Characterization of *Vibrio cholerae* Isolated from Oysters

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Of 790 samples of oyster shellstock freshly harvested during a 12-month survey, 111 (most of which were harvested from June through August) contained *Vibrio cholerae* non-O1 (611 strains), and seven contained O1 Inaba (11 strains) organisms. None of the *V. cholerae* strains isolated were enterotoxigenic by immunological and biological tests.

A man from Abbeville, La., became ill on 10 August 1978, presenting symptoms of severe gastroenteritis, diarrhea, and dehydration. *Vibrio cholerae* O1, which causes epidemic cholera, was isolated from his stool (6). Subsequent investigation led to the detection of 10 other cases in which crabs were the vehicle incriminated epidemiologically and confirmed bacteriologically (4). The toxigenic strain common to all cases was identified as *V. cholerae* biotype El Tor serotype Inaba, which was essentially identical to the strain isolated from a single case of cholera reported from Port Lavaca, Tex., in 1975 (23). Before these outbreaks, the last documented case of domestically acquired cholera occurred in 1911 (20). Public health authorities were concerned that the two recent outbreaks might indicate the establishment of an endemic focus of classical *V. cholerae* O1 in the coastal regions of the United States. Long-term persistence of the pathogen could be accomplished by a life cycle mechanism involving sequential contaminations of humans, water, shellfish, and humans. Alternatively, toxigenic *V. cholerae* O1 might make intermittent appearances in the estuarine environment as a consequence of the serotypic or toxigenic transformation of nontoxic *V. cholerae* non-O1-agglutinable strains. The Food and Drug Administration conducted a surveillance of live crabs for interstate shipment taken from Louisiana waters during the 2 months immediately after the 1978 outbreak. They failed to detect *V. cholerae* O1 organisms in any of the 1,110 crabs (4).

Nevertheless, the serious implications for the public health and the shellfish industry of either of these mechanisms prompted the Food and Drug Administration to sample oyster shellstock at the wholesale level and to determine whether that product contained *V. cholerae*. Oysters were chosen because, unlike crabs, these shellfish are often consumed raw, thus magnifying the potential hazard. From 10 June 1979 through 25 May 1980, 15 to 30 samples of freshly harvested shellstock oysters were collected each week (for a total of 790) from oyster shellstock shippers and packers. The samples were collected through the combined efforts of nine Food and Drug Administration District laboratories. Each sample consisted of five subsamples of 16 oysters in the shell. Each subsample represented a different lot from a different growing area. Oyster samples were shipped to the laboratory for analysis in plastic bags under ice, taking care to avoid direct contact between the ice and the oysters. Upon arrival, shellstock was cleaned and shucked according to Recommended Procedures for the Examination of Sea Water and Shellfish (1).

Each sample was examined for the presence of *V. cholerae* according to Food and Drug Administration procedures (11). Portions (25 g each) from each subsample were blended in alkaline peptone water and in gelatin-phosphate-salt medium. After an 8-h incubation at 35 ± 2°C, 5-mm loopfuls of surface growth from each enrichment were streaked onto thiosulfate-citrate-bile salts-sucrose agar, gelatin agar, and gelatin-phosphate-salt agar plates. Three suspect colonies that exhibited appearances typical of *V. cholerae* were subcultured from each plate. Presumptive *V. cholerae* isolates, determined by an examination of appropriate biochemical characteristics, were screened for enterotoxin pro-
duction by a microslide gel diffusion procedure sensitive to 5 ng of cholera toxin per ml (11). Most of the isolates were reexamined for enterotoxigenicity by Y-1 mouse adrenal cell (11) and microtiter solid-phase radioimmunoassays (13) that were able, in our hands, to detect 0.1 ng of cholera toxin equivalent per ml. In the screening procedure, overnight broth culture supernatants were precipitated with zinc acetate or ammonium sulfate before gel diffusion. In the latter assays, culture supernatants were filtered through 0.45-μm membranes before testing.

Of the 790 samples of freshly harvested oyster shellstock, 111 were found to contain organisms identified biochemically and serologically as V. cholerae non-O1. Seven samples contained V. cholerae O1 Inaba; in five of these samples, both O1 and non-O1 organisms were found. The frequency distribution of positive samples by month of harvest is shown in Table 1. Of the 111 samples that were positive for non-O1 organisms, 82 were harvested in the months of June through August. In July alone, more than 38% of the samples were positive. Of the 611 non-O1 isolates from the 111 samples, nearly half were recovered from July samples. All seven of the oyster samples yielding the 11 V. cholerae O1 Inaba strains recovered in this study were taken in the warm months of May through August.

The percentages of isolates giving positive reactions in six different biochemical tests were tabulated (Table 2) for 486 V. cholerae non-O1 strains isolated from 76 of the 111 positive oyster shellstock samples randomly selected from Atlantic coast and Gulf coast harvesting areas. These tests were chosen either because they have been considered critical to diagnosis or because they have often given variable reactions (11). No consistent association between month or location of oyster sample harvest and biochemical trait or combination of traits could be made for the V. cholerae non-O1 isolates. Nevertheless, a difference in the percentage of isolates from the Atlantic and Gulf coasts with positive reactions in the indole and Voges-Proskauer tests were noted (significant at the α = 0.05 level).

None of the 611 V. cholerae non-O1 or the 11 V. cholerae O1 Inaba isolates were positive when screened for toxin production by the gel diffusion procedure. Furthermore, when the majority (486 non-O1 and 11 O1 strains) were tested by the Y-1 mouse adrenal cell and radioimmunoassays, none were toxigenic.

Twenty-eight V. cholerae non-O1 strains randomly selected from the 486 strains tested for enterotoxin were examined further for enteropathogenicity in the infant rabbit (8 to 12 days) model (8, 10). Each test isolate was washed and resuspended to a density of 10^6 organisms per ml in physiological saline. Approximately 0.1 ml of suspension was injected intrajejunally, and the rabbit was observed after 18 to 24 h for gross intestinal fluid accumulation, marked diarrhea, or death. Of the 28 cultures, 2 (one from the Atlantic and one from the Gulf coast) caused marked intestinal swelling and fluid accumulation when examined at autopsy. The 11 V. cholerae O1 Inaba strains were similarly tested and found nonpathogenic in the infant rabbit model.

From a potential 71,190 colonies that would have been recovered from the 790 oyster samples examined in this study, only 622 nontoxigenic V. cholerae were isolated. Of these, 11 were O1 Inaba isolates, and the 611 remaining were non-

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**Table 1. Frequency distribution of oyster samples positive for V. cholerae by month of harvest**

<table>
<thead>
<tr>
<th>Month of harvest</th>
<th>No. of samples</th>
<th>No. of V. cholerae&lt;sup&gt;Δ&lt;/sup&gt; isolates O1</th>
<th>Non-O1</th>
<th>No. of V. cholerae strains isolated O1</th>
<th>Non-O1</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>73</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>February</td>
<td>68</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (4.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>March</td>
<td>68</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>April</td>
<td>64</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (7.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>May</td>
<td>53</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (13.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>June</td>
<td>62</td>
<td>1 (1.9)</td>
<td>0 (0)</td>
<td>2 (3.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>July</td>
<td>104</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (3.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>August</td>
<td>28</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (12.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>September</td>
<td>93</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (11.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>October</td>
<td>53</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>November</td>
<td>40</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>December</td>
<td>27</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>Δ</sup> Percentages are given within parentheses.

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**Table 2. Positive biochemical reactions of 486 V. cholerae non-O1 isolates from 76 oyster shellstock samples randomly selected from Atlantic coast and Gulf coast harvesting areas**

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>% of isolates with positive reactions in the following month of harvest</th>
<th>Gulf (163)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Atlantic (163)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole production</td>
<td></td>
<td>98.7</td>
<td>87.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td></td>
<td>53.3</td>
<td>33.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td></td>
<td>80.8</td>
<td>84.7</td>
</tr>
<tr>
<td>Sucrose fermentation</td>
<td></td>
<td>100.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Mannitol fermentation</td>
<td></td>
<td>96.6</td>
<td>94.5</td>
</tr>
<tr>
<td>Arabinose fermentation</td>
<td></td>
<td>14.6</td>
<td>7.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of isolates is given within parentheses.
<sup>b</sup> Significant at the overall test level of α = 0.05.
O1 strains. These results demonstrate that oyster contamination by *V. cholerae* occurs at a relatively low level that fluctuates seasonally, apparently in response to changes in water temperature.

The *V. cholerae* O1 Inaba strains that we recovered from oysters could be the product of mutational transformation in serotype. In fact, antigenic shifts in serotype have been reported to occur in vivo (12, 21, 22) and in vitro (3, 17). However, the frequency of O1 isolation encountered in this study and the resulting proportional numerical relationship of O1 to non-O1 strains appear far higher than mutational frequencies might allow.

The *V. cholerae* O1 Inaba strains could have appeared as a result of some form of life cycle mechanism involving sequential contamination of water and shellfish by pathogenic *V. cholerae* O1.

Evidence exists for the relatively long-term survival of *V. cholerae* in seawater (19; B. K. Boutin, J. G. Bradshaw, and W. H. Stroup, personal communication). In addition, it has been suggested that shellfish that were incriminated in previous cholera outbreaks can concentrate these bacteria from seawater (2, 7, 14, 15). Nonetheless, the *V. cholerae* O1 Inaba strains isolated from this study were non-oxigenic, a finding that casts doubt on their hypothetical origin from human patients and carriers.

Despite the lack of toxigenic capability demonstrated for all of the *V. cholerae* isolated from oysters and our inability to resolve the very important epidemiological question of their origin, the public health hazard presented by this level of oyster shellstock contamination remains. On one hand, the possible transformation of non-oxigenic organisms to toxigenic is very relevant, since noncholera vibrios (and *V. cholerae* non-O1) have been shown to produce enterotoxin (18, 24) and cause acute gastroenteritis (5, 9, 16). On the other hand, the demonstration that 2 of 28 randomly selected *V. cholerae* non-O1 cultures were pathogenic in the infant rabbit model suggests that an as yet unknown virulence factor exists among the species.

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**LITERATURE CITED**


