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SHORT COMMUNICATION

ENTEROTOXIGENIC PROPERTIES OF VIBRIO FLUVIALIS (GROUP F VIBRIO) ISOLATED FROM CLINICAL AND ENVIRONMENTAL SOURCES

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Abstract

Toxigenic *Vibrio fluvialis* (formerly Group F vibrio) have been isolated from both clinical and environmental sources in Bangladesh and the United States. Phenotypic and toxigenic characteristics of strains isolated from patients and the environment were similar. Ninety percent of the clinical isolates and 70-80% of the environmental isolates were toxigenic, when concentrated filtrates were tested by the ileal loop assay. *V. fluvialis* appears to be associated with other potentially pathogenic vibrios in the environment, and can be isolated from the aquatic environment of widely diverse geographical areas.

Key words : *Vibrio fluvialis*; Enterotoxins.

Introduction

Vibrio-like organisms, originally designated EF6 or Group F, have been isolated from gastroenteritis patients in various parts of the world (1,2,3), and were identified as the causative agent of an epidemic of diarrhoea in Bangladesh in 1976-77 (4). Organisms identified as members of this group have been isolated from estuarine water and shellfish samples, as well as from household contacts and waters used for drinking and bathing (3,5).

In one of our studies, three strains of EF6 organisms were isolated from 106 Chesapeake Bay (U.S.A.) water samples screened for the presence of vibrios. Phenotypic and biochemical tests were performed to verify the identity of these strains. Some of these isolates were confirmed to be Group F vibrios, by Dr. J.V. Lee (personal communication), who subsequently classified these and other Group F vibrios isolated elsewhere throughout the world, as *Vibrio fluvialis* (6).

Tests were conducted to determine the potential pathogenicity of the environmental isolates. Comparison of clinical and environmental isolates employed three methods for detection of toxigenicity, the results of which are reported in this preliminary communication.

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Materials and methods

Strains employed

A total of 100 strains of *V. fluvialis* were included in the study. Of these, 50 were isolated from diarrhoea patients at the ICDDR,B and 50 from the environment of Chesapeake Bay in the U.S.A. and from Bangladesh. To test for toxigenicity, 8 strains were randomly-selected from the 50 environmental isolates — 3 from the Chesapeake Bay and 5 from Bangladesh; and another 8 strains similarly were chosen from clinical isolates of patients showing moderate-severe dehydration. The environmental strains isolated in Bangladesh were from water sources in the vicinity of households having confirmed *V. fluvialis* diarrhoea cases. Two of the 3 Chesapeake Bay strains selected for toxin testing were from water and 1 was from copepods. These were not associated with clinical cases or disease outbreaks in the surrounding communities. The strain used as the positive control for toxigenicity study was *V. cholerae* 569B, which was processed in a way similar to that for *V. fluvialis*. All strains were maintained on peptone agar stab cultures.

Media used for toxin production

Several media for testing *V. fluvialis* for toxin

production were compared and, from these, two were selected: a modification of the medium of Lockwood *et al.* (7) for toxin production in the rabbit ileal loop (8); and trypticase soy broth (Difco Laboratories, Detroit, Michigan, U.S.A.), supplemented with yeast extract (TSB-YE) for toxin assay employing CHO and Y_1 adrenal cell assays. Stock cultures were grown overnight in the above medium, and 0.25 mL of this grown culture was inoculated into 500 mL of the same medium in 4 L Erlenmeyer flasks, and was incubated at 30°C for 18 h in a gyratory shaker bath.

Preparation of filtrates

After incubation, cultures were centrifuged at 12,000 rpm for 20 min, and the supernatant fluid was filtered through 0.45 μm membrane filters. The filtrates were examined for sterility; and a portion of the sterile filtrate was retained for assay, while the rest was transferred to dialysis bags. The filtrates were concentrated 50X and were stored at 4°C until assayed, in 4-5 days.

Toxigenicity tests

Aliquots of filtrates and concentrated filtrates were tested in the rabbit ileal loop (8), Y_1 adrenal cell (9) and CHO cell (10) assay systems. *V. cholerae* 569B was used as a positive control, while gel-borate buffer solution (formula) and uninoculated 50X concentrated broth served as negative controls.

Antisera against the clinical isolates were raised, by injecting varying amounts of heat-killed, followed by live cultures of the organisms into rabbits, on days, 1,3,7 and 14. Test bleeding was done on day 21 and, if a significantly high titer was observed, the animal was bled to death and serum was separated. The final titer was seen against homologous organisms, by using standard tube agglutination methods.

Results

V. fluvialis exhibit phenotypic traits characteristic of the genus vibrio, but also are similar in several characteristics to *Aeromonas*. Biochemically, all strains included in this study were identified as *V. fluvialis*. The *V. fluvialis* strains from clinical cases belonged to biovar 1 and the environmental isolates to biovars 1 and 2. All strains were lysine-and ornithine decarboxylase-negative and arginine dihydrolase-positive. These strains also were motile, oxidase-positive, and did not produce urease, indole, or hydrogen sulphide. They were sensitive to 150 μg/mL of the vibriostatic compound 0 129, and grew in alkaline-peptone water supplemented with 7% sodium chloride. Seventy-six percent of the clinical isolates and 42% of the environmental isolates were Kanagawa-phenomenon positive, i.e., they produced heat-stable hemolysin on Wagatsuma agar.

In preliminary experiments, neither heat-labile nor heat-stable toxin was detected in crude filtrates of selected strains, in accordance with earlier findings of Lockwood *et al.* (7) and as opposed to those of Agarwal and Sanyal (11).

Results of the rabbit ileal loop assays with the *V. fluvialis* strains isolated from patients and the environment appear in Tables I and II. The strains produced none or very little toxin, as measured by fluid accumulation in rabbit ileal loops when exposed to crude culture filtrates. However, when the filtrates were concentrated 50X, fluid accumulation was detected in most instances. Filtrates prepared with clinical isolates showed signifi-

TABLE I—RABBIT ILEAL LOOP ASSAY OF CULTURE FILTRATES OF *V. FLUVIALIS* STRAINS ISOLATED FROM PATIENTS*

Sample No.	Remarks†	Ratio‡ (v/L)
Q — 3815	CF	0.3
	50X	1.2
Q — 3912	CF	1.3
	50X	2.0
Q — 3998	CF	0.08
	50X	0.34
Q — 3553	CF	0.37
	50X	1.2
Q — 3757	CF	0.30
	50X	0.52
Q — 3498	CF	0.05
	50X	0.10
T — 9381	CF	0.30
	50X	0.44
T — 12266	CF	0.50
	50X	0.98
569-B (positive control)	CF	2.22
Uninoculated broth	—	0
Buffer	—	0

*Eight strains were tested. (see Materials and methods.)

†CF = crude filtrate; 50X = concentrated filtrate (see Materials and methods).

‡Average value of two loops, each in a different animal; (v/L = volume/length).

TABLE II–RABBIT ILEAL LOOP ASSAY OF CULTURE FILTRATES OF *V. FLUVIALIS* ISOLATED FROM THE ENVIRONMENT*

Sample No.	Filtrate type†	Ratio‡ (v/L)
D — 17459	CF	0.13
	50X	0.38
D — 17376	CF	0.29
	50X	1.05
D — 18459	CF	0.15
	50X	0.82
M — 17575	CF	0
	50X	0.11
M — 17652	CF	0.32
	50X	0.88
H — 5	CF	0.32
	50X	0.40
H — 60	CF	0.92
	50X	1.33
H — 62b	CF	0.07
	50X	0.46
569B (positive control)	CF	2.32
Uninoculated broth	—	0
Buffer	—	0

*Eight strains were examined. (see Materials and methods.)

†CF = crude filtrate: 50X = concentrated filtrate (see Materials and methods).

‡Average value of two loops, each in a different animal; (v/L = volume/length).

cantly greater volume/length (v/L) ratios than did the environmental strains.

Most of the crude TSB-YE culture filtrates induced a cytotoxic effect on CHO and Y_1 assay systems, while only 4 filtrates did not have any such effect. None of the test filtrates produced the unequivocal cytotonic reaction observed with the positive control. Furthermore, when heated at 56°C for 1 h, they were not active in any of the assays, and thus were concluded to be heat-labile.

Six of the clinical strains were used to produce antisera in rabbits. Strains yielded titers of 1:320-1:1280 by tube agglutination. The six antisera were tested against each of the 16 isolates; and all 16 strains examined were found to be immunologically homologous. Nine of the strains uniformly agglutinated at a titer of 1:320 against all six antisera, and other five showed agglutination at a higher titer (1:480) against all six antisera. Two strains agglutinated at a titer of 1:640.

Discussion

Since 1977, when the first set of organisms now recognized as *V. fluvialis* was isolated from patients in Bahrain and Bangladesh, additional isolates have been found worldwide, and have been cultivated from shellfish and waters of marine and estuarine environments. Furthermore, these organisms have been implicated as pathogens in patients in Bangladesh, where, during 1977-1980, more than 900 cases were reported at the ICDDR,B cholera treatment hospital in Dhaka. Interestingly, during 1980-1984, fewer than 100 cases per year were recorded.

The *V. fluvialis* strains from the Chesapeake Bay were isolated from water samples collected in June 1979, at the same time as strains of *V. cholerae* non-01 also were isolated from the same water samples. Although 106 water samples and copepods cultured at that time were positive for one or more *V. cholerae* non-01, these samples yielded only 3 *V. fluvialis* isolates. The other vibrio spp. isolated during this sampling included *V. cholerae* non-01, Heiberg groups I, III, IV and V, and *Vibrio parahaemolyticus*.

Experiments done in our laboratories and reported here, show that approximately 90% of *V. fluvialis* strains isolated from clinical sources in Bangladesh and 70-80% of those from environmental samples collected both in Bangladesh and the United States produced variable amounts of toxin. The amount of toxic activity detected in crude culture filtrates was very low, and was not always detectable. Significant results, i.e. readily-available detectable toxin as measured by the ileal loop assay, were obtained with concentrated filtrates. The effect in both the Y_1 adrenal and CHO cell assays also was significant, but the reaction was not dramatic.

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