%) calculated horizontally

Age in Months	EPEC (%)	ETEC (%)	EAEC (%)	DAEC (%)
0-12	10 (18.5)	-	-	8 (21.0)
13-24	16 (29.6)	2 (33.3)	-	-
25-36	16 (29.6)	2 (33.3)	2 (16.7)	16 (42.1)
37-48	04 (7.4)	-	-	-
49-60	08 (14.8)	2 (33.3)	10 (83.3)	14 (36.9)
Total	54 (49.1)	6 (5.5)	12 (10.9)	38 (34.5)

Table 4: Isolation of Diarrhoeagenic strains of E. coli in Various Age Groups

Diarrhoeagenic	Distribution of parasites (n = 19)			
E. coli	Trichuris trichiura (%)*	Ascaris Hymenolepis lumbricoides (%)* nana (%)*		Giardia lamblia (%)*
EPEC [n=54]	2 (3.7)	4 (7.4)	3 (5.6)	2 (3.7)
ETEC [n= 6]	0	5 (83.3)	0	1 (16.7)
EAEC [n= 12]	0	0	0	0
DAEC [n=38]	0	0	0	2 (5.3)
Total [n=110]	2 (1.8)	9 (8.2)	3 (2.7%)	5 (4.5)

Table 5: Co-infection of Diarrhoeagenic E. coli with parasites

Percentages (%) calculated horizontally

Sensitive (%)	Moderate Sensitive (%)	Resistant (%)
44 (36.7)	34 (28.3)	42 (35.0)
90 (75.0)	18 (15.0)	12 (10.0)
70 (58.3)	32 (26.7)	18 (15.0)
106 (88.3)	8 (6.7)	6 (5.0)
14 (11.7)	58 (48.3)	48 (40.0)
58 (48.3)	16 (13.3)	46 (38.3)
4 (3.3)	16 (13.3)	100 (83.3)
	44 (36.7) 90 (75.0) 70 (58.3) 106 (88.3) 14 (11.7) 58 (48.3)	Sensitive (%)         Sensitive (%)           44 (36.7)         34 (28.3)           90 (75.0)         18 (15.0)           70 (58.3)         32 (26.7)           106 (88.3)         8 (6.7)           14 (11.7)         58 (48.3)           58 (48.3)         16 (13.3)

Table 6: Antibiotic Susceptibility Pattern of Isolates

Percentages (%) calculated horizontally out of a total of 120

**DISCUSSION:** The results of the study were based upon the findings of one hundred and twenty (120) samples collected from children below 5 years of age presenting to the Pediatric (both inpatient and outpatient) department. In a study conducted by Dessalegn Met al,<sup>7</sup> the Male: Female ratio was 1.7:1. The overall male to female ratio in our study was found to be 1.2:1.

While little data is available based upon incidence of diarrhea caused by E. coli in recent years, Dessalegn M et al<sup>7</sup> have also reported that only 11.2% of exclusively breastfed children in Ethiopia had diarrhea as compared to 23.5% children who were partially breastfed. In this study, out of total of one hundred and twenty (120) infants and children with diarrhea, percentage of patients were noted among those who were exclusively breastfed for 6 months and those who were mixed fed for first 6 months and it was found that only 36 children (30%) were exclusively breastfed whereas 84 children (70%) were mixed fed.

Our study showed a maximum number of cases were found in summer season (41.7%) followed by autumn (30%) and least in winter (10%).

This is probably due to the fact that diarrhea in tropical and developing countries are mainly caused by bacteria which thrive better in hot and humid conditions.

Flies and other mechanical vectors also thrive better in summer months. Ahmad FS et al<sup>8</sup> in their study on diarrhoeal patients below five years reported that point prevalence was highest during summer months (42.6%) and lowest during winter months (13.5%), which is almost similar to the present study.

Escherichia coli (110/120; 91.7%) was the most common isolate followed by Klebsiella pneumonie (4/120; 3.3%), Proteus vulgaris (4/120; 3.3%) and Shigella flexneri (2/120; 1.7%) respectively. A recent study conducted by Sang W K et al<sup>9</sup> shows E. coli to be the most common pathogen isolated from 115 diarrhoeal stool samples. The percentage of E. coli was 63.48% (73/115) 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 Get al<sup>10</sup> have also reported that E. coli was the most common pathogen (46.88%) isolated by them.

In our study, majority of E. coli were isolated from age group 25-36 months (36/120; 30%) followed by age group 49-60 months (34/120; 28.3%), while the least number of organisms were isolated from age group 37-48 months (4/120; 3.2%). The other organisms that were isolated were Klebsiella pneumonia, 2 strains each (1.7%) from age group 13-24 months and 49-60 months. Likewise Proteus vulgaris was isolated from age group 25-36 months (1.7%) and 49-60 months (1.7%). Shigella flexneri was isolated from age group 13-24 months (1.7%). Bhan MK et al<sup>11</sup> found incidence of persistent diarrhea to be highest in age group 0-11 months (31 per 100 child years). The findings of the study is somewhat different from those of the present study probably because this was a community based study, as compared to the present study which was hospital based.

Mixed infection with parasites and bacterial pathogens were seen in (19/120; 15.8%) of cases with diarrhea. Ascaris lumbricoides was the most commonly encountered parasite (9/19; 47.4%) followed by Giardia lamblia (5/19; 26.3%). Out of the 120 children who presented with diarrhea, Ascaris was seen in (9/120; 7.5%), Giardia in (5/120; 4.2%), Hymenolepis nana in (3/120; 2.5%) and Trichuris trichiura in (2/120; 1.7%) of cases. The reason for finding parasites in cases of bacterial diarrhea could be due to increased gastric and intestinal motility during episodes of diarrhea which helps in flushing out the parasites from the gastrointestinal tract.

Vilchez S et  $al^{12}$  also reported the increased incidence of co-infection of various diarrhoeagenic E. coli with parasites, viruses and other bacterial pathogens were higher in patients with diarrhea than in patients without diarrhea. Addy PAK et  $al^2$  reported parasitic infection in patients with diarrhea in the following order – Ascaris 11.1%, Cryptosporidium 8.0%, Giardia lamblia 3.7%, Trichomonas hominis 1.9%, Trichuris trichiura 0.6% and hookworm in 0.6% of cases. Amongst E. coli isolated (110/120; 91.7%), distribution of various diarrhoeagenic strains of E. coli were as follows, EPEC (54/110; 49.1%), DAEC (38/110; 34.5%).

The percentage of strains of EAEC and ETEC were 10.9%~(12/110) and 5.5%~(6/110) respectively. Nweze EL et al<sup>13</sup> isolated 102 strains of E. coli from 520 stool samples of patients suffering from diarrhea. Out of these, 29.4% were EPEC, 27.4% were EAEC, 21.5% were ETEC and 14.7% were EIEC. Maiya PP et al<sup>14</sup> found EPEC, Salmonella and Shigella as the commonest bacterial isolate in infants and children with acute gastroenteritis.

Addy PAK et  $al^2$  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.

In the present study, 54/110 E. coli isolated were EPEC. Maximum percentage of EPEC (29.6%) was seen in the age group 13-24 months and 25-36 months (32/110) each. Next age group in which EPEC was most common was the age group up to 12 months (18.6%). The serotypes of E. coli encountered in the study were 026, 0111 and 055. 026 was the most common serotype accounting for (22/54; 40.7%) of EPEC. 011 and 055 accounted for (20/54; 37.03%) and (12/54; 22.22%) of EPEC respectively.

Maiya PP et al<sup>14</sup> isolated serotype 026, 0126, 0111 and 0128 from infants and children suffering from acute gastroenteritis in Vellore, India. Toledo MRF et al<sup>15</sup> reported the following serotypes in their study group – 026, 055, 086, 0111ab, 0111ac, 0119, 0125, 0126, 0127, 0128ab and 0128ac. The diverse serotypes encountered by them was probably due to the fact that their study group included 550 children with diarrhea and 129 controls (EPEC was isolated from the control group) which is a much larger sample size as compared to the present study.

The difference in the serotypes isolated could also be due to the fact that the study was conducted in Brazil which is epidemiologically different from India. Addy PAK et al<sup>2</sup> also reported serotype 011, 026 and 055, which were similar to the findings of the present study. The authors however, also reported isolation of additional serotypes viz. 0125, 0119, 0168a, 086a, 0169 and 015. The ETEC serotypes isolated were as follows: 06 in (3/6; 50%) and 015 in (3/6; 50%). Taneja N et al<sup>16</sup> isolated 20 LT – ETEC out of which 40% were serotype 015.

The 12 EAEC isolates in the present study belonged to serotype 044 (8/12; 66.7%) and serotype 0125 (4/12; 33.3%). All DAEC isolates (38) belonged to serotype 01. Meraz MI et al<sup>17</sup> in their retrospective study on isolates of diarrheagenic E. coli identified DAEC in (41/924; 4.4%) of cases. The serotypes of DAEC encountered by them were 01, 0114, 086a, 0116, 08, 028ac, 0143 and 018. The rest of the strains were untypable by "0" antisera. Majority of strains in the present study were resistant to ampicillin (100/120; 83.3%) followed by cefixime (48/120; 40.0%), nalidixic acid (46/120; 38.3%) and ciprofloxacin (42/120; 35.0%).

Resistance to amikacin was only (6/120; 5.0%). Taneja N et al $^{16}$  also reported resistance to amoxicillin, nalidixic acid and ciprofloxacin 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 al $^9$  also reported maximum resistance (95%) to ampicillin and co-trimoxazole.

**CONCLUSION:** Our result shows high rate of diarrhoeagenic E. coli among Indian children presenting with diarrhea particularly in Katihar / Kosi region of Bihar. The finding of diverse E. coli subtypes even amongst the small number of E. coli isolates highlights the importance of pathogenic E. coli in cases of diarrhea and gastroenteritis, and stresses upon need for enhanced surveillance of children with diarrhea in these parts of the globe. Therefore research on diarrhoeagenic E. coli remains an important task to pursue.

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