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# Isolation of Enterotoxigenic *Vibrio cholerae* non-01 from the Buriganga River and Two Ponds of Dhaka, Bangladesh

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## ABSTRACT

***Vibrio cholerae* 01 is usually considered the most toxigenic member of the *Vibrionaceae* and *V. cholerae* non-01 isolated from the environment is non-toxigenic. In our survey of the pollution of some aquatic environments in and around Dhaka, Bangladesh, we wanted to investigate the toxigenicity of *V. cholerae* non-01 isolated from water and sediment samples of the Buriganga river and two ponds in Dhaka, in the rabbit ileal loop (RIL) model. Fluid accumulation was induced by 18 of 28 live cultures and five of 18 cell-free culture filtrates in RIL. Seven of ten *V. cholerae* non-01 which failed to induce fluid in RIL were subjected to repeated passage in rabbit gut. Within two consecutive passages, all the strains could induce fluid in rabbit gut. Both toxigenic and non-toxigenic strains were uniformly sensitive to chloramphenicol and gentamicin but resistant to neomycin, novobiocin, polymyxin-B, streptomycin and vancomycin. Tetracycline sensitivity was found among eight of 17 toxigenic and six of 12 non-toxigenic strains. Sensitivity to trimethoprime-sulfa-methoxazole was noted among seven of 17 toxigenic and six of 12 non-toxigenic strains. Occurrence of enterotoxigenic and drug-resistant *V. cholerae* non-01 in the surface water is a public health hazard.**

*Key words*: *Vibrio cholerae*; Enterotoxins; Environment; Diarrhoea.

## INTRODUCTION

Yajnik and Prasad (1) first report the association of *Vibrio cholerae* non-01 with diarrhoeal disease in man. Five years later, while reviewing various reports of isolation of *V. cholerae* non-01, Politzer (1959) concluded that the organisms are frequently implicated in sporadic cases and outbreaks of diarrhoea in different areas of the world (2). Ecological studies conducted in the USA (3,4) indicated that an aquatic environment is the habitat of *V. cholerae* non-01.

The pathophysiology of diarrhoea caused by *V. cholerae* non-01 is not clear. However, different workers have tested the pathogenicity of *V. cholerae* non-01 isolated from clinical as well as from environmental sources. Gupta (1956) reported fluid accumulation in rabbit gut induced by *Vibrio* strains isolated from clinical sources, whereas strains from aquatic sources failed to induce fluid (5). Mcintyre *et al.* and Spira *et al.* reported enterotoxigenicity of *V. cholerae* non-01 isolated from diarrhoea patients (6,7). But there has been no report of the test of enterotoxigenicity of *V. cholerae* non-01 isolated from the aquatic environments of Bangladesh. The present paper describes the enterotoxigenicity and drug-resistance pattern of *V. cholerae* non-01 isolated from the aquatic environments of Bangladesh.

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## MATERIALS AND METHODS

**Isolation of *V. cholerae non-01***: Water and sediment samples from three points of the Buriganga river (Babubazar ghat, Sadarghat and Farashganj ghat) and from two ponds near the southern bank of the Buriganga river were collected monthly for one year (August, 1982 to July, 1983). Water and sediment samples were streaked onto taurocholate tellurite gelatin agar (TTGA) (8) before and after enrichment in bile peptone broth (9) for six hours. Inoculated plates were incubated at 37°C for 18-24 h. After overnight incubation, typical *Vibrio*-like colonies on TTGA agar plate were tested for cytochrome oxidase. Oxidase positive colonies were identified as *V. cholerae* through conventional biochemical reaction (10). Then the *V. cholerae* strains were tested for slide agglutination with *V. cholerae* 01 polyvalent antiserum. Strains which did not agglutinate with *V. cholerae* 01 polyvalent antiserum were considered *V. cholerae* non-01.

**Preservation of strains.** Well-characterised, *V. cholerae* non-01 strains were streaked on blood agar base slant in a small glass vial. Following overnight incubation at 37°C, the vials were filled with sterile paraffin-oil in order to avoid drying and stored at room temperature.

**Test of enterotoxigenicity.** Strains were subcultured three years (1985) after preservation simultaneously on gelatin agar and TTGA (8). Strains were again checked for their standard carbohydrate fermentation reaction (10) before testing enterotoxigenicity. The enterotoxigenic activity of *V. cholerae* non 01 strains were tested in the rabbit ileal loop model following the method of De and Chatterjee (11). Twenty-eight strains of *V. cholerae* non-01 were tested in two rabbits each.

**Passage in rabbit gut.** Live cultures of those strains which failed to induce fluid accumulation in RIL were subjected to repeated passage in rabbit gut following the standard method (12).

**Test of drug susceptibility.** A test for drug susceptibility was carried out by the standard single-disk agar diffusion method of Bauer *et al.* (13) using disks purchased from BBL (Cockeysville, MD, USA). The antibiotics and chemotherapeutic agents used were (in micrograms, except where indicated otherwise): ampicillin 10, cefamandol 30, chloramphenicol 30, colistin 10, erythromycin 15, gentamicin 10, neomycin 30, novobiocin 30, polymyxin-B 300U, streptomycin 10, tetracycline 30, trimethoprim + sulfamethoxazole 1.25+23.75 and vancomycin 30.

## RESULTS

**Isolation of *V. cholerae* non-01.** During the study period (August 1982 to July 1983), 60 water and 60 sediment samples were collected from the sampling points. *V. cholerae* non-01 strains alone were isolated from all the enriched water and sediment samples. A total of 120 strains of *V. cholerae* non-01 were isolated during the study period and stored in blood agar-base (Difco) slant.

**Enterotoxigenic test.** Twenty-eight strains of *V. cholerae* non-01 were tested for enterotoxigenicity. Live cultures of 18 strains induced fluid accumulation in RIL. Cell-free culture filtrates of only five of these strains induced fluid accumulation. Live cultures and cell-free culture filtrates induced fluid accumulation ranging from 0.6 to 1.2 ml/cm and 0.7 to 1.2 ml/cm of gut, respectively (Table I).

**Passage in rabbit gut.** Seven of ten strains of *V. cholerae* non-01 which failed to induce fluid in RIL were subjected to consecutive repeated passage in rabbit gut. Within two passages all the strains could induce fluid accumulation ranging from 0.6 to 2.3 ml/cm of gut (Table I).

**Drug susceptibility.** Seventeen toxigenic and 12 non-toxigenic strains of *V. cholerae* non-01 were tested for antibiotic sensitivity pattern against 13 antibiotics (Table II). Toxigenic strains were uniformly susceptible to chloramphenicol and gentamicin and resistant to neomycin, polymyxin-B, streptomycin and vancomycin. A variable sensitivity pattern was observed with respect to other antibiotics. The non-toxigenic strains also showed susceptibility patterns similar to toxigenic strains.

Table I. Rabbit Illeal Loop Test of *V. Cholerae* non-01 straints

| Type of strain | No. +ve /No. tested | Range of fluid (ml/cm) induced by | |
|---|---|---|---|
| | | live culture | cell-free culture filtrate |
| Wild type | 17/28 | 0.6-1.2 | 0.7-1.2 |
| Passage | 4[a]/4 | 0.6-2.3 | ND |
| Passage | 3[b]/3 | 0.9-1.7 | ND |
| *V. cholerae* 569B | 1/1 | 1.0 | 1.2 |
| *E. coli* K-12 | 0/1 | 0 | 0 |

ND = Not done
a = Positive after 1st passage.
b = Positive after 2nd passage.

## DISCUSSION

Diarrhoea caused by *V. cholerae* non-01 is less severe than typical cholera cases, however, it (*V. cholerae* non-01) has been associated with "full-blown cholera syndrome" in India and Bangladesh (14,15). In the case of an active infection with *V. cholerae* non-01, a significant rise in serum agglutination titre against homologous strains and an antitoxin titre rise of significant levels were observed in the case of infection with toxigenic *V. cholerae* non-01 in some patients in Bangladesh (16). These findings provide an indication of the pathogenicity of *V. cholerae* non-01 in human diarrhoea. Moreover, *V. cholerae* non-01 associated diarrhoea showed definite seasonality in Bangladesh (17) and the rate of isolation from the environment correlated with the incidence of hospitalised *V. cholerae* non-01 cases (18). None of the above authors tested *in vitro* toxigenicity of *V. cholerae* non-01.

Association of *V. cholerae* non-01 with human diarrhoea is established through experimental studies carried out with human isolates in animal model. It is assumed that strains isolated from environmental sources are mostly non-pathogenic and hence cannot cause disease. This idea became more prominent when Gupta *et al.* (1956) first demonstrated that vibrios isolated from human are capable of causing fluid accumulation in RIL, whereas those isolated from the environment were unable to induce fluid accumulation in RIL (5). Kaper *et al.* (1979) reported enterotoxigenicity of a few isolates of *V. cholerae* non-01 from the Chesapeake Bay (4). Shimada and Sakazaki (1982) reported that 16% of 476 strains isolated from the environment of Japan were toxigenic (19). In the present investigation a significant number (18 of 28) of *V. cholerae* non-01 demonstrated toxigenicity. This indicates pathogenic potential of environmental isolates to induce human diarrhoea.

Pathogenic microorganisms can lose their pathogenicity when preserved in the laboratory. It is assumed that some sort of repression occurs in the toxin gene during preservation of strains in the artificial medium (20). Similar events may happen in the aquatic environment where pathogenic microorganisms lose virulence and may become avirulent. Such avirulent strains become virulent through repeated passages in the animal model. In the present investigation, increased fluid accumulation through repeated passage supports the acquisition of virulence through repeated passage. It may happen in the community constantly where people ingest *V. cholerae* non-01 through faecally polluted water and discharge the same organism into the environment through defecation. Thus constant circulation of the same organism between man and the environment may enhance virulence of the organism. Community-wide outbreak may result in this way.

Table II. Antibiotic susceptibility pattern of toxigenic and non-toxigenic strains of *V. cholerae* non-01

| Antibiotics | Antibiogram of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Toxigenic strain (n=17) | | | | Non-toxigenic strain (n=12) | | | |
| | Sensitive | | Resistance | | Sensitive | | Resistance | |
| | | (%) | | (%) | | (%) | | (%) |
| Ampicillin | 3 | (18) | 14 | (82) | 2 | (17) | 10 | (83) |
| Cefamendol | 8 | (47) | 9 | (53) | 10 | (83) | 2 | (17) |
| Chloramphenicol | 17 | (100) | 0 | | 12 | (100) | 0 | |
| Colistin | 1 | (6) | 16 | (94) | 0 | | 12 | (100) |
| Erythromycin | 1 | (6) | 16 | (94) | 1 | (8) | 11 | (92) |
| Gentamicin | 17 | (100) | 0 | | 12 | (100) | 0 | |
| Neomycin | 0 | | 17 | (100) | 1 | (8) | 11 | (92) |
| Novobiocin | 3 | (18) | 14 | (82) | 3 | (25) | 9 | (75) |
| Polymyxin B | 0 | | 17 | (100) | 0 | | 12 | (100) |
| Streptomycin | 0 | | 17 | (100) | 0 | | 12 | (100) |
| Tetracycline | 8 | (47) | 9 | (53) | 6 | (50) | 6 | (50) |
| Trimethoprim + Sulfamethoxazole | 7 | (41) | 10 | (59) | 6 | (50) | 6 | (50) |
| Vancomycin | 0 | | 17 | (100) | 0 | | 12 | (100) |

Treatment of diseases with antibiotics and chemotherapeutic agents is responsible for the occurrence of drug-resistance among enteric pathogens and the majority of the drug-resistant bacteria carry transferable resistance (R) factor (21). Through discharge of human and animal wastes, drug-resistant bacteria are distributed in the sewage and surface water where exchange of R-plasmids can occur under certain physical, chemical and biological conditions (22). Thus, drug-resistant bacteria can spread in the environment where man and animal may acquire infection with bacteria-carrying, drug-resistant plasmids (23). In Bangladesh, there is clear evidence of antibiotic abuse (24) which may be responsible for the occurrence of drug resistance in the pathogenic bacteria. Further study is needed to explain the mechanism of resistance to uncommon antibiotics, such as neomycin, novobiocin, polymyxin B, and vancomycin, which are rarely used for the treatment of disease in Bangladesh.

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