Vibrio mimicus with multiple toxin types isolated from human and environmental sources

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Summary. A collection of 13 strains of Vibrio mimicus, including both clinical and environmental isolates from different geographic regions, was examined for various toxins. One strain of environmental origin produced cholera-like toxin (CT) which was completely absorbed with anti-CT immunoglobulin G, five strains produced a haemolysin that cross-reacted with the thermostable direct haemolysin of V. parahaemolyticus and DNA from two strains hybridised with a DNA probe specific for the heat-stable enterotoxin of V. cholerae non-O1. Culture supernates of all strains produced a factor that was cytotoxic to Vero and Chinese hamster ovary cells. In this study, we were able to identify strains of V. mimicus that produced, or had the genetic potential to produce, several toxin types simultaneously. The role of these strains as genetic reservoirs is discussed.

Introduction

The species Vibrio mimicus was first proposed by Davis et al.1 for a group of "biochemically atypical" strains of V. cholerae. V. mimicus can be differentiated from V. cholerae by negative sucrose, Voges-Proskauer, corn oil and Jordan tartrate reactions and by sensitivity to polymyxin.1 A single serotyping system is applicable for both V. cholerae and V. mimicus because the two species are indistinguishable serologically.2 Several studies, notably those conducted in Bangladesh3-5 have examined the aetiological role of V. mimicus as a causative agent of gastroenteritis. Apart from gastrointestinal infections, V. mimicus has occasionally been isolated from various other human infections.6 In attempts to determine the virulence factor responsible for disease, previous investigations have documented the ability of strains of V. mimicus to produce either a cholera toxin (CT)-like enterotoxin,7,8 a heat-stable enterotoxin9,10 or a thermostable direct haemolysin (TDH)-like haemolysin.11 However, these studies have investigated the presence of single toxins only. We have looked for all toxin types in each of the strains obtained from several geographic areas. Such an approach has permitted us to locate strains of V. mimicus that produce, or have the genetic potential of producing, several toxin types simultaneously.

Materials and methods

Of the 13 strains of V. mimicus investigated, two (VM1 and VM2) were isolated as co-cultures from cholera patients admitted to the Infectious Diseases Hospital in Calcutta. Eight strains (M-1301, W26768, 7910/91, 74/51, M-33, M-35, 17/10 and M-57) were of clinical origin from Bangladesh, and three strains (VM-4053, VM-4197 and VM-4208) were isolated from environmental sources in LA, USA. All the V. mimicus strains were maintained in nutrient agar stab cultures at room temperature. The serovars of the V. mimicus strains were determined by the somatic O antigen serogrouping scheme developed at the National Institute of Health, Tokyo, Japan.

All media used in this study were from Difco Laboratories. The broth medium used for assessing production of CT-like enterotoxin was Casamino-acid Yeast Extract Medium (CAYE) supplemented with lincomycin (Sigma) 90 mg/L. For tissue culture assay, Tryptic Soy Broth containing yeast extract 0.6% was used. The test strains were cultivated at 37°C for 24 h in a rotatory shaker (Firstek Scientific, USA) set at...
Table. Toxin profiles of 13 *V. mimicus* strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Serovar</th>
<th>Bead-ELISA</th>
<th>NAG-ST DNA probe</th>
<th>Cytotoxin* titre</th>
<th>Toxin profile</th>
</tr>
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<tbody>
<tr>
<td>VM-1</td>
<td>UT</td>
<td>+</td>
<td></td>
<td>8</td>
<td>TDH, Cyt</td>
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<tr>
<td>VM-2</td>
<td>041</td>
<td>+</td>
<td></td>
<td>16</td>
<td>TDH, NAG-ST, Cyt</td>
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<tr>
<td>M-1301</td>
<td>032</td>
<td></td>
<td></td>
<td>16</td>
<td>TDH, Cyt</td>
</tr>
<tr>
<td>W26768</td>
<td>034</td>
<td></td>
<td></td>
<td>8</td>
<td>TDH, Cyt</td>
</tr>
<tr>
<td>7910/91</td>
<td>034</td>
<td></td>
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<td>4</td>
<td>Cyt</td>
</tr>
<tr>
<td>74/51</td>
<td>034</td>
<td>+</td>
<td></td>
<td>2</td>
<td>TDH, Cyt</td>
</tr>
<tr>
<td>M-33</td>
<td>08</td>
<td></td>
<td></td>
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<tr>
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<td>8</td>
<td>Cyt</td>
</tr>
<tr>
<td>17/10</td>
<td>041</td>
<td>+</td>
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<td>16</td>
<td>TDH, Cyt</td>
</tr>
<tr>
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<td></td>
<td>16</td>
<td>Cyt</td>
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<tr>
<td>VM-4053</td>
<td>0126</td>
<td>+</td>
<td></td>
<td>4</td>
<td>CT, Cyt</td>
</tr>
<tr>
<td>VM-4208</td>
<td>032</td>
<td></td>
<td></td>
<td>16</td>
<td>NAG-ST, Cyt</td>
</tr>
<tr>
<td>VM-4197</td>
<td>034</td>
<td></td>
<td></td>
<td>64</td>
<td>Cyt</td>
</tr>
</tbody>
</table>

UT, untypable with available antisera.
TDH, thermostable direct haemolysin; Cyt, Cytotoxin; NAG-ST, heat-stable enterotoxin of *V. cholerae* non-O1; CT, cholera toxin-like enterotoxin.

* Results obtained on Vero cell monolayers.

With the exception of one strain, all the strains examined in this study could be serotyped; there were five strains of serovars 034, and two strains each of serovars 032, 041 and 0126, and one of serovar 08. One strain (VM4053) of environmental origin produced CT-like toxin (> 10 ng/ml) which could be completely absorbed with anti-CT IgG, and DNA from two strains (VM2 and VM4208) hybridised with the NAG-ST DNA probe. In the TDH bead-ELISA, five strains produced a haemolysin that cross-reacted with the TDH haemolysin of *V. parahaemolyticus*.

Culture supernates of all strains produced a factor that was cytotoxic to Vero (titres of 4–64) and CHO cells (titres of 4–32). Only nine strains produced a factor that was cytotoxic to HeLa cells with titres of 2–16. A summary of these assays and the virulence profiles of the strains examined are given in the table.

CT, NAG-ST and TDH are virulence factors first found to be associated with *V. cholerae* 01, *V. cholerae* non-O1, and *V. parahaemolyticus*, respectively. An enteric micro-organism is presumed to be an enteropathogen if it produces any of these toxin types. In this study, we identified strains of *V. mimicus* that produced several toxin types simultaneously, e.g. strain VM2 produced TDH-like haemolysin, a cytotoxic factor active against HeLa and Vero cells and DNA from the strain also hybridised with the NAG-ST DNA probe. This strain would probably represent a highly virulent isolate if toxin-producing ability was the measure of virulence. Interestingly, strain VM 2 was isolated as a co-culture along with a CT-producing strain of *V. cholerae* O1 (biotype Eltor serotype
Ogawa) from a patient suffering from clinical cholera. Thus, the role of *V. mimicus* is uncertain. Irrespective of the source, all 13 strains of *V. mimicus* examined in this study produced a cytotoxic factor and, more importantly, seven of them produced more than one type of toxin.

Although *V. mimicus* can produce multiple toxins this enteropathogen is rarely isolated from hospitalised patients with acute diarrhoea in the Infectious Diseases Hospital in Calcutta (< 0.05%) and elsewhere,

when specific efforts are made to find it. This indicates either that these organisms are not significant pathogens in these settings, or the lack of suitable media for their isolation and differentiation. The significance of *V. mimicus* isolates bearing multiple toxin types remains to be determined. Clearly, production of toxin is not in itself sufficient to cause disease and other factors are necessary to precipitate diarrhoea. This has been elegantly documented in a human volunteer study in which strains of *V. cholerae* non-O1 that produced NAG-ST but did not colonise, or that could colonise but did not produce NAG-ST, were not enteropathogenic; only strains that possessed both attributes could cause diarrhoea. A recent study has indicated that among the vibrios, *V. mimicus* is an important reservoir for the heat-stable enterotoxin. A significant percentage of *V. mimicus* isolates from the aquatic environs of Bangladesh, a region hyper-endemic for diarrhoeal diseases, expressed toxic activity. What then could be the role of multiple toxin types of *V. mimicus*? Rather than acting as significant pathogens in diarrhoeal illness, these organisms may constitute a genetic reservoir of virulence factors or be the recipients of gene transfers. Evidence supporting this hypothesis is emerging, as it has recently been reported that the *tdh* genes of *V. mimicus* are flanked by insertion sequence-like elements or related sequences, or both, which may play a role in the transfer of the *tdh* gene, perhaps as a transposon. Further molecular studies are required to determine whether *V. mimicus* act as a “courier” of virulence factors among vibrios.

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References


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