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Enteroaggregative *Escherichia coli* Infections in Bangladeshi Children: Clinical and Microbiological Features

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ABSTRACT

Escherichia coli **isolated from 389 children, less than 1 - year of age with diarrhoea, were analyzed for the presence of Enteroaggregative *E. coli* (EAggEC) by their pattern of adherence to HeLa cells. EAggEC were isolated from 58 (14.8%) children either as a sole pathogen or in combination with other enteric pathogens. In 60% of these children EAggEC infection occurred in the second half of infancy (7 - 12 m). Thirty - eight of 47 children having EAggEC as a sole pathogen had watery diarrhoea along with vomiting (87%) and dehydration (74%). In contrast, 9 of the 47 cases had mucoid diarrhoea with infrequent vomiting and dehydration and frequent abdominal pain. Children infected with EAggEC were successfully rehydrated with oral rehydration solution (ORS) alone. Seventy - one percent of the EAggEC strains were resistant to more than three antibiotics. It was evident by phage pattern that various EAggEC strains were present in the population. The results indicated that infections with EAggEC may have a role in the development of diarrhoea among children less than 1 - year of age in Bangladesh.**

Key words: Enteroaggregative *E. coli*; Childhood diarrhoea; Diarrhoea; Microbiology; Causative organism; Aetiology.

INTRODUCTION

Enteroaggregative *Escherichia coli* (EAggEC) is a putative new category of diarrhoeagenic *E. coli*, characterised by a definite aggregative pattern ("stacked brick - like") of adherence in which the organisms line up in a pattern around the tissue culture cells, as well as on the vacant space between the cells (1). In humans, this group of *E. coli* has been isolated more frequently from diarrhoeal stools than from non - diarrhoeal stools (2 - 4).

A prospective study of acute and persistent diarrhoea in a cohort of rural children in India has shown that EAggEC is significantly more often associated with persistent diarrhoea than with acute diarrhoea (5). The mechanism(s) by which these bacteria cause diarrhoea is not clear. In rabbits and rats, EAggEC induces a distinct histopathologic lesion in the intestine, suggesting mucosal damage (6). In this paper, we describe the clinical and microbiological features of EAggEC infection in Bangladeshi children with diarrhoea.

MATERIALS AND METHODS

At the Treatment Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) more than 70,000 patients are treated each year. A surveillance system was introduced in 1980 to carry out a thorough investigation of these patients, of whom every 25th patient is sampled. Of the patients enrolled in the surveillance system between March and August of 1988, 389 children aged 0 - 12 months were included in this study.

After selection, the patient was examined by a physician, and a stool and/or a rectal swab sample (RS) was collected for laboratory investigation. The patient's height, weight, and mid - upper - arm circumference (MUAC) were measured and recorded. Stools were examined microscopically for parasites, and stools or rectal swab samples were cultured for isolating bacterial pathogens (8). Three

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colonies that are typical of *E. coli* from each patient's stool were stored on blood agar base slants. *E. coli* colonies were tested for the presence of pathogenic *E. coli* using DNA probes for Shiga-like toxins (SLTI and SLTII), heat-stable toxin (ST), heat-labile toxin (LT), enteropathogenic *E. coli* (EPEC) adhesiveness factor (EAF), diffuse adhesiveness (DA), and invasiveness. Colony blot hybridization was carried out with the above radio-labelled probes under stringent conditions as described by Escheverria *et al.* (9). All *E. coli* colonies were tested for adherence capability to HeLa cells (10) according to methods described by Nataro *et al.* (1). All clinical and microbiological data were collected. Statistical analysis was carried out using the Fisher's exact test.

All EAggEC strains were tested for susceptibility to antimicrobial drugs (11) using commercially available disks (BBL, Microbiology System) at the following concentrations (in micrograms per disk): tetracycline 30, ampicillin 10, chloramphenicol 30, streptomycin 10, nalidixic acid 30, trimethoprim-sulfamethoxazole (TMP-SMX) 1.25 and 23.75 respectively, kanamycin 30, gentamicin 10, and cephalothin 30.

The phage sensitivity of EAggEC strains was tested with 31 phages as described by Monsur *et al.* (12). The phage patterns were determined to identify the different strains of EAggEC, based on the assumption that colonies giving an identical phage pattern belong to the same strain (13).

RESULTS

EAggEC were isolated from 58 (15%) of 389 children, aged 0-12 months, of whom 47 (81%) children had EAggEC as the only pathogen. In the remaining 11 cases, EAggEC was isolated along with other pathogens: *Shigellae* (1.7%), *V. cholerae* (3.4%), *Aeromonas* (1.7%), diffuse adherent *E. coli* (10.3%), and enteropathogenic *E. coli* (1.7%). None of these EAggEC isolates hybridized with the probes for other diarrhoeagenic *E. coli* and none of the probe-positive *E. coli* strains showed aggregative adherence to HeLa cells. No parasite was detected by microscopic examination of stools. Data of children who excreted only EAggEC were analyzed for several demographic, clinical, and laboratory features. Table I shows the classification of the patients according to the appearance of their stools (into two groups): watery diarrhoea, i.e. without mucous or blood, and mucoid diarrhoea, i.e. diarrhoea with mucous and/or blood.

Eighty-one percent of the patients with EAggEC infection presented with watery diarrhoea and 19% with mucoid diarrhoea. Patients with mucoid diarrhoea differed from the group with watery diarrhoea in several respects (Table I). Significantly more children at the age of 7-12 months had mucoid diarrhoea ($p<0.05$). The watery diarrhoea patients had illnesses commonly marked by significantly more dehydration ($p<0.05$) and vomiting ($p<0.001$) than those of the patients with mucoid diarrhoea. Abdominal pain was significantly more pronounced ($p<0.02$) in children with mucoid diarrhoea. Children with watery diarrhoea had to be admitted for rehydration more often than those with mucoid diarrhoea ($p<0.04$).

Table I. Demographic, Clinical, and Laboratory Features of Patients with EAggEC Infection by Gross Stool Appearance

Features	Watery diarrhoea (n=38)	Mucoid diarrhoea (n=9)	
	Percentages		
Age group (months)			
0-6	47.3	11.1	
7-12	52.6	88.8	$p<0.05$
Duration of diarrhoea (days)			
<7	71.0	89.0	
≥7	29.0	11.0	
Dehydration status			
None	26.3	77.7	
Mild	55.3	11.1	
Moderate	15.8	11.1	
Severe	2.6	0.1	$p<0.05$
Vomiting			
Yes	86.8	33.3	$p\leq0.002$
Abdominal pain			
Yes	16.2	55.6	$p<0.02$
Body temperature			
>37.8 °C	28.9	11.1	
Wt/Ht (% median of NCHS standards)			
<90%	37.0	57.1	
≥90%	63.0	42.9	
MUAC			
<11 cm	21.0	0.0	
≥11 cm	79.0	100.0	
Feeding status			
With breast-milk	86.5	87.5	
Without breast-milk	13.5	12.5	
Stool microscopy			
Red cells 0/HPF	93.3	66.7	
1-10/HPF	6.6	33.3	
Leucocytes 0-10/HPF	43.3	50.0	
10+/HPF	56.6	50.0	
Macrophage 0-5/HPF	6.6	0.0	
5+/HPF	93.3	100.0	
Disposition			
Ambulatory	28.9	66.7	$p<0.04$
Admitted	71.1	33.3	

NCHS = National Center for Health Statistics
MUAC = Mid-upper arm circumference
HPF = High power field

No difference in the duration of diarrhoea, fever, and nutritional and feeding status was noted between the watery diarrhoea and mucoid diarrhoea groups. Stool microscopy showed the presence of a similar number of leucocytes in patients with watery diarrhoea and mucoid diarrhoea. Significantly more macrophages were present in the stools of children infected with EAggEC.

Table II shows that breast-fed infants had a significantly shorter duration of diarrhoea than those who were not breast fed ($p < 0.03$). Similarly, well-nourished children had shorter durations of diarrhoea compared with their under-nourished counterparts ($p < 0.04$).

Table II. Effect of Various Parameters on the Duration of Diarrhoea Before Coming to Hospital

Features		Diarrhoeal illnesses: Less than 7 days (n=35)	Diarrhoeal illnesses: More than 7 days (n=12)	
		Percentages		
Breastfed		94.0	64.0	$p < 0.03$
Non-breastfed		6.0	36.0	
Wt/Ht (% median of NCHS std)	<90%	32.0	67.0	$p < 0.04$
	≥90%	68.0	33.0	
MUAC				
	<11 cm	14.0	25.0	
	≥11 cm	86.0	75.0	

Fisher's exact test.

Table III shows that 39 (85%) children were markedly improved with rehydration therapy alone and in 6 (13%) children diarrhoea persisted for more than 14 days. Of the 39 children who improved, 82% were successfully rehydrated with ORS alone, and 15% were treated with ORS first and then given intravenous infusions. Illness continued in 6 children who received antimicrobial agents, such as ampicillin, tetracycline, or metronidazole empirically before coming to the treatment centre. However, the antibiotic susceptibility pattern has shown that 70.7% of the strains were resistant to 3 or more antibiotics (Table IV).

The phage sensitivity pattern showed that EAggEC strains isolated from this group of children did not have any consistent phage patterns. However, in 8 children *E. coli* colonies isolated from the same patient showed similar phage patterns, but patterns varied from patient to patient. EAggEC strains isolated from 14 children were not susceptible to any of the 31 phages used.

DISCUSSION

This study showed that EAggEC was associated with 15% of diarrhoeal episodes during infancy either as a sole agent or in combination with other enteropathogens. Bhan *et al.* (5) and Cravioto *et al.* (14) have shown that EAggEC infection is more often associated with persistent diarrhoea than acute diarrhoea. In our study, analysis of clinical findings of infants infected with EAggEC showed that 10% of the children had persistent diarrhoea (i.e. diarrhoea that continued for ≥15 days). However, more than 54% of children in the studies of Bhan *et al.* (5) and Cravioto *et al.* (14) were more than 1-yr of age, whereas the present study was conducted on children less than 1-yr of age.

Table III. Outcome of Rehydration in Infants Infected with EAggEC

Outcome	Total (n=47) (%)	% of children rehydrated with: ORS and IV (n=6)	ORS alone (n=40)	Regular food and drink (n=1)
Cured	39 (83)	15	82	3
Illness continued	6 (13)	0	100	0
Refused treatment	2 (4)	0	100	0

We observed that EAggEC was associated with both watery and mucoid diarrhoeas. Similar clinical findings had also been reported by others. Bhan *et al.* (5) and Cravioto *et al.* (14) have shown that children infected with EAggEC had gross blood in their stools. We observed that 19% of children had mucoid diarrhoea. Clinical observations of mucoid diarrhoea and abdominal pain suggested the invasive nature of this organism, which is also supported by the evidence of histopathological findings in animals indicating by mucosal damage (6). Although, EAggEC has been shown to be associated with only persistant diarrhoea in the studies conducted in northern India (5) and Mexico (14), the study that was carried out in Chile (3) found an association of the organisms with acute diarrhoea. Although a case-control study might shed more light on the association of EAggEC with acute diarrhoea, we believe that EAggEC could be a cause of acute diarrhoea in our children because they were the only putative pathogens isolated: we did not look for *cryptosporidium* and rotavirus in these children. However, our previous experience at the ICDDR,B suggests that the prevalence of *cryptosporidium* and rotavirus infection is less during the summer, when this study was done (16).

Table IV. Antimicrobial Susceptibility Patterns of EAggEC Strains

Antibiotics	Percentage resistant
Ampicillin	91.4
Tetracycline	100.0
Streptomycin	82.8
Gentamicin	0.0
Kanamycin	1.7
Trimethoprim – sulfamethoxazole	51.7
Nalidixic acid	10.3
Cephalothin	52.2
Chloramphenicol	52.2

NOTE : Resistant to <3 antibiotics = 17 strains (29.3%)
Resistant to ≥3 antibiotics = 41 strains (70.7%)

We have found the presence of leucocytes in stools in similar proportions of patients with acute diarrhoea and mucoid diarrhoea. This finding may seem anomalous, but may be related to the fact that even healthy people in the tropics suffer from tropical colonopathy (17) which might contribute to the shedding of leucocytes in the stool. Some of our data suggest that patients with proven cholera have leucocytes in their stools (unpublished observation).

Our study showed that 60% of the children infected with EAggEC were 7 – 12 months of age. It was observed that children who were breast – fed (94%) and well – nourished had shorter durations of diarrhoea (< 7 days). A similar paucity of infection with *Shigella* and rotavirus diarrhoea has been observed in young infants (< 7 monts) in developing countries, and breast – feeding has been postulated to provide protection (18). In the present study, dehydration (74%) and vomiting (87%) were important features among children with EAggEC associated watery diarrhoea. It is important to note that most of the patients with EAggEC infection were successfully rehydrated with ORS alone. Studies of antimicrobial susceptibility patterns suggested that many EAggEC strains (71%) were resistant to many antimicrobials. Moreover, recent evidence has shown that the use of inappropriate and inadequate drugs may enhance the disease process resulting in prolongation of illness (19). This study indicated that EAggEC – associated diarrhoea in our children was due to many different strains of EAggEC as indicated by the phage susceptibility patterns of the isolates. Therefore, a surveillance system to monitor the antibiotic susceptibility of different diarrhoeagenic strains of EAggEC needs to be instituted.

A considerable amount of epidemiological data has been published by several investigators (3,4,5,14) to implicate EAggEC as a cause of diarrhoea in certain geographic regions, namely India (4,5). Mexico (14), and Chile (3). In the present study, identification of a large number of EAggEC from children with diarrhoea suggests that this organism may be an important aetiological agent of diarrhoea.

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