

SHORT COMMUNICATION

TOXIGENICITY AND DRUG SENSITIVITY OF *VIBRIO MIMICUS* ISOLATED FROM FRESH WATER PRAWNS IN BANGLADESH

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Abstract

Eight isolates of *Vibrio mimicus* from 20 prawns (*Macrobrachium malcolmsonii*) obtained from fresh water in Bangladesh were tested for enterotoxin production and antibiotic sensitivity. Six of the eight isolates (75%) induced fluid accumulation in rabbit ileal loops. All isolates were resistant to kanamycin and streptomycin and sensitive to chloramphenicol, gentamicin and trimethoprim-sulfamethoxazole; 75% were sensitive to ampicillin. The proportion of isolates producing toxin of all isolates and their pattern of sensitivity to antimicrobial drugs resembled human isolates of *V. mimicus*.

Introduction

In 1936 Heiberg (1) reported the existence of strains of *Vibrio cholerae* which did not ferment sucrose. He proposed a scheme for grouping *V. cholerae* on the basis of whether or not they were able to ferment sucrose, mannose and arabinose; strains which fermented mannose but not sucrose and arabinose were assigned to Heiberg Group V. More than 50 strains which did not ferment sucrose were then studied and on the basis of their DNA hybridisation and biochemical characteristics designated as a new species, *V. mimicus* (2, 3). The salient characteristics of this organism are reviewed in Table I. This pathogen has since been isolated in the United States, Japan, Bangladesh, New Zealand and Canada (2) and is associated with diarrhoea, otitis media and food poisoning (4). In Japan, *V. mimicus* has been isolated from rivers, sea water and fish (5) and has also been associated with cases of food poisoning (6, 7). In Bangladesh, Sanyal and colleagues studied the pathogenicity of *V. mimicus* isolated from patients with diarrhoea (8, 9), and the organism has recently been isolated from a case of traveller's diarrhoea

(10). In 1984 Spira *et al.* finally established the pathogenicity of *V. mimicus* when they isolated a toxin similar to cholera toxin (11).

Like other vibrios, *V. mimicus* is transmitted by water (12,13) and has been isolated from shellfish and from patients with diarrhoea who had eaten uncooked or unprocessed food (14). *V. mimicus* is generally susceptible to many drugs with the exception of sulfadiazine, to which about 87% of strains are resistant (2,8,9,15). A few strains have also shown resistance to penicillin, colistin, nalidixic acid and kanamycin (16).

It is now believed that *V. mimicus* strains inhabiting the aquatic environment of Bangladesh, like *V. cholerae*, are acquiring resistance to several antibiotics (17). This paper examines the resistance to commonly used antibiotics of *V. mimicus* isolated from fresh water prawns in Bangladesh.

Materials and methods

Twenty dead prawns (*Macrobrachium malcolmsonii*) which had been preserved in ice were bought from fish vendors at a local market in Dhaka, Bangladesh in January 1986. Specimens were collected from the gills and under the carapace of the prawns using dry sterile plain cotton swabs. Swabs were plated in the field on thiosulfate citrate

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TABLE I—CHARACTERISTICS OF *V. MIMICUS*

Oxidase	+	Citrate utilization	+
Gelatinase	+	Motility	+
VSC 0/129:		Indole	+
10 mcg	S		
150 mcg	S		
Glucose (Acid)	+	Urease	—
Glucose (Gas)	—	<i>V. cholerae</i> -polyvalent	
Mannose (Acid)	+	O antisera :	—
Sucrose (Acid)	—	String test	++
Mannitol	+	L-ornithine	+
Arabinose	—	L-lysine	+
H ₂ S (KIA)	—	L-arginine	—
		Voges-Proskauer	—
		Growth in APW	
		(with % of added NaCl) :	
		0%	+
		3%	+
		6%	V(62.5)
		8%	—
		10%	—

S = sensitive;

V () = % positive.

VSC = *vibrio* static compound (2, 4-diamino-6, 7 diisopropylpteridine)

APW = alkaline peptone water (with added NaCl), pH = 8.2.

KIA = Kligler's iron agar

++ = string length 3.4 mm

bile-salt sucrose (TCBS) agar. After incubation at 37°C for 24 hours, 8 plates showed *Vibrio*-like organisms which were later identified by standard methods (2) as *V. mimicus*. The enteropathogenicity of live, whole cells of *V. mimicus* (10^8 – 10^{10}) was tested using rabbit ileal loops (RIL) (18). *V. cholerae* 569B and *Escherichia coli* K-12 were used as positive and negative controls for loop activity; a ratio of ≥ 0.5 was considered as positive (18).

The sensitivity of the strains to several common antibiotics was tested by the single disk agar diffusion method of Bauer *et al.* (19). The antibiotics and their concentrations per disk (in micrograms) were : ampicillin 10, chloramphenicol 30, gentamicin 10, kanamycin 30, streptomycin 10, trimethoprim-sulfamethoxazole 1.25 + .23.75 and tetracycline 30.

Results

Of twenty prawns, we found *V. mimicus* in eight. No other vibrios were found in the samples examined. In the RIL test six of them caused significant fluid accumulation in a duplicated test (Table II).

All *V. mimicus* isolates were resistant to kanamycin and streptomycin while 6 were resistant to ampicillin and 5 to tetracycline. All the isolates were

TABLE II — RESULTS OF RIL TESTS WITH LIVE CELLS OF *V. MIMICUS* ISOLATED FROM PRAWNS

Strain No.	Fluid accumulation* / length of loop (ml/cm)
<i>V. cholerae</i> 569B (positive control)	1.85
4P1	1.76
6aP	1.80
7P	0.00
8P	1.19
13P	0.95
16P	1.29
17P	1.00
18P	0.30
<i>E. coli</i> (negative control)	0.00

* Mean fluid accumulation in loops of two rabbits. ≥ 0.50 considered as positive.

sensitive to chloramphenicol and trimethoprim-sulfamethoxazole.

Discussion

We reported earlier the isolation in abundance of *V. mimicus* from aquatic environments (20). This is the first report of the isolation of *V. mimicus* from prawns in Bangladesh and it seems that prawns became infected in their natural habitat.

Investigators in the USA and Japan have also found this organism in prawn samples (2,5) and the latter have likewise suggested contamination of fish by environmental vibrios (5).

V. mimicus is widely distributed in the aquatic environment of Bangladesh and is also associated with clinical diarrhoea. Our findings with prawns suggest that they may have a role in the transmission of *V. mimicus* infections. Contamination of prawns by this organism is ubiquitous especially during the hot season when environmental counts of *V. mimicus* rise to a maximum. We assume, that as with other vibrios, at least 10^5 viable organisms are required to establish *V. mimicus* infection (5). The high dose may in part account for the relatively infrequent reports of disease associated with this organism. In addition, food preparation in Bangladesh normally entails complete cooking of most foods. However, some individuals are known to eat uncooked prawns and the health hazard to them is particularly high.

Our findings showed that most isolates of *V. mimicus* from prawns were enterotoxigenic as well as resistant to several common antibiotics. The pattern of antibiotic resistance in prawn isolates resembled our previous findings with environmental isolates (21). These resistance patterns may have diagnostic and taxonomic significance if there are distinct "environmental" and "clinical" phenotypes.

In summary, we have found *V. mimicus* in aquatic environments in Bangladesh and in prawns meant for human consumption. Most prawn isolates produced enterotoxin and were therefore hazardous if not properly handled or processed. Their antibiotic resistance patterns may help differentiate environmental and clinical strains.

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