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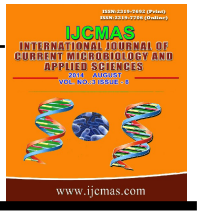

Original Research Article

E. coli pathotypes and their antibiotic resistance in young children with diarrhea in Hyderabad, India

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A B S T R A C T

The study aims to isolate, identify and characterize the *E. coli* pathotypes and look for their antibiotic resistance in young children with diarrhea in Hyderabad, India. Children in the age group of 6 months to 5 years suffering from acute diarrhea and attending outpatient clinic or admitted in Niloufer Hospital, Hyderabad were enrolled for the study. Examination of stool samples from 502 children in between 6 months to 5 years of age indicated that 229 (45.6%) children harbored one or more of the *E. coli* pathotypes. Among 229 serotypes, Enteropathogenic *E. coli* (EPEC) accounted for 35%, Enterotoxigenic *E. coli* (ETEC) 11%, Shiga toxigenic *E. coli* (STEC) 30%, *E. coli* O157:H7 24% and non diarrheagenic *E. coli* 55.8%. Antibiotic sensitivity assay in a subsample indicated that more than 70% of the *E. coli* isolates were resistant to Norflaxacin, Amoxycillin, Co-Trimoxazole, Ampicillin, Ceftrioxone, Cefotaxime and Metranidazole. Emerging bacterial pathogen like *E. coli* O157:H7 was isolated from diarrheal patients during the study. In general *E. coli* pathotypes were resistant to Ampicillin, Amoxycillin, Metranidazole and Co-Trimoxazole. Further study on the prevalence of other pathotypes of *Escherichia coli* and their antibiotic resistance among diarrheal patients is required.

Keywords

Acute diarrhea; *E. coli* O157:H7; Entero-pathogenic *E. coli;* Shiga toxigenic *E .coli*

Introduction

Acute diarrheal diseases are an important health problem among children under five in developing countries (Rodas *et al*., 2011). It has been reported that diarrheal diseases cause approximately 3 million deaths worldwide per year (Jafari *et al*., 2008). More than 20 viral, bacterial, and parasitic enteropathogens are currently associated with acute diarrhea in children (Nataro and Kaper, 1998). *Rotavirus* and diarrheagenic

Escherichia coli being the most common cause of acute diarrhea in children. The reported cases and deaths due to acute diarrheal diseases in India during 2004 are 9575112 and 2855 (Health information of India, 2004).

Among the bacterial pathogens *E. coli* plays an important role in causing diarrhea in below five year children. EPEC (Enteropathogenic *E. coli*) is an important category of diarrheagenic *E. coli* which has been linked to infant diarrhea in the developing world (Al-Hilali and Almohana, 2011). In India the status of STEC (Shiga toxigenic *E. coli*) and *E. coli* O157:H7 prevalence and contribution to disease is uncertain. In 2002, researchers in Calcutta, India, reported presence of non-O157 STEC isolates in stool samples of 1.4% of humans suffering from bloody diarrhea (Khan *et al*., 2002).

The bacterium responsible for 50% of the cases of persistent diarrhea in India was reported from Delhi and named as Enteroaggregative *E. coli* (AIIMS, 1994). A recent study on incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India indicated that *Escherichia coli* constituted 75.5% including 13.35 pathogenic *E. coli* (Samal *et al*., 2008).

Antibiotic treatment of common bacterial infections plays a crucial role in reducing morbidity and mortality due to these diseases; however, overuse and misuse of antibiotics in the treatment of diarrhea could lead to increased antibiotic resistance (Jafari *et al*., 2009). The progressive increase in antibiotic resistance among enteric pathogens in developing countries is a research priority of the Diarrheal Disease Control program of the World Health Organization (Rahbar et al., 2007; Mansouri and Shareifi, 2002; WHO, 2011). In this context a study was undertaken to isolate, identify and characterize the *E. coli* pathotypes and their antibiotic resistance in children less than 5 years with diarrhea in Hyderabad, India.

Materials and Methods

Diarrhea was defined as at least three loose stools in 24 h, any number of watery stools, or one or two loose stools in 24 h accompanied by at least one of the symptoms like nausea, vomiting, abdominal cramps or fever of 38^0 C. Acute diarrhea was defined as diarrhea that lasted 14 days or less at the time of presentation.

Study site and subjects

This study was carried out at Niloufer Hospital, Hyderabad which is one of biggest government pediatric hospitals in Andhra Pradesh, during 2010-2011, India. Children in the age group of 6 months to 5 years suffering from acute diarrhea and attending outpatient clinic or admitted in Niloufer Hospital, Hyderabad were enrolled for the study.

Ethical approval

Ethical approval was obtained from the National Institute of Nutrition, Hyderabad, the Indian Council of Medical Research, Ministry of Health and Family Welfare of Govt. of India

Sample collection

Stool specimens were collected from the children with diarrhea under 5 years of age over a period of one year. The samples were inoculated into Carry Blair transport medium and transported to the laboratory.

Sample analysis

The specimens were checked microscopically (Direct smear, Gram staining), each specimen was cultured according to the standard method. In order to evaluate the role of *Escherichia coli* serotypes all samples were cultured initially on MacConkey Agar. Then the suspected colonies were sub cultured on Eosin Methylene Blue (EMB) agar to see the metallic sheen color of the *Escherichia coli*. The isolates of *E. coli* were initially identified and confirmed by biochemical tests (carbohydrate fermentation test, Indole test, Urease test, Nitrate reduction test, Ornithine test, Lysine test, H_2S production test citrate utilization test, MRVP test) and then by Polymerase Chain Reaction (PCR).

DNA isolation and Polymerase Chain reaction Assay

E. coli DNA isolation was done by using commercial DNA isolation kit provided by Bioserve Biotechnologies Pvt India Ltd. DNA templates were subjected to PCR using four sets (F and R) of primers targeting virulence properties genes listed in Table 1. The reaction mixture contained Master mix which is premixed ready to use solution containing *Taq* DNA polymerase dNTP, $Mgcl_2$, and according to Bioserve Biotechnologies procedure, the reaction mixtures were prepared in 0.2ml eppendorf tube with 25 µl reaction volumes. The amplification was performed with thermocycler (PCR system, Applied Biosystem) For STEC (stx1 & stx2 Primers), ETEC(st primers) 95°C (5 min, 1 cycle); and then 39 cycles of 95°C for 30sec, 52°C for 30 sec and 68°C for 45 sec and a final extension step for 7 min at 68°C . For ETEC (lt primers) & EPEC (eae A& bfpA Primers) 95°C (5 min, 1 cycle); and then 39 cycles of 95°C for 30sec, 55°C for 30 sec and 68°C for 1min and a final extension step for 7 min at 68°C. For EHEC (O157 H7) 95°C (5 min, 1 cycle); and then 39 cycles of 95°C for 30sec, 58 or 60°C for 40 sec and 68°C for 1min and a final extension step for 7 min at 68°C. The amplified PCR products were detected by agarose gel electrophoresis and visualized staining with ethidium bromide using gel documentation system.

Antibiotic sensitivity assay

Antibiotic sensitivity assay was performed by using Himedia antibiotic discs for commonly used antimicrobial agents including Gentamycin (G), Norfloxacin (NX), Amicacin (AK), Amoxycillin (AM), Furazolidone (FR), Co-Tromoxazole (COT), Ampicillin (A), Ceftriaxone (CTR), Cefotaxime (CTX) and Metranidazole (MT).

Statistical analysis

The data were analyzed using SPSS 15.0 version computer package. Associations between categorical variables were obtained using Chi-square and Fishers exact tests.

Results and Discussion

A total of 502 stool samples form under five aged children were collected during the study period. More than 54% of children were below one year of age and only 3% belonged to more than 4 years of age (Table 2). The major symptoms reported were diarrhea, fever and vomiting. Examination of stool samples showed that 229 (45.6%) children harbored one or more of the *E. coli* pathotypes. Among 229 serotypes, EPEC accounted for 35%, ETEC accounted for 11%, STEC accounted for 30%, *E. coli* O157:H7 accounted for 24% and the non diarrheagenic *E. coli* accounted for 55.8%. The *E. coli* pathotypes like EIEC *(Entero*

invasive E. coli) and EAEC *(Entero aggregative E. coli*) were not detected in the stool samples. The prevalence of *E.coli* serotypes was higher in children below one year than the older age group. Seasonal distribution of the *E. coli* pathotypes showed that majority of the cases of diarrhea due to *E. coli* occurred in summer (Table 3).

Antibiotic sensitivity assay showed that more than 70% of the *E. coli* isolates were resistant to Norflaxacin, Amoxycillin, Co-Trimoxazole, Ampicillin, Ceftrioxone, Cefotaxime and Metranidazole. Results of the antibiotic susceptibility testing of Shigella toxigenic *E. coli* revealed that, more than 80% of isolates were resistant to Norfloxacin, Amoxicillin, Ampicillin, Cefotaxime and Metronidazole but these cultures were sensitive to CoTrimoxazole and Amikacin (n=22). The susceptibility pattern of Enteropathogenic *E. coli* revealed that more than 90% of the isolates were resistant to Amoxicillin, Ampicillin, and Metronidazole and sensitive to Gentamycin, Furanidazole and Amikacin. More than 60% of the *E. coli* O157:H7 isolates were resistant to Metronidazole and Amoxicillin. The susceptibility pattern of *Enterotoxigenic E. coli* indicated that more than 80% of the isolates were resistant to CoTrimoxazole, Amoxicillin, Ampicillin and Amikacin.

Acute diarrhea is an important public health problem in developing countries. *E. coli* has been identified as important cause of infantile diarrhea in all the developing countries where it has been looked for, but the incidence has varied greatly in different countries. This is the first report of a systematic surveillance study on the prevalence of *E. coli* pathotypes isolated from diarrhea patients from a health care centre in Hyderabad. The present study revealed that the prevalence of EPEC (35%) in diarrheal stool samples was higher than the other serotypes of *E. coli*. This is consistent with other studies reported from India. For example, a study conducted on incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India indicated that, among the pathogenic *E. coli* spp. isolated most were EPEC (40.6%), followed by ETEC (52.7%), and EAEC (Enteroaggregative *E. coli*) (40.6%) (Samal *et al*., 2008). Pie *et al*. reported that EAEC was responsible for 55% of the diarrhea cases in a diarrheal epidemic in South India (Pie *et al*., 1997). Similar study in Iran indicated 12.4% of EPEC to be the causative agent (Jafari *et al*., 2008). Another study conducted by Dallal et al, indicated that the highest isolation rate was observed for EPEC (19.7%) in Tehran (Dallal and Khorramizadeh, 2006). A high frequency of EPEC was also observed in Korea (56%) and Brazil (34.0%) (Gomes et al., 1991).

The prevalence of *E. coli* serotypes was more in children less than 1 year old. Similar kind of study done in Iran indicated that more than 27% of EPEC isolates were from children of 1 year or under, 67% from those under 2 years and more than 82% were from children under under 3 years (Dallal and Khorramizadeh, 2006).

Seasonal distribution of the pathogenic *E. coli* showed that most of the cases of diarrhea caused *by E. coli* serotypes occurred in summer probably owing to the increase in temperature. A study conducted by Jafari et al. indicated that highest acute diarrhea incidence was seen in children of 12-24 months of age during the dry seasons (Jafari *et al.,* 2009). Similar type of study conducted by Dallal et al. indicated that most of the cases of diarrhea caused by *E. coli, Shigella, Salmonella* and *Campylobacter* Spp. occurred in summer (Dallal and Khorramizadeh, 2006).

In our study, for the first time in Hyderabad we have isolated *E.coli* O157; H7 from diarrheal stool samples of children below 5 year of age. *Escherichia coli* O157:H7 is an important agent of haemorrhagic colitis and haemolytic ureamic syndrome in children less than 5 years old and elderly people (Rajii *et al*., 2008).

In the present study *Escherichia coli* O157:H7 was isolated from 24% of diarrheal stool samples. The only study conducted in India by Paul *et al*, indicated that *E. coli* O157:H7 which do not produce verocytotoxins was isolated from a diarrheal patient in Kolkata (Paul *et al.,* 1991). A study conducted in Kolkata indicated that 4.81% of shiga toxin producing *E.coli* including O157:H7 were isolated from animal handlers, animal products and admitted diarrheic children (Chattopadhyay *et al*., 2003). Similar kind of study conducted on prevalence and characterization of verocytoxin producing *Escherichia coli* O157 from diarrheal patients in Morogoro, Tanzania indicated that 21% of the patients were harboring the pathogenic bacteria (Rajii *et al*., 2008).

Resistance to currently used antimicrobial agents among enteric pathogens has increased dramatically worldwide during the past decade (Salmanzdeh-Ahrabi *et al*., 2007; Temu *et al*., 2007; Woodward and Rodgers, 2000). The antibiotics like trimethoprim-sulfamethoxazole, ampicillin and amoxicillin are widely used in developing countries to treat diarrhea because of their availability over the counter (Nguyen *et al*., 2005). In this study, most of the *E. coli* isolates were resistant to Norflaxacin, Amoxycillin, Co-Trimoxazole, Ampicillin, Ceftrioxone, Cefotaxime and Metronidazole. Other studies from India have also reported antibiotic resistance. For instance, in a study from North India, pathogenic *E. coli* strains were resistant to Ampicillin, Gentamicin, Cefotaxime, and Ciprofloxacin and sensitive to Amoxicillin, Nalidixic acid, Trimethoprim, and Chloromphenicol (Taneja *et al*., 2006). A study conducted in Bolivia indicated that More than 67% of the ETEC strains were resistant to one or several antimicrobial agents like Ampicillin, Ampicillin Sulbactum, Tetracycline and Chloromphenicol (Rodas *et al.,* 2011). A study done in Brazil indicted that ETEC isolates were highly resistant to Ampicillin, Sulfonamides and Tetracycline (Garcia *et al*., 2011). Similar kind of study done in Iran indicated that diarrheagenic *E. coli* showed a high incidence of resistance to tetracycline (63%), ampicillin (62%), streptomycin (56%), amoxicillin/clavulanic acid (44.5%), trimethoprim/sulfamethoxazole (39.5%), and cephalothin (37%) (Aslani *et al*., 2008).

This study thus highlights the high prevalence of an emerging bacterial pathogen like *E. coli O157:H7* in children under 5 with diarrhea in Hyderabad. *E. coli* serotypes were resistant to commonly used antimicrobial agents like Ampicillin, Amoxycillin, Metranidazole and Co-Trimoxazole which is of clinical significance. Further study on the prevalence of other pathotypes of *Escherichia coli* is required. Active surveillance and control strategies are needed for reducing the number of diarrheal cases in this region.

Table.1 Primer sequences and size of amplified products from selected genes

E.coli pathotype Product size bp	Primer name	DNA sequences
ETEC 450	*LT-A*	F- 5'-GGCGACAGATTATACCGTGC-3' R- 5'-CGGTCTCTATATTCCCTGTT-3'
EPEC 324	*bfpA*	F -5´-AAT GGT GCT TGC GCT TGC TGC- 3´ R- 5´-GCC GCT TTA TCC AAC CTG GTA -3´
STEC 225	*stx2*	F-5´-GGC ACT GTC TGA AAC TGC TCC -3´ R-5´-TCG CCA GTT ATC TGA CAT TCT G -3´
E.coli 57:H7 657	*Flich 7*	F -5´- GCGCTGTCGAGTTCTATCGAGC 3´ R -5´- CAACGGTGACTTTATCGCCATTCC-3´

Table.2 Age distribution of *E.coli* pathotype positive children with gastrointestinal infection

Age (Years)	*E.coli*		*EPEC*		*ETEC*		*STEC*		*E.coli 0157:H7*	
	No	%	No	%	No	%	No	%	No	%
<1	106	46.3	8	32.0	3	37.5	13	61.9	9	52.9
1 to <2	68	29.7	10	40.0	3	37.5	3	14.3	6	35.3
2 to <3	33	14.4	5	20.0	2	25.0	4	19.0	1	5.9
3 to <4	9	3.9	0	0	0	0	0	0	0	0
4 to 5	13	5.7	2	8.0	0	0	1	4.8	1	5.9
Total	229	100	25	100	8	100	21	100	17	100

*EPEC – Enteropathogenic *E.coli*
ETEC – Enterotoxigenic *E.coli*
STEC – Shigatoxigenic *E.coli*

Table.3 Seasonal distribution of *E.coli* cultures from children with gastrointestinal infection

Season	**No. of cultures**	***E.coli***	***EPEC***	***ETEC***	***STEC***	***E.coli 0157:H7***
Summer	136	95 (41.5)	15(60)	6(75)	12(57.1)	8(47.1)
Rainy	100	78(34.1)	9(36)	2(25)	9(42.9)	2(11.8)
Winter	64	56(24.5)	1(4)	0	0	7(41.2)
Total	300	229	25	8	21	17

*EPEC – Enteropathogenic *E.coli;* ETEC – Enterotoxigenic *E.coli;*
STEC – Shigatoxigenic *E.coli*

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References

Al-Hilali, S.A. and Almohana, A.M., 2011.Occurrence and molecular characterization of enteropathogenic *Escherichia coli* serotypes from children with diarrhea in Najaf, Iraq. *Indian Journal of Medical Microbiology*., 29: 383-388.

All India Institute of Medical Sciences. Annual Report (AIIMS). 1994. Available from: www.aiims.ac.in/ aiims/research.

Aslani, M.M., Ahrabi, S.S., Alikhani, Y.M., Jafari, F., Zali. R.M. and Mani, M., 2008. Molecular detection and antimicrobial resistance of diarrheagenic *Escherichia coli* strains isolated from diarrheal cases. *Saudi Med. J.*, 29: 388-92.

Chattopadhyay, U.K., Gupta, S., Dutta, S., 2003. Search for shiga toxin producing *Escherichia coli* (STEC) including 0157:H7 strains in and around Kolkata. *Indian J. Med. Microbiol.*, 21: 17-20.

Dallal, M.M.S., Khorramizadeh, M.R. and Ardalan, M., 2006. Occurrence of enteropathogenic bacteria in children under 5 years with diarrhea in south Tehran. *Eastern Mediterranean Health Journal*., 12*:* 792-797.

Garcia, P.G., Silva, V.L. and Diniz, C.G. 2011. Occurrence and antimicrobial drug susceptibility patterns of commensal and diarrheagenic Escherichia coli in fecal microbiota from children with and without acute diarrhea. *J. Microbiol*., 49:46-52

Gomes, T.A.T., Rassi, V., MacDonald, S.R., Ramos, L.R., Trabulsi, M.A. and Veiera, B.E. 1991. Enteropathogens associated with acute diarrheal diseases in urban infants in Sao Paulo, Brazil. *J Infec. Dis*., 164:331-7

Health Information of India. Population Health statistics. Govt. of India, Central bureau of Health Intelligence of India, ministry of health and Family welfare. 2004. Available from: http://www.cbhidghs.nic.in/.

Jafari, F., Hamidian, M. and Rezadehbashi. 2009 Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran. *Can. J. Infec. Dis. Med. Microbiology*., 20: 56-62.

Jafari, F., Shokrzadeh, L., Hamidian, M., Slamanzadeh-Ahrabi, S. and Zali, M.R. 2008. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. *Jpn. J. Infec. Dis*., 61: 269-273.

Khan, A., Das, S.C., Ramamurthy, T., Sikdar, A., Khanam, J., Yamasaki, S., Takeda, Y. and Nairk, G.B. 2002. Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. *J. Clin. Microbiol*., 40:2009-2015.

Mansouri, S. and Shareifi, S. 2002. Antimicrobial resistance pattern of

Escherichia coli causing urinary tract infections, and that of human fecal flora, in the Southeast of Iran. *Microbial Drug Resistance*., 8:123-8.

Nataro, J.P., Kaper, J.B. 1998. Diarrheagenic Escherichia coli. *Clin. Microbiol. Rev*. 11:142-201.

Nguyen, T.V., Le, P.V., Le, C.H. and Weintraub, A. 2005. Antibiotic resistance in diarrheagenic *Escherchia coli* and *Shigella* strains isolated from children in Hanoi, Vietnam. Antimicrob. Agents Chemother., 49: 816-9.

Paul, M., Ghosh, A.R., Nair, G.B., Bhattacharya, S.K., Pal, S.D. and Sen, D. 1991. Diarrhea associated with *Escherichia coli* 0157:H7 which do not produce verocytotoxins. *J. Diarrheal Dis. Res*., 9: 1234-1239.

Pie, M., Kang, G., Ramakrishna, B.S., Venkataraman, A. and Muliyil, J. 1997. An epidemic of diarrhea in south India caused by enteroaggregative *Escherichia Coli*. *Indian J. Med. Res*., 106: 7-12.

Rajii, M.A., Minga, U.M. and Machangu, R.S. 2008. Prevalence and characterization of verocytoxin producing *Escherichia coli* 0157 from diarrhea patients in Morogoro, Tanzania. *Tanzan J. Health Res*. 10: 151-158.

Rahbar, M., Deldari, M. and Hajia, M. 2007. Changing prevalence and antibiotic susceptibility patterns of different *Shigella* species in Tehran, Iran. *Int. J. Microbiol.* Available. from: http://www.ispub.com/ostia/index.php ?xmlFilePath=journals/ijmb/vol3n2/sh igella.xml.

Rodas, C., Mamani, R., Blanco, J., Blanco, J.E., Wiklund, G., Svennerholm, A., Sjoling, A. and Iniguez. 2011. Enterotoxins, colonization factors, serotypes and antimicrobial resistance of enterotoxigenic *Escherichia coli* (ETEC) strains isolated from hospitalized children with diarrhea in Bolivia. *Braz J. Infect. Dis*., 15:132-137.

Salmanzdeh-Ahrabi, S., Jafari, F., Habibi, E., Irajian, G.R., Aslani, M.M. and Baghbani-Arani, F. 2007. Serotype distribution and antimicrobial resistance rates of *Shigella* spp. isolates in Tehran, Iran. *Mikrobiyol. Bul*. 41: 453-457.

Samal, S.K., Khunti, H.K., Nanda, P.K., Sathapathy, C.S., Nayak, S.R., Sarangi, A.K., Sahoo, N., Pattnaikk, S.K., Chhotray, G.P. and Pal, B.B. 2008. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. *Jpn. J. Infec .Dis*. 61: 350-355.

Taneja, N., Rao, P., Rao, D., Singh, M. and Sharma, M. 2006. Enterotoxigenic *Escherichia coli* causing cholerogenic syndrome during an interepidemic period of cholera in North India. *Jpn. J. Infec.dis.* 59: 245-248.

Temu, M.M., Kaatano, G.M., Miyaye, N.D., Bhuhalata, S.N., Shushu, M.L. and Kishamawe, C. 2007. Antimicrobial susceptibility of *S. flexneri* and *S. dysenteriae* isolated from stool specimens of patients with bloody diarrhoea in Mwanza, Tanzania. Tanzan. Health Res. Bull. 9*:* 186-189.

World Health Organization. Shigella. 2011. Available from: www.who.int/vaccineresearch/ disease/-shigella/en.

Woodward, D.L. and Rodgers, F.G. 2000. Surveillance of antimicrobial resistance in *Salmonella*, *Shigella* and *Vibrio cholerae* in Latin America and the Caribbean: A collaborative project. *Can. J. Infect. Dis.*, 11:181-186.