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LETTER TO THE EDITOR

# Enterotoxigenicity of *Vibrio fluvialis* Strains Isolated from Fresh Water Environment

Sir,

*Vibrio fluvialis* is a member of the family *Vibrionaceae* (1). It was initially designated as EF6 or Group F Vibrio (2). It has been isolated from patients with gastroenteritis from different parts of the world (2,3). Clinical symptoms of diarrhoea due to *V. fluvialis* resembled those of typical cholera (4). The present letter describes the enterotoxigenicity, haemolytic activity and drug resistance pattern of *V. fluvialis* isolated from fresh water prawn (*Macrobrachium malcolmsonii*) and water samples of the Buriganga river of Dhaka, Bangladesh.

Strains of *V. fluvialis* were mostly isolated from prawn samples of local fish market and from water samples of the river Buriganga. The strains were preserved on blood agar slant overlaid with sterile paraffin oil and stored at room temperature for four years till use.

Enterotoxigenicity of *V. fluvialis* was tested in rabbit ileal loop (RIL) assay (5). Cell-free culture filtrate was prepared in Richardson's medium. Live cultures and cell-free culture filtrates of eight strains of *V. fluvialis* were tested for enterotoxigenicity in two rabbits. Live cultures and cell-free culture filtrates of *V. cholerae* 569B and *E. coli* K-12 were used as positive and negative controls respectively. In RIL assay, fluid accumulation equal to or more than 0.5 ml/cm of gut was considered a positive reaction.

Test strains were streaked onto blood agar (5% sheep blood) plate and incubated aerobically at 37°C overnight. After incubation, haemolytic zone around each colony was carefully investigated.

Sensitivity of *V. fluvialis* to different antibiotics was tested by the agar diffusion method described by Bauer *et al.* (6) with disks purchased from bioMérioux, France. The antimicrobial agents and their concentrations per disk were as follows: ampicillin 10 µg; chloramphenicol 30 µg; colistin 10 µg; erythromycin 15 µg; gentamicin 10 µg; neomycin 30 µg, novobiocin 30 µg; polymyxin-B 300 U; streptomycin 10 µg; tetracycline 30 µg; trimethoprim-sulfamethoxazole 1.25 µg + 23.75 µg and vancomycin 30 µg.

Eight strains (four strains from river water and equal number from prawn samples) were selected at random. Both live cultures and cell-free culture filtrates of each strain were tested for enterotoxigenicity in the ileal loops of two rabbits. Only four strains (two from river water and two from prawn samples) were positive in RIL assay. The mean volume of fluid secretion induced by live culture and cell-free culture filtrate ranged from 1.1 to 1.2 ml/cm and from 0.7 to 1.8 ml/cm of gut respectively.

All eight strains that were tested for enterotoxigenicity, were also tested for their haemolytic activity on sheep blood (5%) agar plate. Only five strains produced prominent zone of haemolysis around each colony.

Four toxigenic strains were tested for sensitivity to 12 antimicrobial agents. Strains were uniformly sensitive to chloramphenicol and gentamicin. Variable sensitivity patterns were observed with respect to other antimicrobial agents.

*V. fluvialis* has been identified as one of the causative agents of diarrhoea because of its isolation from patients with cholera-like diarrhoea syndrome (4). Moreover, various pathogenic factors, enterotoxigenic activity and elongation of Chinese hamster ovary (CHO) cells have been detected in the cell-free culture-filtrate of *V. fluvialis* strains (7). In this study, we have demonstrated fluid accumulation in RIL model by live-cultures and cell-free culture filtrates of *V. fluvialis* strains. Therefore, our results provide further evidence regarding the enterotoxigenic potentials of *V. fluvialis*.

Out of six haemolytic strains, live cultures and cell-free culture filtrates of four strains induced fluid accumulation in RIL. Like *Aeromonas* strains (8), haemolytic activity of *V. fluvialis* strains may reflect enterotoxigenic activity. Further study is needed to confirm any correlations between haemolytic activity and enterotoxigenicity of *V. fluvialis*.

Drug-resistant bacteria of human and animal origin are distributed in sewage and sewage contaminated surface waters where exchange of R-plasmid can occur (9). Therefore, from the polluted environment, man and other animals may acquire infection with bacteria carrying plasmid-mediated drug resistance (10). In Bangladesh clear evidence of antibiotic abuse was

demonstrated (11) which may be responsible for the occurrence of drug resistance in the pathogenic bacteria. The occurrence of multiple drug resistance among the toxigenic strains of *V. fluvialis* may reflect the effect of drug abuse in Bangladesh. This observation together with our similar earlier findings (12) indicate the need for continuing surveillance of drug-resistance and proper use of antimicrobials in the community.

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