

Original Article

Incidence of Bacterial Enteropathogens among Hospitalized Diarrhea Patients from Orissa, India

Surya Kanta Samal, Hemant Kumar Khuntia, Prafulla Kumar Nanda¹, Chandra Sekhar Satapathy¹, Sudeep Ranjan Nayak², Ashok Kumar Sarangi³, Nilamani Sahoo⁴, Sanjay Kumar Pattnaik⁵, Guru Prasad Chhotray and Bhibhuti Bhusan Pal*

Regional Medical Research Centre; ¹Department of Biotechnology, Majhighariani Institute of Technology and Sciences; ³Department of Biotechnology, Trident Academy of Creative Technology; ⁴Department of Microbiology, Centre for Post-Graduate Studies, Orissa University of Agriculture and Technology;

⁵Department of Microbiology, MPC Autonomous College, North Orissa University, Orissa; and

²Department of Microbiology, Allahabad Agriculture Institute (Deemed University), Uttar Pradesh, India

(Received January 30, 2008. Accepted June 6, 2008)

SUMMARY: Bacteriological analysis of 1,551 stool/rectal swabs from all age groups of diarrhea patients of different hospitals of Orissa from January 2004 to December 2006 was carried out using standard procedures. Among all enteropathogens isolated in 886 culture-positive samples, *Escherichia coli* constituted 75.5%, including 13.2% pathogenic *E. coli*; *Vibrio cholerae* O1 constituted 17.3%; *V. cholerae* O139, 1%; *Shigella* spp., 4.5% (*Shigella flexneri* type 6, 2.9%, *S. dysenteriae* type 1, 0.7%, *S. sonnei*, 0.6%, and *S. boydii*, 0.3%); *Salmonella* spp., 0.7%; and *Aeromonas* spp., only 2.0%. The isolation of bacterial enteropathogens was highest during July 2005, followed by September 2006. The prevalence of shigellosis in this region was relatively low. Cholera cases were more frequent during the rainy seasons. The dominance of *V. cholerae* O1 Inaba over Ogawa serotypes was observed in 2005, whereas this trend was reversed in 2006. The resistance profile of *V. cholerae* O1 was co-trimoxazole (Co), furazolidone (Fr), and nalidixic acid (Na); for *Aeromonas* spp., it was ampicillin (A), Fr, ciprofloxacin (Cf), Na, norfloxacin (Nx), and Co. Pathogenic *E. coli* strains were resistant to A, Fr, Co, streptomycin (S), Cf, Na, Nx, and neomycin (N); *Shigella* spp. were resistant to Fr, Na, Co, and S; and *Salmonella* spp. were resistant to A and Fr. Active surveillance should be continued among diarrhea patients to look for different enteropathogens and to define the shifting antibiogram patterns in this region.

INTRODUCTION

Diarrhea is thought to be one of the most common causes of morbidity and mortality among infants and children in developing countries (1). Acute diarrhea is a devastating disease with worldwide distribution and has a significant impact on public health. Diarrheal diseases are the cause of almost 3 million deaths annually, mainly among children younger than 5 years of age (2). Globally, 1.3 billion cases of acute diarrhea occur in children below 5 years annually, of which more than 3 million are fatal; 80% of these deaths are in children below 2 years of age (3). Approximately 35% of the deaths can be attributed to acute non-dysenteric diarrhea, and an estimated 45% occur in children with persistent diarrhea (4). More than 20 viral, bacterial, and parasitic enteropathogens are currently associated with acute diarrhea. Among the viral and bacterial enteropathogens, rotavirus and diarrheagenic *Escherichia coli* are those most commonly responsible for acute diarrhea in children. *Shigella*, *Salmonella*, *Campylobacter jejuni/coli*, *Vibrio cholerae*, *Aeromonas* and *Plesiomonas* spp. and other infections caused by protozoa and helminths occur more commonly among low-income groups in poorer areas, where environmental sanitation is significantly deteriorated (5). Cholera is an acute diarrheal illness caused by the toxigenic bacteria *V. cholerae* serogroup O1 and O139 and is associated with rapid loss of body fluids

leading to dehydration, electrolyte disturbances and hypovolemic shock; without treatment, death can occur within hours (6). *V. cholerae* poses a significant threat and is endemic in different parts of India. The state of Orissa has a long coastal tract which experiences cyclones or flooding almost every year, followed by diarrheal outbreaks accounting for a high rate of morbidity and mortality in the state. There are published reports of outbreaks due to *V. cholerae* O1 and O139 serogroups in Orissa (7,8). So far, however, the reports on the bacterial etiology of acute diarrhea among hospitalized diarrhea patients from Orissa are scarce. Hence, the present study was undertaken to document the incidence of different bacterial enteropathogens associated with hospitalized diarrhea patients of all age groups from this region.

MATERIALS AND METHODS

Specimen collection: The surveillance was conducted for a period of 3 years from January 2004 to December 2006 among diarrhea patients in three Orissa hospitals: Capital Hospital in Bhubaneswar, Infectious Disease Hospital in Puri, and Sishu Bhaban in Cuttack. A total of 1,551 stool/rectal swabs were collected from all age groups of diarrhea patients. The samples were collected once a week from hospitalized diarrhea patients before the administration of antibiotics and carried to the laboratory in Cary-Blair transport medium.

Bacteriology and serology: The stool/rectal swabs were subcultured on thiosulphate-citrate-bile salt sucrose (TCBS) agar (Becton and Dickinson Co., [BD], Sparks, Md., USA) for the isolation of *Vibrio* spp., MacConkey agar (BD) for

*Corresponding author: Mailing address: Microbiology Division, Regional Medical Research Centre (ICMR), Bhubaneswar-751023, Orissa, India. E-mail: bbpal_rmrc@yahoo.co.in

Table 1. PCR primer sequences and conditions used for the detection of genes specific for *V. cholerae* isolates

Group/organism	Target gene or encoding region	Primer sequences (5'-3')	Amplicon size (bp)	PCR conditions ¹⁾	Reference
<i>V. cholerae</i>	<i>ctxA</i>	CTCAGACGGGATTTGTTAGGCACG	301	94°C 1.0 min 60°C 1.5 min 72°C 1.5 min	11
		TCTATCTCTGTAGCCCTATTACG			
	<i>tcpA</i> (Classical)	CACGATAAGAAAACCGGTCAAGAG	617		
ACCAAATGCAACGCCGAATGGAG					
ETEC	<i>tcpA</i> (El Tor)	GAAGAAAGTTTGTAAGAAGAACAC	471		
		GAAGGACCTTCTTTCACGTTG			
EPEC	<i>elt</i>	GCGGACAGATTATACCGTGC	450	94°C 1.0 min 55°C 1.5 min 72°C 1.5 min	12
		CGGTCTCTATATCCCTGTT			
EAggEC	<i>eae</i>	AAACAGGTGAAACTGTTGCC	454	94°C 1.0 min 55°C 1.5 min 72°C 1.5 min	13
		CTCTGCAGATTAACCCCTCTGC			
EAggEC	<i>ast</i>	CACAGTATATCCGAAGGC	94	94°C 1.0 min 53°C 1.0 min 72°C 1.0 min	14
		CGAGTGACGGCTTTGTAG			

¹⁾: 30 cycles consisting of denaturation, annealing, extension.

ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EAggEC, enteroaggregative *E. coli*.

the isolation of *E. coli*, Hektoen enteric agar (BD) for the isolation of *Shigella* and *Salmonella* spp. and Rimler-Shotts agar (Hi-Media, Mumbai, India) for the isolation of *Aeromonas* spp. The method followed the protocol of the 1987 World Health Organization manual (9). Serotyping of *Shigella* spp. and *V. cholerae* was done with antisera (BD) according to the instructions provided.

Antibiotic susceptibility test: Antibiotic susceptibility testing of *Salmonella*, *Shigella*, *V. cholerae*, pathogenic *E. coli*, and *Aeromonas* was carried out by the disk diffusion technique (10) using a commercially available disc (Hi-Media). The antibiotics used were ampicillin (A, 10 µg), co-trimoxazole (Co, 25 µg), chloramphenicol (C, 30 µg), ciprofloxacin (Cf, 5 µg), furazolidone (Fr, 50 µg), gentamicin (G, 10 µg), nalidixic acid (Na, 30 µg), norfloxacin (Nx, 10 µg), streptomycin (S, 10 µg), tetracycline (T, 30 µg), and neomycin (N, 30 µg).

DNA template preparation and polymerase chain reaction (PCR) assay for *V. cholerae* and *E. coli*: A multiplex PCR assay was employed to determine the presence of A-subunit cholera toxin gene (*ctxA*) and to biotype the *V. cholerae* strains by targeting *tcpA* (encoding the major structural subunit of the toxin co-regulatory pilus), which is specific for El Tor and classical strains by the method described by Keasler and Hall (11). *E. coli* strains were screened for the presence of a variety of virulent genes such as *elt* (encoding heat-labile toxin) (12) for enterotoxigenic *E. coli* (ETEC); *eae* (gene for enterocyte attachment and effacement) (13) for enteropathogenic *E. coli* (EPEC), and *ast* (encoding stable toxin produced by enteroaggregative *E. coli* [EAggEC]) (14) for EAggEC. Template DNA was prepared from the culture grown in Luria broth (LB) overnight by boiling in a water bath for 10 min and instantly cooling on ice. PCR amplification was done with appropriate volumes of 10× amplification buffer (500 mM KCl, 100 mM Tris-HCl, 15 mM MgCl₂, (pH 8.3), 2.5 mM each deoxynucleoside triphosphate, 10 pmol of each primer, 1.25 unit of *Taq* DNA polymerase (Bangalore Genei, Bangalore, India) and 5 µl of template DNA. The reaction volume was adjusted to 25 µl using sterile triple-distilled water. Simplex and multiplex PCRs were performed in an automated thermocycler (MG96G; LongGene, Hangzhou,

China) for 30 cycles using conditions described in Table 1.

RESULTS

A total of 1,551 stool/rectal swabs obtained from patients with acute diarrhea were analyzed from January 2004 to December 2006. Out of 886 (57.1%) culture-positive samples, 669 (75.5%) were *E. coli*, of which 117 (13.2%) were pathogenic, and 153 (17.3%) were *V. cholerae* (O1 Ogawa strain, 74 [8.3%]; and O1 Inaba strain, 70 [7.9%]; and O139 strain, 9 [1%]); in addition, 40 (4.5%) were *Shigella*, 6 (0.7%) were *Salmonella* spp., and 18 (2.0%) were *Aeromonas* spp. (Table 2). Among the pathogenic *E. coli* spp. isolated, most were EPEC ($n = 40$, 6%), followed by ETEC ($n = 52$, 7.8%), and EAggEC ($n = 25$, 3.7%), respectively. Among the *Shigella* spp. isolated, most were *S. flexneri* type 6 ($n = 26$, 2.9%), followed by *S. dysenteriae* type 1 ($n = 6$, 0.7%), *S. sonnei* ($n = 5$, 0.6%), and *S. boydii* ($n = 3$, 0.3%), respectively. During the year 2004, the vast majority of the isolates from diarrhea patients were *E. coli* (217, 93.1%), followed by *Shigella* spp. (4.4%) and *V. cholerae* O1 Ogawa (2.1%). In the years 2005 and 2006 the isolates were mostly *E. coli* (73.3 and 62.2%), followed by *V. cholerae* (20.9 and 25.7%) and *Shigella* spp.

Table 2. Spectrum of bacterial enteropathogens isolated from diarrhea patients during January 2004 to December 2006

	2004 $n = 373$ no. (%)	2005 $n = 653$ no. (%)	2006 $n = 525$ no. (%)	Total $n = 1,551$ no. (%)
Culture Positive	233 (62.5)	412 (63.1)	241 (45.9)	886 (57.1)
Culture Negative	140 (37.5)	241 (36.9)	284 (54.1)	665 (42.9)
<i>E. coli</i>	217 (93.1)	302 (73.3)	150 (62.2)	669 (75.5)
Pathogenic <i>E. coli</i>	36 (15.5)	53 (12.9)	28 (11.6)	117 (13.2)
<i>V. cholerae</i>	5 (2.1)	86 (20.9)	62 (25.7)	153 (17.3)
<i>V. cholerae</i> O1 Ogawa	5 (2.1)	34 (8.3)	35 (14.5)	74 (8.4)
<i>V. cholerae</i> O1 Inaba	0 (0.0)	50 (12.1)	20 (8.3)	70 (7.9)
<i>V. cholerae</i> O139	0 (0.0)	2 (0.5)	7 (2.9)	9 (1.0)
<i>Shigella</i>	10 (4.4)	19 (4.6)	11 (4.6)	40 (4.5)
<i>Salmonella</i>	1 (0.4)	5 (1.2)	0 (0.0)	6 (0.7)
<i>Aeromonas</i>	0 (0.0)	0 (0.0)	18 (7.5)	18 (2.0)

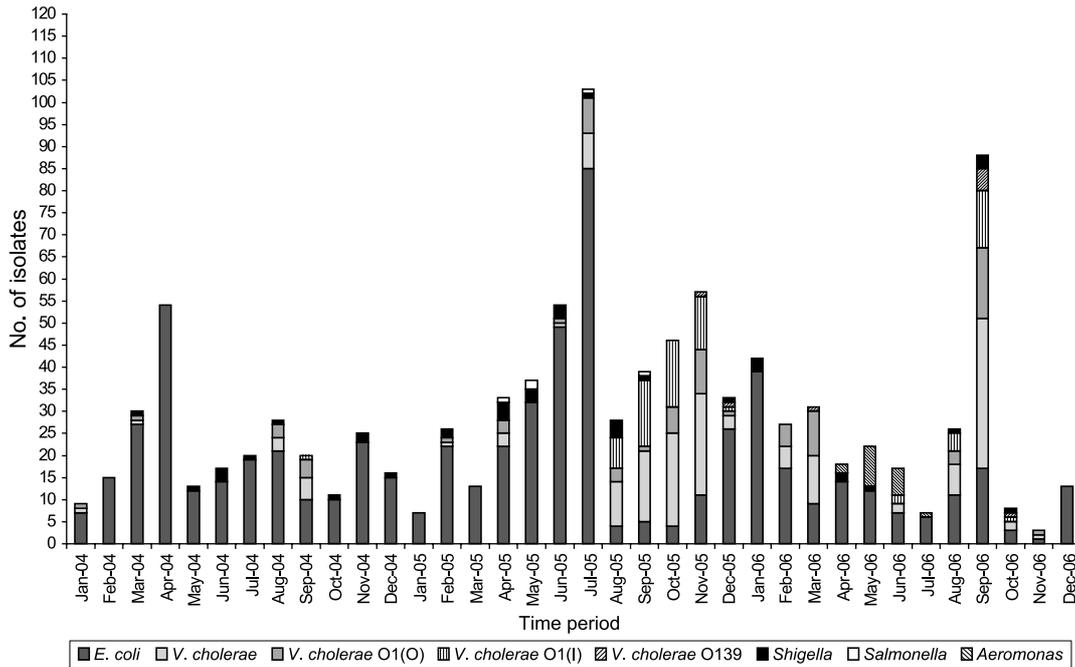


Fig. 1. Month wise isolation of different bacterial enteropathogens isolated from diarrhea patients from January 2004 to December 2006.

(4.6%). Among the total culture-positive patients (886), 516 were male and 370 were female patients belonging to different age groups with a variety of clinical symptoms.

Seasonal analysis: Children were more likely to be brought for treatment during the rainy season. Each year a higher prevalence of diarrhea patients was reported from July to October, the rainy season in this region. It gradually decreased towards the month of November, and continued at a low rate through February (winter), with prevalence reappearing from March through June (summer). A high prevalence of infection due to *V. cholerae* was observed from July to October almost every year (Fig. 1).

Age specific distribution of bacterial enteropathogens: Out of the total samples analyzed, 28.6% ($n = 253$) of those from the age group <5 years were positive for at least one bacteria and 8.2% ($n = 73$) of those from 5-14 years. However, the rate increased up to 32.2% ($n = 285$) in people 15-40 years of age and 31% ($n = 275$) in cases of adults age >40 years. Males were more frequently infected than females in the <5 years, 5-14 years, and >40 years age groups whereas a higher prevalence was observed in females in the 15-40 years age group (Fig. 2).

Antibiotic susceptibility pattern: An antibiotic susceptibility test was done for *V. cholerae* strains, which showed resistance to Co, Fr, and Na. The overall resistance profiles were as follows: *E. coli* strains, A, Fr, Co, S, Cf, Na, Nx, and N; *Shigella* spp., T, S, Na, Co, N, and Fr; and *Salmonella*, Fr and A. The susceptibility pattern of *Aeromonas* spp. revealed resistance to A, Fr, Cf, Na, Nx, and Co (Fig. 3). The *V. cholerae* O1 Ogawa and Inaba isolates were sensitive to Nx, Cf, S, G, T, C, N, A, and azithromycin. The pathogenic *E. coli* strains were sensitive to C, G, and T. All 144 *V. cholerae* O1 isolates were 94.1% resistant to Na, 88.2% to Co, and 80.4% to Fr. The *Aeromonas* spp. were 83.4% resistant to A, 77.8% to T, 72.3% to Co and 61.2% to Na, respectively. *Shigella* spp. were 75.6% resistant to Fr, 75.2% to Na, 60.1% to Co, and 55.9% to S. Similarly, *Salmonella* spp. were 60.4% resistant to A and 59.7% to Fr. The virulent *E. coli* strains were 85.4%

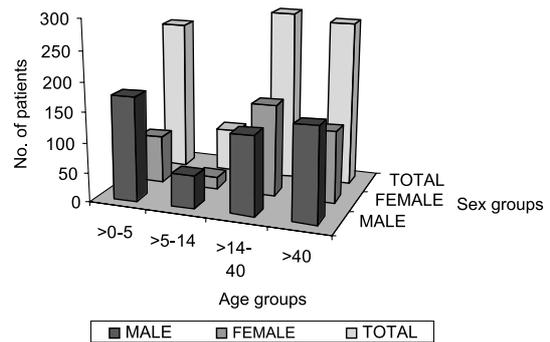


Fig. 2. Age and sex wise distribution of culture positive patients during January 2004 to December 2006.

resistant to A, 93.7% to Fr, 64.6% to Co, 58.3% to S, 79.2% to Cf, 85.4% to Na, 66.7% to Nx, and 60.4% to N, respectively.

PCR assay of *V. cholerae* and *E. coli*: All the *V. cholerae* O1 Ogawa ($n = 74$), *V. cholerae* O1 Inaba ($n = 70$) and *V. cholerae* O139 ($n = 9$) strains isolated during the study period were positive for *ctxA* and *tcpA* genes, showing the El Tor biotype as revealed by PCR assay. Most of the EPEC ($n = 40$), ETEC ($n = 52$), and EAaggEC ($n = 25$) strains isolated were positive for *eae*, *elt*, and *ast* genes, respectively, as revealed by PCR assay. During the years 2004, 2005, and 2006, EPEC cases numbered 10 (4.5%), 21 (7.0%) and 9 (6.2%), ETEC cases were 20 (9.2%), 21 (6.8%) and 11 (7.0%), and EAaggEC cases were 6 (2.8%), 11 (3.7%) and 8 (5.3%), respectively.

DISCUSSION

This is the first report of a systematic surveillance study on the prevalence of different bacterial enteropathogens isolated from hospitalized diarrhea patients from Orissa. In this investigation five bacterial enteropathogens were isolated from hospitalized diarrhea patients. The prevalence of cases

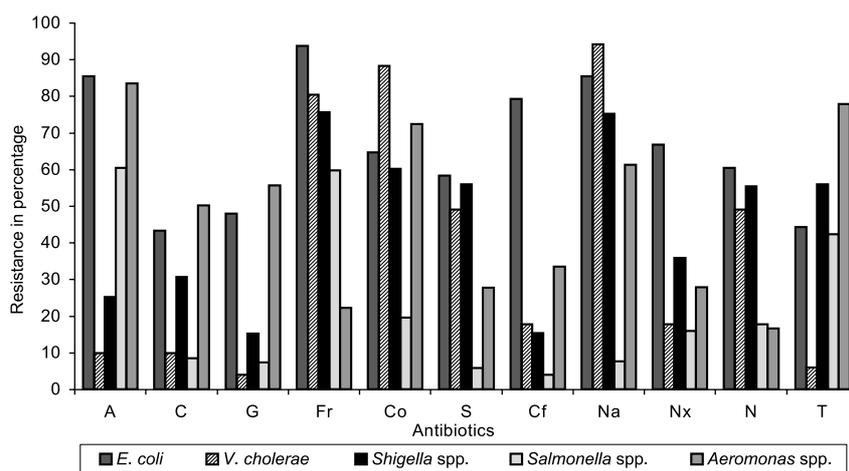


Fig. 3. Antibiotic resistance pattern of different bacterial enteropathogens. A, ampicillin; C, chloramphenicol; G, gentamicin; Fr, furazolidone; Co, co-trimoxazole; S, streptomycin; Cf, ciprofloxacin; Na, nalidixic acid; Nx, norfloxacin; N, neomycin; T, tetracycline.

of diarrhea in Ifakara, Tanzania with a known etiology was 71.8% in the dry season and 63.1% in the rainy season. Diarrhea in children in developing countries other than Tanzania has been reported to have a known etiology in 50 to 60% of the diagnosed cases (15,16); the main difference between these reports is the inclusion of diarrheagenic *E. coli*, which increased the frequency of cases of diarrhea with known etiology to 34.6 and 28.0% in the dry and the rainy seasons, respectively. Pai et al. (17) reported that EAggEC was responsible for 55% of the diarrhea cases in a diarrheal epidemic in South India. *Shigella* spp. were isolated at a lower frequency during dry and rainy seasons, whereas Vargas et al. (18) from Ifakara, Tanzania reported high prevalence of *Shigella* in dry season. Infection with diarrheagenic *E. coli*, *S. flexneri*, *Yersinia enterocolitica* and *Giardia* were observed at lower numbers in Italy in 1996 (19). As in the present investigation, *Shigella* spp. were also isolated from a low percentage of diarrhea patients. Jindal et al. (20) has reported isolates of *E. coli* (21.1%), *Salmonella* (8.6%), *Shigella* (4%), and *Campylobacter* (0.7%) among children suffering from diarrhea in Amritsar, India during 1995, but we obtained a higher percentage of *E. coli* (75.5%) and lower percentage of *Salmonella* (0.7%) infection during the same study period. A slightly higher percentage of *E. coli* and lower percentage of *Salmonella* and *Shigella* were isolated from diarrheal children in Lagos, Nigeria in 1989 - 1990, whereas no *V. cholerae*, *Cryptosporidium*, or *Plesiomonas* were detected (21). Mangia et al. (22) reported the high prevalence of *E. coli* responsible for childhood diarrhea in Brazil. Cholera is known to be highly seasonal in North India, starting from April and lasting up to November every year (23,24). We detected a greater number of cholera cases in the months of August to November during the years 2005 and 2006. However, few cholera cases were reported during the months of January and March of 2004, February of 2005, and February to March of 2006. Also a greater number of *V. cholerae* isolates appeared in 2005 and 2006 in comparison to those during 2004, which might have been due to the scanty rainfall of 2004 in comparison to that in 2005 and 2006. A study conducted in Lahore, Pakistan during 1985 to 1991 showed a high prevalence of bacterial infection among children under the age of 5 years in all seasons (25). Basak et al. (26) from Kolkata reported *E. coli* and *V. cholerae* among hospitalized patients with secretory diarrhea in all age groups of patients during 1992. The

male:female ratio remained 1.4:1 in their study period and is agreement with the earlier report of Sharma et al. (27). A low incidence of bacterial enteropathogens among children of age group of 5 - 14 years old was observed in the present study, which might be due to a low number of hospital admissions of diarrhea patients from this age group. Sinha et al. (28) from Kolkata, India reported 6.5 and 3.1% prevalence of *Aeromonas* spp. during 2000 and 2001, respectively; whereas a lower percentage (2%) of *Aeromonas* spp. was isolated from the diarrhea patients in the present region.

Cholera was found to be more common during the rainy season in this region. Sixty-eight *V. cholerae* O1 Ogawa and only one Inaba strain were reported from Kolkata during 1996 (29). In the present investigation, 153 (17.3%) *V. cholerae* strains, including 74 (8.4%) O1 Ogawa, 70 (7.9%) O1 Inaba, and 9 (1%) O139 strains were isolated. We also found a very low percentage of *Salmonella* spp. (<1%), *Shigella* spp. (4.5%), and *Aeromonas* spp. (2.0%) from hospitalized diarrhea patients. Dutta et al. (30) from Kolkata analyzed 402 *V. cholerae* isolates from 2004 and 2005, among which 43.3 and 56.7% were identified as Ogawa and Inaba serotypes, respectively. A study conducted in Dhaka, Bangladesh during 1996 revealed the prevalence of cholera; out of 242 cholera cases, 132 patients were infected with *V. cholerae* O139 (54.5%) strains and 110 were infected with *V. cholerae* O1 strains (31). By comparison, a very lower number of *V. cholerae* O139 (0-2.9%) strains were isolated from 2004 to 2006 in the present study. The presence of cholera toxin genes like *ctxA* and *tcpA* for the El Tor biotype was also found among *V. cholerae* strains by PCR assay.

Prevalences of different pathogenic *E. coli* have been reported among the diarrhea patients from different countries as follows: 23.5% ETEC from North India (32); 15% EPEC and 11.5% EAggEC in bloody diarrhea patients from Baghdad, Iraq (33); 14% EPEC from Kuala Lumpur (34); 18% ETEC and 1% EAggEC in Jakarta, Indonesia (35); 6.5% ETEC, 5.2% enteroinvasive *E. coli* (EIEC), 3.9% EAggEC, and 2.6% EPEC in Beirut, Lebanon (36); 5.5% EPEC and 0.5% EAggEC in Athens, Greece (37); 12.8% EPEC, 10.2% EAggEC, 5.7% ETEC, and 1.5% EIEC in Irbid, Jordan (38); and 8.0% ETEC, 2.0% EAggEC, and EPEC and EIEC <1% in Sweden (39), respectively. In the present investigation there were low prevalences of EPEC (6%), ETEC (7.8%), and EAggEC (3.7%) among hospitalized diarrhea patients from

Orissa. The virulent *E. coli* isolates varied from 15.5 to 11.6% during the study period of 2004-2006 (Table 2).

Profuse watery diarrhea with rice water stool and vomiting were the clinical symptoms among the cholera cases infected with *V. cholerae*. *Shigella* infection was occasionally associated with blood and/or mucus in stools; abdominal cramping with mild fever was also noted.

Early institution of therapy using appropriate intravenous fluid is lifesaving for cholera patients; however, therapy with appropriate antimicrobial agents significantly shortens the duration of hospitalization, and reduces the volume of stools and rehydration fluids required. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing in developing as well as developed countries. The resistance pattern of *V. cholerae* was Na and Co; among *Shigella* overall resistance to Na, Co, and Fr was observed. The *V. cholerae* O1 isolates reported from West Bengal during 2004 were resistant to Co, Fr, and Na (41). In the present investigation, *V. cholerae* isolates were resistant to Co, Fr, and Na, but the profile for *Shigella* was T, S, Na, Co, N, and Fr resistance. That of *Salmonella* was Fr and A resistance whereas non-typhoidal *Salmonella* spp. reported from Chandigarh during 2004 were resistant Cf only (40). Na resistance may be a forerunner of resistance to fluoroquinolones, and occurrence of such resistance may create a therapeutic challenge in the future. In North India, pathogenic *E. coli* strains were resistant to A, G, cefotaxime, and Cf and sensitive to amoxycillin, Na, trimethoprim, and C during an interepidemic period of 2001 and the epidemic period of 2002 (32). Oyofu et al. (42) reported that ETEC and EAEC strains from Alexandria, Egypt in 1995 were sensitive to Nx, Cf, and Na but were resistant to A, T, C, and sulfamethoxazole. Santos et al. (43) reported that the strains of *Shigella* spp., *Salmonella* spp., *E. coli*, and *Klebsiella pneumoniae* from Latin America were resistant to Fr, C, A, and G. In the present study, it is reported that the pathogenic *E. coli* strains were resistant to A, Fr, Co, S, Cf, Na, Nx, and N. Rationalization of therapy for cholera cases is important in any cholera outbreak. *Aeromonas* showed resistance to A, Fr, Cf, Na, Nx, and Co. Of children's diarrhea cases in Tehran, Iran, 4.5% were *Aeromonas* spp. isolates (44). Sinha et al. (28) reported that majority of *Aeromonas* strains isolated from hospitalized diarrhea cases in Kolkata during 2004 exhibited multidrug resistance.

Active surveillance and control strategies are needed for reducing the number of cholera and diarrhea cases in this region. Additionally, reporting of cholera cases and monitoring of the antibiotic resistance pattern are required for long-term surveillance of the changing and obscure epidemiology of diarrheal disorders in Orissa.

ACKNOWLEDGMENTS

We are thankful to Dr. S. K. Kar, Director, R.M.R.C., Bhubaneswar for his constant support, help, and valuable suggestions throughout the study period. We are also thankful to Mr. C. R. Samantra, Lab assistant, for collection of samples.

REFERENCES

- Clarke, S.C. (2001): Diarrhoeagenic *Escherichia coli*: an emerging problem? *Diagn. Microbiol. Infect. Dis.*, 41, 93-98.
- Guerrant, R.L., Kosek, M., Lima, A.A., et al. (2002): Updating the DALYs for diarrhoeal disease. *Trends Parasitol.*, 18, 191-193.
- World Health Organization (1999): Health Situations in the South-East Asia Regions 1994-1997. Regional Office of SEAR, New Delhi.
- Murray, C. and Lopez, A. (1996): The global burden of disease. A

comprehensive assessment of mortality and disability from diseases, injuries and risk factor in 1990 and projected to 2020. Cambridge, MA. Harvard School of Public Health.

- O'Ryan, M., Prado, V. and Pickering, L.K. (2005): A millennium update on pediatric diarrhoeal illness in the developing world. *Semin. Pediatr. Infect. Dis.*, 16, 125-136.
- Sack, D.A., Sack, R.B., Nair, G.B., et al. (2004): Cholera. *Lancet*, 363, 223-233.
- Chhotray, G.P., Pal, B.B., Khuntia, H.K., et al. (2002): Incidence and molecular analysis of *V. cholerae* associated with cholera outbreak subsequent to the super cyclone in Orissa, India. *Epidemiol. Infect.*, 128, 131-138.
- Pal, B.B., Khuntia, H.K., Samal, S.K., et al. (2006): Emergence of *Vibrio cholerae* O1 biotype El Tor serotype Inaba causing outbreaks of cholera in Orissa, India. *Jpn. J. Infect. Dis.*, 59, 266-269.
- World Health Organization (1987): Manual of Laboratory Infections of Acute Enteric Infections. CDD/83.3. World Health Organization, Geneva.
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C., et al. (1966): Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45, 493-506.
- Keasler, S.P. and Hall, R.H. (1993): Detecting and biotyping *Vibrio cholerae* O1 with multiplex polymerase chain reaction. *Lancet*, 341, 1661.
- Stacy-Phipps, S., Mecca, J.J. and Weiss, J.B. (1995): Multiplex PCR assay and simple preparation method for stool specimens to detect enterotoxigenic *Escherichia coli* DNA during course of infection. *J. Clin. Microbiol.*, 33, 1054-1059.
- Yu, J. and Kaper, J.B. (1992): Cloning and characterization of the *eae* gene of enterohaemorrhagic *Escherichia coli* O157:H7. *Mol. Microbiol.*, 6, 411-417.
- Yamamoto, T. and Nakazawa, M. (1997): Detection and sequences of enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene in enterotoxigenic *E. coli* strains isolated from piglets and calves with diarrhea. *J. Clin. Microbiol.*, 35, 223-227.
- Mertens, T.E., Wijenayake, R., Pinto, M.R., et al. (1990): Microbiological agents associated with childhood diarrhoea in the dry zone of Sri Lanka. *Trop. Med. Parasitol.*, 41, 115-120.
- Georges, M.C., Wachsmuth, I.K., Meunier, D.M., et al. (1984): Parasitic, bacterial, and viral enteric pathogens associated with diarrhea in the Central African Republic. *J. Clin. Microbiol.*, 19, 571-575.
- Pai, M., Kang, G., Ramakrishna, B.S., et al. (1997): An epidemic of diarrhoea in south India caused by enteroaggregative *Escherichia coli*. *Indian J. Med. Res.*, 106, 7-12.
- Vargas, M., Gascon, J., Casals, C., et al. (2004): Etiology of diarrhoea in children less than five years of age in Ifkara, Tanzania. *Am. J. Trop. Med. Hyg.*, 70, 536-539.
- Caprioli, A., Pezzella, C., Morelli, R., et al. (1996): Enteropathogens associated with childhood diarrhoea in Italy. The Italian Study Group on Gastrointestinal Infections. *Pediatr. Infect. Dis.*, 15, 876-883.
- Jindal, N., Arora, R., Bhushan, B., et al. (1995): A study of infective etiology of chronic diarrhoea in children in Amritsar. *J. Indian Med. Assoc.*, 93, 169-170.
- Ogunsanya, T.I., Rotimi, V.O. and Adenuga, A. (1994): A study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria. *J. Med. Microbiol.*, 40, 10-14.
- Mangia, A.H., Duarte, A.N., Duarte, R., et al. (1993): Aetiology of acute diarrhoea in hospitalized children in Rio de Janeiro. *J. Trop. Pediatr.*, 39, 365-367.
- Mohanty, S., Kapil, A. and Das, B.K. (2004): Seasonality and antimicrobial resistance pattern of *Vibrio cholerae* in a tertiary care hospital of North India. *Trop. Doctor*, 34, 249-251.
- Das, S. and Gupta, S. (2005): Diversity of *Vibrio cholerae* strains isolated in Delhi, India, during 1992-2000. *J. Health Popul. Nutr.*, 23, 44-51.
- Khalil, K., Lindblom, G.B., Mazhar, K., et al. (1993): Early child health in Lahore, Pakistan: VIII. Microbiology. *Acta Pediatr.*, Suppl., 390, 87-94.
- Basak, S., Chakraborty, P.S., Chandy, J., et al. (1992): An etiological studies on hospital inpatients with secretory diarrhoea in Calcutta. *J. Indian Med. Assoc.*, 90, 14-16.
- Sharma, N.C., Mandal, P.K., Dhillon, R., et al. (2007): Changing profile of *V. cholerae* O1, O139 in Delhi and its periphery (2003-2005). *Indian J. Med. Res.*, 125, 633-640.
- Sinha, S., Shimada, T., Ramamurthy, T., et al. (2004): Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *J. Med. Microbiol.*, 53, 527-534.
- Mukhopadhyay, A.K., Garg, S., Nair, G.B., et al. (1995): Biotype traits

- and antibiotic susceptibility of *Vibrio cholerae* serogroups O1 before, during and after the emergence of the O139 serogroup. *Epidemiol. Infect.*, 155, 427-434.
30. Dutta, B., Ghosh, R., Sharma, N.C., et al. (2006): Spread of cholera with newer clones of *Vibrio cholerae* O1 El Tor serotype Inaba, in India. *J. Clin. Microbiol.*, 44, 3391-3393.
 31. Dhar, U., Benish, M.L., Khan, W.A., et al. (1996): Clinical features, antimicrobial susceptibility and toxin production in *Vibrio cholerae* O139 infection: comparison with *V. cholerae* O1 infection. *Trans. R. Soc. Trop. Med. Hyg.*, 90, 402-405.
 32. Taneja, N., Rao, P., Rao, D.S.V.R., et al. (2006): Enterotoxigenic *Escherichia coli* causing cholera-like syndrome during an interepidemic period of cholera in North India. *Jpn. J. Infect. Dis.*, 59, 245-248.
 33. Shebib, Z.A., Abdul Ghani, Z.G. and Mahdi, L. Kh. (2003): First report of *Escherichia coli* O157 among Iraqi children. *East. Mediterr. Health J.*, 9, 159-166.
 34. Lee, W.S. and Puthuchery, S.D. (2002): Bacterial enteropathogens isolated in childhood diarrhoea in Kuala Lumpur- the changing trend. *Med. J. Malaysia*, 57, 24-30.
 35. Oyoyo, B.A., Subekti, D., Tjaniadi, P., et al. (2002): Enteropathogens associated with acute diarrhoea in community and hospital patients in Jakarta, Indonesia. *FEMS Immunol. Med. Microbiol.*, 34, 139-146.
 36. Matar, G.M., Abdo, D., Khneisser, I., et al. (2002): The multiplex-PCR-assay detection and genotyping of diarrhoeagenic *Escherichia coli* in diarrhoeal stools. *Ann. Trop. Med. Parasitol.*, 96, 317-324.
 37. Kafetzis, D.A., Maltezou, H.C., Zafeiropoulou, A., et al. (2001): Epidemiology, clinical course and impact on hospitalization costs of acute diarrhoea among hospitalized children in Athens, Greece. *Scand. J. Infect. Dis.*, 33, 681-685.
 38. Youssef, M., Shurman, A., Bougnoux, M., et al. (2000): Bacterial, viral and parasitic enteric pathogens associated with acute diarrhoea in hospitalized children from northern Jordan. *FEMS Immunol. Med. Microbiol.*, 28, 257-263.
 39. Syenungsson, B., Lagergren, A., Ekwall, E., et al. (2002): Enteropathogens in adult patients with diarrhoea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clin. Infect. Dis.*, 35, 1452-1453.
 40. Taneja, N., Mohan, B., Khurana, S., et al. (2004): Antimicrobial resistance in selected bacterial enteropathogens in north India. *Indian J. Med. Res.*, 120, 39-43.
 41. Sur, D., Dutta, S., Sarkar, B.L., et al. (2007): Occurrence, significance and molecular epidemiology of cholera outbreaks in West Bengal. *Indian J. Med. Res.*, 125, 772-776.
 42. Oyoyo, B.A., Peruski, L.F., Ismail, T.F., et al. (1997): Enteropathogens associated with diarrhoea among military personnel during operation Bright Star 96, in Alexandria, Egypt. *Mil. Med.*, 162, 396-400.
 43. Santos, J.I., De la Maza, L. and Tanaka, J. (1989): Antimicrobial susceptibility of selected bacterial enteropathogens in Latin America and worldwide. *Scand J. Gastroenterol. (Suppl.)*, 169, 28-33.
 44. Soltan Dallal, M.M. and Moezardalan, K. (2004): *Aeromonas* spp. associated with children's diarrhoea in Tehran: a case control study. *Ann. Trop. Paediatr.*, 24, 45-51.