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RESEARCH ARTICLE

A STUDY OF BACTERIAL ENTEROPATHOGENS CAUSING ACUTE DIARRHEA IN CHILDREN UNDER 5 YEARS WITH REFERENCE TO DIARRHEAGENIC *E.COLI*

***[1]Dr. Shwetha, J. V., [1]Dr. Sneha, K. C., [2]Deepa, S. and [1]Dr. Ambica, R.**

[1]Department of Microbiology, Bangalore Medical College and Research Institute, Bangalore, Karnataka, India
[2]MBBS student, Bangalore Medical College and Research Institute, Bangalore, Karanataka, India

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ABSTRACT

Purpose: Diarrhea remains a major public health problem in the developing world. Diarrhea is an important cause of morbidity and mortality in children. This study investigated the bacterial enteropathogens causing acute diarrhea in Children Under 5 years with special reference to Diarrheagenic E.Coli.

Materials and Methods: The study was carried at a tertiary care hospital, children under 5 years of age presenting with acute diarrhea were evaluated. The stool sample of the cases were investigated for parasitic and bacterial pathogens. The E. coli isolated from these samples were tested by molecular method to detect diarrheagenic E.coli.

Results: Out of 50 stool samples investigated, 16 (32%) sample yielded E.coli, 5 (10%) were Vibrio cholerae, 3 (6%) were Salmonella species and 2 (4%) were Shigella species. The Giardia species was seen in 5 (10%) cases, 4 (8%) showed eggs of A.lumbricoides and 3 (6%) showed eggs of hookworm and 1 (2%) showed E.histolytica cysts. Mixed infection was
seen in 3 cases. Other stool samples showed only normal commensals. All 16 E.coli isolates were negative for diarrheagenic E.coli by molecular method typing.

Conclusion: In this study, E.coli was the most common bacteria isolated from stool samples of acute diarrhea cases in children. But caution has to be exercised while reporting it as a pathogen as none of the E.coli isolates in this study were diarrheagenic E.coli. The misinterpretation may result in inadvertent use of antibiotics contributing to drug resistance.

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INTRODUCTION

Diarrhea remains a major public health problem in the world. Diarrhea is an importantcause of morbidity and mortality in children from developing countries. It is the second leadingcause of death in children under five years old. Globally, every year there are nearly 1.7 billioncases and 760 000 deaths of children under five due to diarrhea (www.who. int/mediacentre/factsheets/fs330/en/). In India every year almost 10% infants and 14% in the age group of 0-4years die due to diarrhea. (Isalkar, 2010) The main etiology of the diarrhea is various bacteria (such as *Escherichia coli, (E.coli), Salmonella species, Shigella species., Vibrio cholerae (V.cholerae), Campylobacter jejuni, Yersinia enterocolitica* and *Aeromonas spp*.), enteroparasites (*Giardia spp*, *Cryptosporidium spp*, and *Entamoeba.histolytica*) andviruses (Adenovirus, Norwalk virus, and Rotavirus). (Guerrant *et al*., 1990) After rotavirus, diarrheagenic *Escherichia coli* (DEC) are the second most commoncause of diarrhea in children less than five years. (www.who.int/mediacentre/factsheets/ fs330/en/) There are six categories of DEC., these areenterohemorrhagic (EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAggEC) anddiffusely adherent (DA) *E. coli*. They differ mainly in their virulence genetic makeup. (Nataro and Kaper, 1998) Etiological diagnosis of DEC is not available in routine microbiology laboratories. Thereis paucity of data regarding their prevalence in this region. The knowledge of the relativeimportance and prevalence of the different pathogens in different regions is essential for theproper management of outbreaks, planning and implementation of control measures. (Usein *et al*., 2009)

Though antibiotics are not the first line of management in acute diarrhea, they playsignificant role in management of severe cases and in outbreaks. (Forbes *et al*., 2007) Antibiotic susceptibility pattern of organisms causing acute diarrhea may vary depending on study population and antibiotic usage in a hospital. The knowledge of recent regional patterns is critical for therapeutic decision making.

Corresponding author: Dr. Shwetha, J. V.
Bangalore Medical College and Research Institute, Bangalore, Karnataka, India.

MATERIALS AND METHODS

This study was carried out in 2014at a tertiary care hospital attached to a Medical College. Children under 5 years of age presenting with acute diarrhea (WHO definition) were evaluated under this study after obtaining the informed consent from the guardians/parents. History and the findings of clinical examination including the severity of dehydration were documented using proforma. The hospitalized children were followeduntil discharged. Ethical clearance was obtained from the institutional ethics committee before conducting the study.

Inclusion Criteria

All children under 5 years presenting with complaints of acute diarrhea were included Acute diarrhea defined as the passage of three or more loose stools within the previous 24 hour that conformed to the shape of the container (www.who.int/ mediacentre/factsheets/fs330/en/).

Exclusion Criteria

- Children above the age of 5 years
- Children less than 5 years with acute diarrhea but treated with antibiotics in the recent past or after the onset of diarrhea
- Children less than 5 years with persistent or chronic diarrhea

Freshly passed stool samples were collected in a clean screw-capped wide mouthed container and transported to the microbiology laboratory without delay. A transport medium such as Cary-Blair was used if a delay in transport to laboratory was anticipated. (Forbes *et al*., 2077)In the microbiology laboratory, samples were processed as follows (Forbes *et al*., 2077):

- The gross examination of the stool sample for consistency, colour and presence of visible blood, mucus or segments of parasites was done.
- The wet mount with saline and iodine were done to detect pus cells, RBCs and ova or cyst of parasites.
- Hanging drop preparation was done in case of liquid stools to detect darting motility which can suggest the presence of *V. cholera*.
- Direct culture: The stool sample were plated on 5% sheep blood agar, MacConkey agar, sorbitol-MacConkey agar, xylose-lysine–deoxycholate agar (XLD) and thiosulphate citrate bile salt agar (TCBS) (from Hi Media, Mumbai).
- Enrichment culture: Fresh feces were introduced into liquid culture medium such as selenite-F broth/ tetrathionate broth (incubated for 12-18 hours) / alkaline peptone water (6-8 hours).

Following this subculture were made on the solid culture medium used for direct plating.

- All the culture plates were incubated under aerobic condition at 37 ^{o} C for 24 hours.
- After 24 hours, culture plates were examined.
- All the bacterial isolates thus obtained were characterized and identified up to species level by studying their cultural and morphological features, results of Gram stain and standard biochemical tests. (Forbes *et al*., 2077)
- Antimicrobial susceptibility testing were carried out by modified Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI 2013) guidelines using Muller Hinton agar plate (from Hi Media, Mumbai). (CLSI 2013)

Table 1. Molecular Identifiction of Diarrheagenic *E.coli*

S. No.	Ref No.	PCR									Molecular Identification
		est	*elt*	*east*	*bfpa*	*eaf*	*eagg*	*eae*	*stx1*	*stx2*	
1	DCHIP125	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
2	DCHIP126	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
3	DCHIP127	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
4	DCHIP128	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
5	DCHIP129	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
6	DCHIP130	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
7	DCHIP131	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
8	DCHIP132	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
9	DCHIP133	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
10	DCHIP134	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
11	DCHIP135	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
12	DCHIP136	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
13	DCHIP137	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
14	DCHIP138	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
15	DCHIP139	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*
16	DCHIP140	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	Non-pathogenic *E.coli*

The single *E.coli* isolates obtained by culture were sent Regional medical Research centre (ICMR), Belgaum for further molecular typing for virulence genes to categorize into different pathotypes of DEC. (Yu and Kaper, 1992; Gunzburg *et al*., 1995; Franke *et al*., 1994)

RESULTS

Out of 50 children recruited under the study, 14 were male and 36 were female. 20 out of 50 were less than 1 year and 30 were between 1-5 years. Apart from diarrhea, fever was the most common symptom and it was seen in all the cases. 18 children had vomiting along with fever and diarrhea. None of the cases had bloody diarrhea. Out of 50, 12 (24%) had severe malnutrition on clinical examination and dehydration was seen

in 48 (96%) cases. 12 (24%) had mild dehydration, 32 (64%) had moderate dehydration and 4 (8%) had severe dehydration. On microbiology examination, in wet mount, the Giardia species was seen in 5 (10%) cases, 4 (8%) showed eggs of *Ascaris.lumbricoides* and 3 (6%) showed eggs of hookworm and 1 (2%) showed *Entameba.histolytica* cysts. Mixed infection was seen in 3 cases. Two cases had both Giardia species and *A.lumbricoides* eggs in the stool sample. One case had both *A.lumbricoides* and hookwormin the stool sample. out of 50 stool samples cultured, 16 (32%) sample yielded *E.coli*, 5 (10%) were *V. cholerae*, 3 (6%) were Salmonella species and 2 (4%) were Shigella species. Other stool samples grew only normal commensals. None of the *E.coli* tested fermented sorbital in sorbital MacConkey agar. The 16 (32%) sample which yielded single isolates of *E.coli* were tested by molecular methods for identification of genes present in diarrheagenic *E.coli. N*one of the *E.coli* isolates tested were positive for DEC (Table 1). The antimicrobial susceptibility testing of *E.coli i*solates showed that 80% were resistant to ampicillin, 68% were resistant to cephalosporins, 35% were resistant to ciprofloxacin and 15% were resistant to trimethoprim-sulfamethoxazole. The 2 Shigella isolates were sensitive to ampicillin, ciprofloxacin and trimethoprim-sulfamethoxazole.

DISCUSSION

In this study, the predominant organism isolated was *E.coli* followed by *V.cholerae*, Salmonella species and Shigella species. Parasites were seen in 22% of the cases. In a study from Burkino Faso, Bonkoungou *et al* showed that viral etiology for diarrhea was more common in children less than 2 years of age and bacterial pathogens were isolated in all age groups. The diarrhea due to virus was more commoner in dry seasons whereas due to bacteria was commoner in rainy seasons. (Bonkoungou *et al*., 2013)In the present study, viral etiology was not investigated. Around 50% of the cases in our study were below 2 years, viral etiology would be the commoner cause of diarrhea in them as most of the culture isolates showed non-pathogenic *E.coli* or the normal commensals. In other studies, the enteric pathogens were detected in stool samples from both diarrhea cases and non-diarrhea cases. Gascon *et al* undertook case-control study among diarrhea cases in children under five years of age from Ifakara, Tanzania. They concluded that there were high rates of enteropathogens isolated in stool samples from children without diarrhea (52.23%). In their study, Shigella species were the only pathogen statistically related with diarrhea ($p < 0.029$). Enterotoxigenic, Enteroaggregative or Enteropathogenic strains of *E.coli* were not related with diarrhea neither were Giardia or Salmonella species. (Gascón *et al*., 2000) Though in this study, controls were not studied, isolates of *V.cholerae* can be related to diarrhea. Prevalances of different pathogenic *E.coli* have been reported among diarrhea patients from different countries as follows: 23.5 % ETEC from North India, 15% EPEC and 11.5% EAggEC in bloody diarrhea patients from Baghdad, Iran; 14% EPEC from Kuala Lumpur; 18% ETEC and 1% EAggEC in Jakartha, Indonesia; 8% ETEC, 2% EAggEC and EPEC <1% in Sweden. (Taneja *et al*., 2006; Shebib *et al*., 2003; Lee and Puthucheary, 2002; Oyofo *et al*., 2002; Svenungsson *et al*., 2000) The low prevalance of EPEC (6%), ETEC (7.8%) and EAggEC (3.7%) among hospitalized diarrhea patients from Orissa. (Samal *et al*., 2008) Though the predominant organism isolated in this study was *E.coli*, none of them were positive for markers of DEC. The limitations of the study are less sample size and shorter duration of the study. Most of the isolated strains of non-pathogenic were also resistant to many antibiotics. In a study by Literak *et al*, detected antimicrobial resistance in fecal E. coli isolates from healthy urban children of two age groups in relation to their antibiotic therapy. The study was performed in the Czech Republic during 2007 to 2009. Of E.coli isolates from 275 children aged 6 weeks, 36% (n = 177) were resistant to 1 to 7 antibiotics. Of isolates from 253 children aged 6 to 17 years, 24% (n = 205) were resistant to 1 to 5 antibiotics. There was no significant difference in the prevalences of antibiotic-resistant E.coli isolates between these groups of children, even though the consumptions of antibiotics were quite different. (Literak *et al*., 2011)

Conclusion

In this study, *E.coli* was the most common bacteria isolated from stool samples of acute diarrhea cases in children. But caution has to be exercised while reporting it as a pathogen as none of the *E.coli* isolates in this study were DEC. The misinterpretation may result in inadvertent use of antibiotics contributing to drug resistance.

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