

Original Article

Bacteriological Etiology of Flood Affected Diarrhoeal Patients Admitted in Dhaka Medical College Hospital and Antibioqram of the isolates

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Abstract

Diarrhoea is a self-limiting disease caused by various enteropathogens and antimicrobial therapy is an important adjunct to fluid therapy in the management of the cases. We have studied the flood-affected diarrhoeal (post-flood diarrhoea) patients admitted in Dhaka Medical College Hospital during August to September, 2007. A total of 114 faecal samples were collected from the patients of all age groups, and investigated for bacterial enteropathogens. Specimens were cultured in appropriate media and identification of the organisms showing growth was done by relevant biochemical tests. In addition, the isolates of *Vibrio cholerae* were confirmed using polyvalent antisera. Sensitivity of the isolated organisms was done by disc diffusion method. Among the isolated bacterial enteropathogens, majority were *V. cholerae* (46, 40.3%). Other isolates included *Escherichia coli* (18, 23.0%), *Aeromonas* species (8, 10.0%) and *V. parahaemolyticus* (5, 7.0%). No pathogenic bacteria was isolated from 27 of the cases. No *Salmonella* or *Shigella* was detected in any case. Majority of the cases (24, 52.0%) infected with *V. cholerae* were children having less than 10 years of age. Most of the isolates of *V. cholerae* (77.7%) were sensitive to Ciprofloxacin. Almost all of the *V. cholerae* isolates (97.9%) were resistant to Tetracycline, followed by Co-trimoxazole (93.5%) and Nalidixic acid (89.2%). All of the *E. coli* isolates (100.0%) were sensitive to Ceftriaxone, followed by Ceftazidime (88.8%) and Ciprofloxacin (77.7%). Thus it can be concluded that Ciprofloxacin and Ceftriaxone can be considered as the drugs of choice for the treatment of post-flood diarrhoea along with proper rehydration therapy.

Key words: Post-flood diarrhoea, Enteropathogens, *V. cholerae*, *E. coli*

Introduction

Acute diarrhoea is a common cause of morbidity and mortality throughout the world. Usually, the most severe cases as well as the most frequently occurring forms of the disease in developing countries are of bacterial etiology.^{1,2} Cholera, caused by *Vibrio cholerae*, is a severe dehydration-causing fatal diarrhoeal disease of worldwide importance.³ It remains an important cause of diarrhoeal illness and death in

Asia, Africa and Latin America.⁴ Its home is in the valleys of the Ganges and other great rivers of the Far East where high humidity and population density have maintained the disease.⁵ The bacteria *V. cholerae* can survive for up to 2 weeks in fresh water and 8 weeks in saline water.⁵ It is endemic and follows a distinct seasonality in Bangladesh.⁶ In 1982, the classical Vibrios began to re-establish itself in Bangladesh. A new pandemic began with a unique serotype O139 in the country in 1992.⁵ There was a subsequent re-emergence of *V. cholerae* O1 of the El tor biotype in Bangladesh since 1994.⁷ During two epidemics of diarrhoea in Bangladesh in October, 2004 and December, 2005, 953 and 344 strains of *V. cholerae* O1 were isolated from the cases admitted into the Dhaka hospital and Matlab hospital of

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ICDDR,B respectively.⁸

Cholera is a self-limiting, non-invasive disease,³ the powerful exotoxin (enterotoxin) of the causative bacteria *V. cholerae* stimulates the adenylyl cyclase-adenosine monophosphate pathway of the mucosa resulting in an outpouring of small bowel fluid.⁵ Almost all cases of diarrhoea being self-limiting, antimicrobial therapy is only an adjunct to fluid therapy in the management of the patients.⁴ In diarrhoea endemic areas like Bangladesh, people have some degree of acquired immunity against the disease, and patients developing symptoms of cholera usually seek services from the primary healthcare facilities available nearby.⁹

Waterborne diarrhoea is not unusual after the flood in Bangladesh. The recent flood in July to August, 2007 was also followed by diarrhoea and accordingly a 'diarrhoea cell' was set up in Dhaka Medical College Hospital (DMCH) for specialized patient care. The purpose of this study was to find out the bacterial agents causing diarrhoea among the flood affected patients admitted into DMCH and to see sensitivity pattern of the isolated microorganisms against commonly prescribed antimicrobial agents.

Methods

This cross-sectional and analytical study extended for a period of 40 days from 12 August to 22 September, 2007. A total of 114 stool samples/ rectal swabs were collected from flood affected patients admitted into Dhaka Medical College Hospital. The samples were transported immediately to the Microbiology laboratory of Dhaka Medical College and processed as early as possible.

The specimens were inoculated on MacConkey's Agar, TCBS agar, SS agar and Peptone water. The organisms showing growth were identified by colony morphology, microscopic examination, motility test and other relevant biochemical tests.¹⁰ *V. cholerae* was further identified by indole production, methyl red reaction, VP test, citrate utilization, oxidase and urease production tests. Final identification of the isolated Vibrios was done by slide agglutination test with specific antisera (*Vibrio cholerae* O1 polyvalent, Remel Inc. 12076 Santa Fe Drive Lenexa, KS 66215, USA). All the bacteria isolated were tested for sensitivity against a panel of antimicrobial agents, following standard procedures of the Kirby and Bauer disc diffusion method.¹¹

Results

One hundred and fourteen stool samples were cultured, of which 78 (68.42%) yielded growth of pathogenic bacteria, majority being *V. cholerae* (46/78, 58.97%), followed by *E. coli* (18/78, 23.08%) and other organisms. (Table I)

Table I: Pathogenic bacteria isolated from the study population

Organisms isolated	Number	Percentage
<i>Vibrio cholerae</i>	46	58.97%
<i>Escherichia coli</i>	18	23.08%
Aeromonas species	8	10.26%
<i>V. parahaemolyticus</i>	5	6.41%
Klebsiella species	1	1.28%
Total	78	100.0%

Among the 46 isolated *V. cholerae*, majority were sero-positive for *V. cholerae* O1 (26, 56.52%) while the rest 20 (43.48%) were sero-negative with *V. cholerae* O1 polyvalent antisera.

The age groups of the patients suffering from diarrhoea due to *V. cholerae* ranged from 6 months to 55 years. However, most of them (24, 52.16%) were under 10 years of age, while a calculated 39.14% of the cases were between 11-30 years, and only 2 (4.35%) were found above 50 years. (Table II)

Table II: Age distribution of the cases yielding *V. cholerae* (n=46)

Age group	Number	Percentage
0-10 years	24	52.16%
11-20 years	9	19.57%
21-30 years	9	19.57%
31-40 years	2	4.35%
41-50 years	0	0%
50+ years	2	4.35%
Total	46	100.0%

Most of the *V. cholerae* isolates (71.7%) were sensitive to Ciprofloxacin. This was followed by Ceftriaxone (63.1%),

Azithromycin (58.6%) and Cefazidime (54.3%). Cephalixin was found sensitive to only 28.2% of the isolates. Tetracycline was found as the most resistant drug (97.9%), followed by co-trimoxazole (93.5 %) and nalidixic acid (89.2%). (Figure 1)

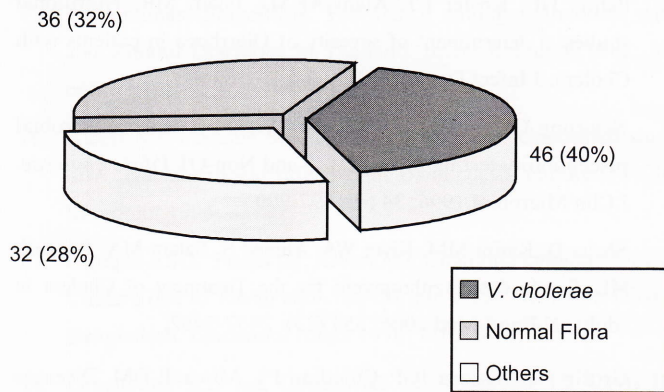


Figure 1: Stool culture result of the study population (n=114)

V. cholerae isolated from 8 cases were resistant to all the antimicrobials used *in vitro*. Among the other bacterial agents, Ciprofloxacin was 77.7% sensitive against *E. coli*, while sensitivity results of this antibiotic for *Aeromonas* species, *V. parahaemolyticus* and *Klebsiella* species were 25%, 80%, and 100%, respectively. Cefazidime and Ceftriaxone were 88.8% and 100% sensitive respectively against *E. coli*. (Table III)

Table III: Rate of sensitivity of enteropathogens other than *V. cholerae* (n=32)

Antimicrobial agents	No. of the organisms found sensitive			
	<i>E. coli</i> (n=18)	<i>Aeromonas</i> species (n=8)	<i>V. para-haemolyticus</i> (n=5)	<i>Klebsiella</i> species (n=1)
Nalidixic Acid	2 (11.7%)	0 (0%)	0 (0%)	0 (0%)
Ciprofloxacin	14 (77.7%)	2 (25%)	4 (80%)	1 (100%)
Cephalixin	1 (5.5%)	1 (12.5%)	2 (40%)	0 (0%)
Azithromycin	2 (11.1%)	1 (12.5%)	0 (0%)	0 (0%)
Cefazidime	16 (88.8%)	0 (0%)	3 (60%)	1 (100%)
Ceftriaxone	18 (100.0%)	3 (37.5%)	1 (20%)	1 (100%)
Cotrimoxazole	4 (22.2%)	0 (0%)	0 (0%)	0 (0%)
Tetracycline	1 (5.5%)	0 (0%)	0 (0%)	0 (0%)

Discussion

In the first surveillance study of diarrhoeal patients from Dhaka published in 1982, few bacterial enteropathogens were isolated which included *Salmonella* species, *Shigella* species, *Vibrio cholerae* O1, *Campylobacter jejuni* and Enterotoxigenic *Escherichia coli* (ETEC).¹² In another study, additional bacteria like *V. cholerae* O139, *Aeromonas* species, *Shigella* species, *E. coli* were isolated as potential pathogens.¹³ In the present study, five types of enteropathogens were isolated from 114 patients of a diarrhoea outbreak following flood. This study differs from other studies, because patients coming from the flood-affected areas were only included. Previously *V. cholerae* O1 & O139, *C. jejuni*, Enterotoxigenic *E. coli* (ETEC), Enterohaemorrhagic *E. coli* (EHEC) were significantly associated with diarrhoea.¹³ In the present study, 46 (58.97%) isolated bacteria were *V. cholerae* and 18 (23%) were *E. coli*. However, many other diarrhoeagenic strains could not be differentiated due to lack of logistic supports.

The role of *Aeromonas* species as a significant agent causing diarrhoeal disease remains controversial. These organisms have been epidemiologically linked to acute Diarrhoea as had been reported in some studies.^{14,15} As reported in previous Cholera epidemics, ETEC Diarrhoea and Rota virus Diarrhoea had seasonal peaks with Cholera peaking in the hot, dry Spring months and again in the hot wet months of August to October.^{12,16} *V. cholerae* re-emerged in 1994¹⁷ as the predominant pathogen causing Cholera as was found again in the current post-flood Diarrhoea cases. Viral agents causing Diarrhoea were not sought in this study due to existing laboratory limitations.

Early administration of ORS and intravenous fluids can save the lives of patients with Diarrhoea. Therapy with an effective antimicrobial agent significantly shortens the duration of Diarrhoea and hospitalization, and reduces the volume of watery stool.¹⁸ Tetracycline and Doxycycline have long been the antibiotics of choice for treatment of severe Cholera in Bangladesh excluding children and pregnant women.^{6,18} In the surveillance update of *V. cholerae* by the ICDDR,B in 2003, sensitivity was 100% for each of Tetracycline and Ciprofloxacin.¹⁹ In 2006, the sensitivity of *V. cholerae* O1 to Ciprofloxacin remained same (100%), while to Tetracycline it was reduced to 54 (03%).²⁰ In Dhaka, Bangladesh, 96% of drug-resistant-strains of *V. cholerae* (resistant to at least one of the tested antibiotics) appeared by 2005, 49% of which

were found resistant to both Erythromycin and Azithromycin.⁸ Previous reports of resistance to Erythromycin could be a marker for resistance to Azithromycin, similarly resistance to Tetracycline is considered as an indicator for resistance to Doxycycline.⁸ *V. cholerae* strains isolated from patients in Eastern India, over the past six years, had shown that the bacilli were resistant to several old as well as new antibiotics including Ampicillin, Tetracycline, Furazolidine, Norfloxacin and Ciprofloxacin.²¹ In the same study, the susceptibility of *V. cholerae* O1 to Ciprofloxacin and Doxycycline was 94% and 73% respectively, while 37% of the isolates were resistant to Tetracycline.²²

With the passage of time, other drugs like Nalidixic acid, Cephalexin, Ceftazidime, Ceftriaxone, Levofloxacin and Azithromycin are being used in treating diarrhoeal cases.²³ The emergence of drug-resistant enteropathogens has been reported worldwide. In a study by Gomi in 2001, Ciprofloxacin and Levofloxacin were highly active against all pathogens. Azithromycin and Ceftriaxone also showed a high *in vitro* sensitivity.²² In the current study, the sensitivity to Ciprofloxacin was 77.7%, 25.0%, and 80.0% against *E. coli*, *Aeromonas* species and *V. parahaemolyticus* respectively, while sensitivity of the same organisms to Ceftriaxone was 100.0%, 37.5%, and 20.0%, respectively.

The sensitivity of *V. cholerae*, *E. coli* and *Aeromonas* species to Azithromycin was 58.6%, 11.1% and 12.5% respectively. Nalidixic acid is still used for dysenteric illness in developing countries^{23,24} and it has been used in a screening test for prediction of fluoroquinolone-resistance.²⁵ In this study, the resistance to Nalidixic acid of *V. cholerae* and *E. coli* were 8.92% and 88.9% respectively. The cross-resistance between these drugs raises concern about the emergence of future fluoroquinolone resistance.²⁶

As in other studies, investigators of the present study also found an association of Diarrhoea with *Aeromonas* species.²⁷ Thus from the present study, it can be concluded that the major bacterial pathogens causing post-flood diarrhoea includes *V. cholerae*, *E. coli*, *Aeromonas* species and *V. parahaemolyticus* and Ciprofloxacin and Ceftriaxone can still be considered as the drugs of choice for treatment of post-flood diarrhoea along with proper rehydration.

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