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Role of Enteropathogenic Escherichia Coli in Paediatric Diarrhoeas in South India

Gandham Pavani Tilak[1], Jyothi lakshmi G Mudaliar[2]
Department of Microbiology, Medical Institute of Medical Sciences, Hyderabad, India[1]
Department of Microbiology, Gandhi Medical College, Hyderabad, India[2]

Corresponding author: Gandham Pavani Tilak, MD. Assistant Professor, Department of Microbiology, Mediciti Institute of Medical Sciences(MIMS), Ghanpur, Medchal Mandal, Ranga Reddy District, Andhra Pradesh, India–501401. Tel : 918418 256201 – 207, E-mail: drpavanic@gmail.com

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ABSTRACT

Background: Enteropathogenic Escherichia coli(EPEC) is a major cause of diarrhoea in children below 5 years of age. Serotyping is classical method for identification of EPEC strains. But serotypic markers are rarely sufficient to reliably identify the strains as Escherichia coli. Introduction of PCR methodology which depends on detection of virulence factors has provided a practical and rapid way of detecting diarrhoeagenic Esch.coli. Multiantibiotic resistant EPEC strains are a common phenomenon with world wide extension. Moreover for the selection of appropriate therapy of dirrhoeas, knowledge of local antimicrobial therapy pattern plays an important role. **Objectives:** To study the role of EPEC in Paediatric diarrhoea by both Serogrouping and Molecular characterisation by PCR and to analyse the antibiotic susceptabililty patterns of EPEC strains in our area. **Materials and methods:** Prospective study of stool samples collected from children with diarrhoea and without diarrhoea who were below 5 years of age was conducted from May to November 2011. Escherichia.coli isolates were identified by Microscopy, Culture and Biochemical reactions. Among the Escherichia coli isolates, EPEC isolates were identified by Serogrouping. Escherichia coli isolates were also subjected to Molecular characterisation by Multiplex PCR assay and those isolates which showed pathogenic genes were futher serotyped. Antibiotic susceptibility pattern of EPEC isolates was determined by CLSI guidelines. **Results:** Among the Escherichia coli isolates 36.8% in the diarrhoeal group and none of them from the nondiarrhoeal group were identified as EPEC by serogrouping. 73.3% of the EPEC isolates were below 2 years of age and no much difference in the sex distribution was observed. Mild to moderate dehydration and feccal leuckocytes were seen in 59.9% and 56.6% of isolates respectively. High resistance to Nalidixic acid, Ampicillin, Cotrimoxazole, Ciprofloxacin and Norfloxacin was observed in the diarrhoeal group and resistance to only ampicillin was seen in the nondiarrhoeal group. In the diarrhoeal group 38.8% of Escherichia coli were EAEC and no other diarrhoeagenic Escherichia coli group was found by molecular characterisation. In the nondiarrhoeal Escherichia coli strains, 46.6% showed EAEC genes. EAEC strains in the diarrhoeal group belonged to multiple serotypes, the most common serotype being ONT and in the nondiarrhoeal group, 85.7% were of a single serotype, the most common isolate being O153. Among the Escherichia coli isolates which agglutinated with EPEC polyvalent antisera, 33.3% were positive for Enteroaggregative genes. **Conclusion:** EPEC is still an important pathogen in paediatric diarrhoeas. O serogrouping can still be relied upon for detection of EPEC. Dehydration is one of the clinical features of EPEC diarrhoea. Fluoroquinolones should only be prescribed in children as second line antibiotics. EAEC are present in classical ' O ' serogroups. Serotype O 153 has an increasing potential for asymptomatic carrier state in children below 5 years of age in our area.

Key words: Andhra Pradesh, Enteropathogenic Escherichia coli (EPEC), Antibiotic Susceptibility, Serotyping, Molecular characterisation, Enteroaggregative Escherchia coli (EAEC).

1. INTRODUCTION

Diarrhoea is major cause of illness in many areas of the world (1). EPEC is a very important pathogen in children with diarrhoea (2). It is a major cause of diarrhoea in children below 5 years of age (3). Incidence of EPEC varies from one locality to another (4). Serotyping is the classical method for identification of EPEC strains (5). But serotypic markers are rarely sufficient to reliably identify a strain as Escherichiacoli (6).

Introduction of PCR methodology which depends on detection of virulence factors has provided a practical and rapid way of detecting diarrhoeagenic Escherichiacoli (7). Resistance of Enterpathogenic bacteria to commonly prescribed antibiotics is increasing both in the developing as well as developed countries and resistance has emerged even to the newer, more potent antimicrobial agents (8).

Total no of Esch.coli	% of Agglutination with EPEC Polyvalent antiserum			
	Diarrhoeal group		Nondiarrhoeal group	
	EPEC	Non EPEC	EPEC	Non EPEC
82	36.8 %	63.4 %	0 %	100 %

Table 1. Results of the research

Multidrug resistant EPEC strains are a common phenomenon in recent researches with world wide extension. More over for the selection of appropiate therapy, knowledge of local antimicrobial therapy pattern plays an important role (9). Thus, keeping in mind all these facts the present study was conducted to assess the role of EPEC in poediatric diarrhoeas in our hospital isolates by both Serotyping and Molecular characterisation and to identify its Antibiotic susceptibility pattern.

2. MATERIALS AND METHODS

A total of 100 stool samples collected from patients suffering from acute diarrhoea and 30 samples from nondiarrhoeal cases in children below 5 years admitted to Gandhi hospital during the period May to November 2011, were processed in Microbiology department, Gandhi Medical College, Musheerabad, AP, India. Approval was obtained from the Institute Research council before commencement of the study. Details of of age, sex, antibiotic usage, clinical signs of dehydration of these children were recorded. Wet films were prepared from the samples and examined under microscope for pus cells. Each sample was inoculated on MacConkey agar, Wilson&Blair and Thiosulphate Citrate Bile Sucrose agar before and after enrichment with Selenite F broth and Alkaline Peptone water. After overnight incubation, plates were examined and organisms were identified by Gram stain, motility, Culture and Standard biochemical reactions as per Mackie McCartney Practical medical Microbiology 14th edition Escherichia coli isolates were subjected to serogrouping using EPEC polyvalent antisera which included antisera to the predominant O antigens of Enteropathogenic strains implicated in paediatric diarrhoeas i.e, O 26, O 55, O 86, O 111, O 114, O 119, O 125, O 126, O 127, O 128 and O 142. Antibiotic sensitivity testing for these EPEC isolates was done by Kirby-Bauer technique on Mueller Hinton agar with Ampicillin (10mcg) Amikacin (30 mcg), Cotrimaxazole (1.25 mg + 23.75 mcg), Nalidixic acid (30mcg), Ciprofloxacin (30 mcg) and Norfloxacin (30 mcg) discs as per CLSI guidelines. All the Esch. coli isolates were sent to National Institute of Cholera & Enteric Diseases (NICED) for Molecular characterisation by Multiplex PCR assay. The Escherichia coli isolates which were reported to contain pathogenic genes were serotyped using O antisera to all Escherichia coli strains by NICED.

3. RESULTS

Among the 100 stool samples from acute diarrhoeal cases analysed, 82 (82%) were Escherichia. coli, 8 (8%) Klebsiella, 5 (5%) Citrobacter and 2 (2%) Enterococci. The isolates in nondiarrhoeal group were Escherichia coli 18 (60%), Klebsiella 9 (30%), Enterococci 2 (6.7%) and Proteus 1 (3.3%) Escherichia coli was the most common isolate in both the diarrhoeal and nondiarrhoeal groups followed by Klebsiella, Proteus and Citrobacter in the diarrhoeal group and in the age matched controls, Klebsiella was the next common pathogen followed by Enterococci. Among the Escherichia coli isolates subjected to serogrouping, 30 isolates (36.8%) agglutinated with EPEC polyvalent antisera. In the control group, none of them showed agglutination.

Of the 30 EPEC isolates, most of them 16 isolates (73.3%) were of the below 2 years age group. Overall the sex distribution of EPEC showed a female preponderance 30 (57.6%). Mild to moderate dehydration was seen in 18(59.9%) of the isolates of EPEC. On microscopy, fecal leukocytes were seen in 17 (56.6%) of EPEC. Antibiotic susceptibility of EPEC isolates showed highest sensitivity to Amikacin (100 %). Sensitivity to Norfloxacin and Ciprofloxacin was 46.6% and 36.7% respectively. Resistance was observed to Cotrimoxazole (34.4%), Nalidixic acid (30%) and Ampicillin (30 %). The results of Multiplex PCR assay revealed that out of the Escherichia coli isolates from the diarrhoeal group, 31 (38.8%) were positive for Enteroaggegative genes. No other diarrhoeagenic Escherichia coli genes were found in this group.

Among the 18 Esch.coli strains in the nondiarrhoeal group, 7 (46.6%) were possitive for Enteroaggegative genes. No other diarrhoeagenic Esch. coli genes were found in this group. In the EAEC strains in the diarrhoeal group, 14 (60.8%) of them were of below 1 year age group. In the EAEC strains in the nondiarrhoeal group, 4 (57.1%) were below 1 year age group. There was a female preponderance in the EAEC isolates in both the diarrhoeal 15 (65.2%) and nondiarrhoeal 5 (71.4%) groups. Mild dehydration was seen only in 6 (26.1%) of EAEC strains in the diarrhoeal group. No dehydration was seen in the nondiarrhoeal group. Fecal leukocytes were seen in 5 (21.7%) of isolates in the diarrhoeal and 1 (7%) in the nondiarrhoeal group. The Serotyping of EAEC isolates in the diarrhoeal group revealed that EAEC belonged to Multiple serotypes. 15 (50 %) strains were nontypable, 7 (30.4%) were of the serotype O 153, 3 (13.1%) each were of the serotypes O 86a and O 127a and 1(4.4%) each was of the serotype O 78 and O 8. Out of the EAEC isolates in nondiarrhoeal group, most of them belonged to a single sero type. 6 (85.7%) were of the serotypes O 153 and 1 (14.3%) was nontypable. Among the 30 Esch.coli isolates which agglutinated with EPEC polyvalent antisera, 10 (33.3%) were positive for Enteroaggregative genes. (Table 1).

4. DISCUSSION

The present study was undertaken to assess the role of EPEC in diarrhoea in children below 5 years. Among the 100 stool samples from the acute diarrhoeal casess analysed, the isolation of Escherichia coli was 82 (82%), Klebsiella 8 (8%), Proteus 5(5 %), Citrobacter 3 (3%) and Enterococci 2 (2%). In the study conducted by C.K. Joshi et al, 73% isolation of Esch. coli, 46% isolation of Klebsiella, 0.8 % isolation

of Enterococci was reported which correlates well with our study (10). Study by K.K. Khanna et al showed as isolation of Escherichia coli 21.1%, Proteus 1.1% and Klebsiella 2.8% which was in contrast to our study (11).

The incidence of EPEC was 30 % in our diarrhoeal group which was comparable with the study of Amela et al which showed an incidence of 54% (12). Our study was in contrast to the studies of Tawfeek et al which showed an incidence of 13% (13). In the nondiarrhoeal group, none of the isolates could be typed as Enteropathogenic strains in the present study. PAK Addy et al reported an incidence of 4.1 %. EPEC in the control group (14). Seropositives only in the diarrhoeal group and no seropositives in the control group suggests that Serogrouping of EPEC is still an important method for detection of EPEC. O serogrouping appears to be still the simplest and useful test for presumptive identification of EPEC (15). 22 (73.3%) cases of EPEC diarrhoea in the present study occured in children below 2 years of age, predominently in below 1 year age group. This correlated well with the studies of C.K Joshi et al (73.8%) and K.K. Khanna (75%) (10, 11). This can be due to a decline in maternally acquired antibodies and the introduction of weaning foods that are potentially contaminated. In addition, crawling usually begins at this age and the risk of ingesting contaminated materials is high (16). Lower incidence of EPEC diarrhoea in children after 2 years may be due to acquisition of antibodies reactive to EPEC virulence associated proteins by infants living in endemic areas (17). Though a slight female preponderance was observed in the sex distribution of our study group, no significant diference was observed in the present study. This compares well with study conducted by Barbara J Stoll et al. (18). Mild to moderate dehydration was seen in 59.9% cases of EPEC diarrhoea which was in accordance with the study by K.K. Khanna et al which showed 64.5% dehydration (11). In a study conducted by Ulysses Fagundes et al, dehydration was noted in 95.2% cases of EPEC diarrhoea which was in contrast to our study (19). Dehydration in EPEC diarrhoea is due to the production of a bacterial toxin in stomach that interacts with the digestive juices and causes the patient to lose large amounts of water through the intestines (20). Fecal leukocytes were observed in 17 (56.6%) of cases of EPEC diarrhoea in our study. This is similar to the observations of K.K. Khanna et al (46.4%) (11). Relatively high frequencies of fecal leukocytes in our study suggest that although EPEC strains are not invasive pathogens, they induce an inflamatory response in the gut epithelium in vivo by triggering the production of cytokines and chemokines which recruit polymorphonuclear leukocytes to the infection site (21). EPEC strains are found to be resistant to several antibiotics. There was high resistance to Nalidixic acid (70%), Ampicillin (70%), Cotrimoxazole (66.6%), Ciprofloxacin (63.3%), Norfloxacin (58.4%). Multidrug resistance was also observed by S.N. Saxena et al. (22). Resistance to antibiotics such as Ampicillin, Cotrimoxazole is found in diarrhoeagenic Esch. coli isolated from children with diarrhoea in developing contries where the over use and misuse of antibiotics is common (23). Resistance to fluoroquinolones i.e Ciprofloxacin and Norfloxacin which is 63.3% and 58.4% respectively shows that resistance is also emerging to these drugs. This is because these antibiotics are widely used as the first choice for treatment of diarrhoea in developing countries (24). Fluoroquinolones should only be prescribed as second line antibiotics in case ofspecific infections or as second line antibiotics in severe bacterial infections with proven resistance to safer drugs (25). In our diarrhoeal group, in the 80 Esch. Coli isolates which were subjected to Multiplex PCR assay, 31(38.8%) were EAEC and no other diarrhoeagenic Escherichia coli group was found. In the study conducted by Kyung Hee Kim et al, EAECwere 14.7%, ETEC were 22.5%,EPEC were 6.5% and EIECwere 1% (26). Terasa Estrada Garcia et al in their study showed EAEC as 26%, ETEC as 27 %, EPECas 16% and EIEC as 3% (27). In comparision to our results of multiplex PCRassay, where 31(38.8%) isolates were positive for EAEC genes, the studies conducted by JavSaranya et al revaealed an EAECpositivity of 15.1% (28). Terasa Estrada Garcia et al showed a positivity of 26.6% of EAEC genes (27). In the 18 nondiarrhoeal Escherichia coli isolates subjected to Multiplex PCR assay, 12 (66.6%) showed EAEC genes in comparision to the study of Jav Saranya et al which showed a positivity of 0.6%. In our diarrhoeal group among the Escherichia coli isolates identified by Multiplex PCR assay as EAEC, the most common serotype was ONT(O Nontypable). This finding is comparable to the study of Soumen K et al. (29). However this serotype ONT is a frequent characteristic of EAECstrains (30). Due to their aggregative phenoytype, many EAECstarins autoagglutinate and are often described as nontypable or as 0-rough. It is also well established that EAEC are highlt heterogenous (31). Therefore we can think thatmost of the EAEC strains in our area are rough strains.In our control group, in the EAEC strains identified by Multiplex PCR assay, most of the strains were of a single serotype, this was in accordance with the study of Angela Christina Rodrigues Gilardi et al. (32). But the predominant serotype in our study was O153, whereas in the above study the predominant serotype was O86. The presence of a single serotype O153 in most of the nondiarrhoeal group EAEC isolates suggests that the serotype O153 has an increasing potential for asymptomatic carrier state in children less than 5 years in our area. Since this serotype is common in cattle and studies have suggested its presence in animal foods like curds and since its transmission from animals to humans is also reported, its presence in controls can be explained (33, 34). Among the 30 Escherichia coli isolates which agglutinated with EPEC polyvalent antisera, 10(33.3%) were positive for EAECgenes. Among these 10 isolates which were positive for EAECgenes 7 isolates (70%) belonged to Enteroaggregative serotypes. This shows that EAEC are present in classical EPEC O Serogrpoups (35, 36).

5. CONCLUSION

EPEC is still an important pathogen in Paediatric diarrhoeas. O Serogrouping appears to be still the simplest and useful test for presumptive identification of EPEC which can be relied upon for detection of EPEC. Dehydration can be considered as one of the clinical features of Paediatric diarhoeas. Fluoroquinolones should only be prescribed to children as second line antibiotics in case of severe bacterial infections only with proven resistance to safer drugs. Most of the EAECstrains in our area are rough strainswhich are

nontypable. Serotype O153 which is present in animal foods like curd in India has an increasing potential for asymptomatic carrier state in children below 5years of age in our area. EAEC are present in classical O Serogroups.

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