

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 BIHARSweta Muni¹, Sangeeta Dey², Krishan Nandan³, Priyanka Paul Biswas⁴, Aninda Sen⁵**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: INTRODUCTION: Diarrhoea is defined as passage of loose, liquid or watery stools. 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. 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 fulfil 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 diarrhoea 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 diarrhoea, the results showed that the Serotype O26, O111 and O55 were the predominant serotypes of EPEC. Among the ETEC strains only 2 serotypes were seen viz. O6 and O15. Serotype O44 and O125 were the only two serotypes of EAEC seen. Among the DAEC, the only serotype was O1. 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 diarrhoea 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 diarrhoea and gastroenteritis and stresses upon the need for enhanced surveillance of children with diarrhoea 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.¹ In the developing countries, diarrhoea remains a wet season disease with bacteria playing a greater role.

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The agents responsible for infantile diarrhoea may be bacteria, viruses, parasites, malnutrition and drugs. These organisms cause diarrhoea alone, or with others.² 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 diarrhoea 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.³ *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 diarrhoea in infants in developing, low-income nations and can cause chronic diarrhoea.⁴

Similarly, Enterotoxigenic strains of *E. coli* (ETEC) are a common cause of traveler's diarrhoea and a very important cause of diarrhoea in infants and children.⁵ Although studies on pathogens causing diarrhoea and gastroenteritis have been carried out elsewhere, there are not many reports about pathogens involved in diarrhoea 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 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 complaints of diarrhoea 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 guidelines⁶ 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.

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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 O26 (40.7%), O111 (37%) and O55 (22.2%) were the predominant serotypes of EPEC. Among the ETEC strains only 2 serotypes were seen viz. O6 and O15 (both 50%). Serotype O44 (66.7%) and O125 (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].

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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].

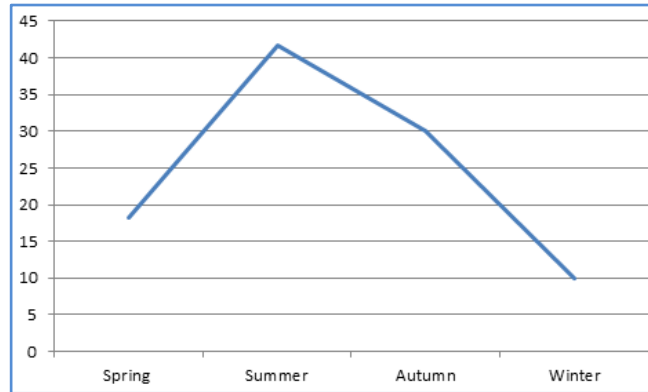


Fig. 1: Seasonal Distribution of Cases with Diarrhoea

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

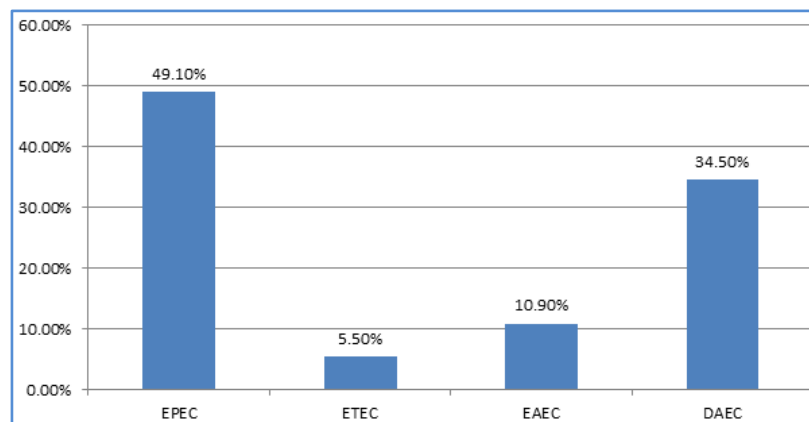


Fig. 2: Distribution of Diarrhoeagenic strains of E. Coli

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Diarrhoeagenic E. coli	No. of strains	Percentage
1. EPEC (n=54)		
Serotype O26	22	40.7
Serotype O55	12	22.2
Serotype O111	20	37.0
2. ETEC (n=6)		
Serotype O6	03	50.0
Serotype O15	03	50.0
3. EAEC (n=12)		
Serotype O44	08	66.7
Serotype O125	04	33.3
4. DAEC (n=38)		
Serotype O1	38	100.0

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

Age-Group (in Months)	Serotypes of Enteropathogenic E. coli (EPEC)			Total
	0111 (%)*	026 (%)*	055 (%)*	
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

*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

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Diarrhoeagenic E. coli	Distribution of parasites (n = 19)			
	Trichuris trichiura (%)*	Ascaris lumbricoides (%)*	Hymenolepis 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

Antibiotics	Sensitive (%)	Moderate Sensitive (%)	Resistant (%)
CIP (Ciprofloxacin)	44 (36.7)	34 (28.3)	42 (35.0)
GE (Gentamicin)	90 (75.0)	18 (15.0)	12 (10.0)
OF (Ofloxacin)	70 (58.3)	32 (26.7)	18 (15.0)
AK (Amikacin)	106 (88.3)	8 (6.7)	6 (5.0)
CXM (Cefixime)	14 (11.7)	58 (48.3)	48 (40.0)
NA (Nalidixic acid)	58 (48.3)	16 (13.3)	46 (38.3)
AMP (Ampicillin)	4 (3.3)	16 (13.3)	100 (83.3)

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 M et al,⁷ 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 diarrhoea caused by E. coli in recent years, Dessalegn M et al⁷ have also reported that only 11.2% of exclusively breastfed children in Ethiopia had diarrhoea 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 diarrhoea, 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%).

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This is probably due to the fact that diarrhoea 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 al⁸ 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 pneumoniae* (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 al⁹ 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 G et al¹⁰ 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 pneumoniae*, 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 al¹¹ found incidence of persistent diarrhoea 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 diarrhoea. *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 diarrhoea, *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 diarrhoea could be due to increased gastric and intestinal motility during episodes of diarrhoea which helps in flushing out the parasites from the gastrointestinal tract.

Vilchez S et al¹² 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 diarrhoea than in patients without diarrhoea. Addy PAK et al² reported parasitic infection in patients with diarrhoea 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%), EAEC (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 al¹³ isolated 102 strains of *E. coli* from 520 stool samples of patients suffering from diarrhoea. Out of these, 29.4% were EPEC, 27.4% were EAEC, 21.5% were ETEC and 14.7% were EIEC. Maiya PP et al¹⁴ found EPEC, *Salmonella* and *Shigella* as the commonest bacterial isolate in infants and children with acute gastroenteritis.

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Addy PAK et al² 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 O26, O111 and O55. O26 was the most common serotype accounting for (22/54; 40.7%) of EPEC. O11 and O55 accounted for (20/54; 37.03%) and (12/54; 22.22%) of EPEC respectively.

Maiya PP et al¹⁴ isolated serotype O26, O126, O111 and O128 from infants and children suffering from acute gastroenteritis in Vellore, India. Toledo MRF et al¹⁵ reported the following serotypes in their study group – O26, O55, O86, O111ab, O111ac, O119, O125, O126, O127, O128ab and O128ac. The diverse serotypes encountered by them was probably due to the fact that their study group included 550 children with diarrhoea 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 al² 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. The ETEC serotypes isolated were as follows: O6 in (3/6; 50%) and O15 in (3/6; 50%). Taneja N et al¹⁶ isolated 20 LT – ETEC out of which 40% were serotype O15.

The 12 EAEC isolates in the present study belonged to serotype O44 (8/12; 66.7%) and serotype O125 (4/12; 33.3%). All DAEC isolates (38) belonged to serotype O1. Meraz MI et al¹⁷ in their retrospective study on isolates of diarrhoeagenic *E. coli* identified DAEC in (41/924; 4.4%) of cases. The serotypes of DAEC encountered by them were O1, O114, O86a, O116, O8, O28ac, O143 and O18. The rest of the strains were untypable by “O” 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 al¹⁶ 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 al⁹ 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 diarrhoea 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 diarrhoea and gastroenteritis, and stresses upon need for enhanced surveillance of children with diarrhoea in these parts of the globe. Therefore research on diarrhoeagenic *E. coli* remains an important task to pursue.

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