Enterotoxicity of clinical and environmental isolates of Aeromonas spp.

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Summary. Of 147 isolates of three species of Aeromonas, 54 were from clinical and 93 from environmental sources. When tested for enterotoxin production, most of the isolates (56%) caused accumulation of fluid in rabbit ileal loops (RILs). Although large proportions of clinical and environmental isolates of A. caviae (55% and 65%, respectively) elicited such a response in RILs, isolates of A. hydrophila and A. sobria produced significantly more fluid (p < 0.05). Furthermore, the environmental strains of A. hydrophila and A. sobria produced more fluid than the clinical isolates (p < 0.05). The strains of Aeromonas spp. that caused little or no fluid accumulation in the initial experiments became enterotoxin producers after 1–3 passages through RILs, regardless of their source, and showed gradual enhancement of fluid outpouring after each passage. The present study suggests that all strains of these species of Aeromonas are potentially enterotoxigenic, whether from clinical or environmental sources.

Introduction

Production of a heat-labile enterotoxin by clinical and environmental isolates of Aeromonas was first demonstrated in an adult rabbit ileal loop (RIL) model.1–3 Subsequently enterotoxin production by strains of Aeromonas was demonstrated in various other animal and tissue-culture assays.4–11 However, it was reported that, whereas clinical isolates of A. hydrophila and A. sobria produced enterotoxin,12–14 few or none of the environmental isolates did so.15,16 Other studies suggested that a smaller proportion of environmental strains of A. hydrophila and A. sobria, compared with those of diarrhoeal origin, are enterotoxigenic.17,18 Furthermore, strains of A. caviae from either clinical or environmental sources were reported to be rarely enterotoxin producers.9,10,15–21 Because these observations caused confusion about the enterotoxigenicity of Aeromonas spp., the present study was undertaken, with a large number of diarrhoeal and environmental isolates, to examine the enterotoxic activity of different species, and to correlate this with the sources of the isolates.

Materials and methods

Strains of Aeromonas spp.

We examined 147 strains (table), 54 isolated from cases of acute diarrhoea and 93 from environmental sources (water and fish). By the criteria of Popoff,22 the strains were identified and classified into three species (A. hydrophila, A. sobria, A. caviae), according to their ability to hydrolyse aesculin, to ferment salicin, and to produce gas, acetoin and H₂S. The organisms were maintained in peptone agar stab cultures at room temperature and did not undergo more than three subcultures before the experiments for enterotoxin production.

Preparation of culture filtrates

Culture filtrates of the strains were prepared by the method of Annapurna and Sanyal.9 Briefly, 10 ml of Brain Heart Infusion Broth (BHIB; Difco) in a 50-ml conical flask was inoculated with 5–6 smooth separate colonies from overnight growth on nutrient agar (NA). The flasks were incubated at 37°C for 16–18 h in a shaking water bath with 80–120 oscillations/min. The cultures were centrifuged at 22 000 g for 20 min at 4°C, and the supernates were filtered through 0.22-μm membrane filters (Millipore) and stored at 4°C. Culture filtrate was prepared from a strain only when live cells caused fluid accumulation in RILs either before or after passage (see below).

Enterotoxicity test

Live bacteria and culture filtrates were tested for enterotoxicity in the adult RIL model by the method of De and Chatterje23 as adopted by Annapurna and Sanyal.9 Bacteria grown in BHIB for 3 h were diluted 10-fold in the same medium and inoculated into RILs in doses of 1 ml containing 10⁵–10⁶ cfu. A BHIB-culture of the toxigenic strain 569B of Vibrio cholerae...
was used as a positive control and unseeded BHIB as a negative control. Culture filtrates (1 ml) were tested in the same way. Each test was done in three rabbits.

**Passage in RILs**

Successive passages of strains that caused little or no accumulation of fluid in the initial test were made in RILs as described by Sanyal et al.\(^24,25\). Briefly, each strain was cultured aseptically from an RIL on NA and incubated overnight; five or six colonies were inoculated into BHIB and incubated for 3 h, and 1 ml of diluted culture was inoculated again into an RIL. The process continued until a good response was obtained.

**Results**

In the initial tests on the three species of *Aeromonas* in RILs, live cells of the majority (56%) of the clinical and environmental isolates caused accumulation of fluid (table). Culture filtrates of these strains caused similar secretory response. With organisms from both sources, there were strain variations in the volume of fluid accumulation, and also variations between individual loops inoculated with the same strain (table).

Accumulation of fluid was caused by the majority of the strains of *A. caviae*—55% of clinical and 65% of environmental isolates; live cells and culture filtrates caused similar fluid accumulation. However, live cells of *A. hydrophila* and *A. sobria* of environmental origin caused significantly more fluid accumulation (p < 0.05) than those of *A. caviae* from either source (fig. 1); similar results were obtained with culture filtrates.

Furthermore, live cells and culture filtrates of *A. hydrophila* and *A. sobria* from environmental sources caused significantly more fluid accumulation (p < 0.05) than the clinical isolates (fig. 1); but with *A. caviae* no significant difference (p > 0.05) was found between strains of clinical and environmental origin.

The non-toxigenic strains of *Aeromonas* (44% of the isolates) belonging to all three species became enterotoxin producers after 1–2 passages through RILs, regardless of their origin (table), though three environmental isolates (one of each species) required three passages before they caused marked fluid accumulation. The initially non-toxigenic strains showed gradual enhancement of fluid outpouring after each passage: the findings with six representative strains (two of each species) are shown in fig. 2.

**Table.** Accumulation of fluid in RILs inoculated with BHIB cultures of three species of *Aeromonas* from clinical or environmental sources

<table>
<thead>
<tr>
<th>Species and source</th>
<th>Number of strains tested</th>
<th>Strains tested before passage in RILs</th>
<th>Strains tested after passage in RILs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number (%) causing accumulation of fluid</td>
<td>Range of volumes of fluid (ml/cm of RIL)*</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td></td>
<td>3 (60)</td>
<td>0.5–0.7</td>
</tr>
<tr>
<td>Clinical</td>
<td>5</td>
<td>5 (56)</td>
<td>0.6–1.7</td>
</tr>
<tr>
<td>Environmental</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. sobria</em></td>
<td></td>
<td>15 (56)</td>
<td>0.4–1.0</td>
</tr>
<tr>
<td>Clinical</td>
<td>27</td>
<td>14 (42)</td>
<td>0.5–1.3</td>
</tr>
<tr>
<td>Environmental</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. caviae</em></td>
<td></td>
<td>12 (55)</td>
<td>0.4–0.9</td>
</tr>
<tr>
<td>Clinical</td>
<td>22</td>
<td>33 (65)</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td>Environmental</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control†</td>
<td></td>
<td>0.8–1.5</td>
<td></td>
</tr>
<tr>
<td>Negative control‡</td>
<td></td>
<td>0.0–0.0</td>
<td></td>
</tr>
</tbody>
</table>

*The stated range of volumes was observed in three RILs inoculated with each strain.
†BHIB-culture of strain 569B of *V. cholerae*.
‡BHIB.
The large number of clinical and environmental strains that caused little or no accumulation of fluid in the initial experiments showed enhancement of their enterotoxic activity after only 1–2 passages through the susceptible rabbit ileum. Similar observations on the effect of passage through the gut of a susceptible host were made earlier, and also with many other organisms such as *V. cholerae* non-O1, *Plesiomonas shigelloides*, *V. fluvialis*, *V. mimicus*, CT-gene-negative *V. cholerae* O1 and *Klebsiella pneumoniae*. Further, it is known that the so-called hypertoxigenic strain 569B of *V. cholerae* O1 also requires passage through rabbit gut for enhancement of toxin production; a mechanism of repression of toxin gene which becomes expressed on passage in a susceptible host may account for this phenomenon. There is every likelihood that toxicogenicity of our fresh isolates may have decreased during 1–3 subcultures *in vitro* before the loop tests. It appears that repression–derepression of a virulence factor in a bacterial strain is a means of its adaptation to a particular micro-environment.

Strains of the three species of *Aeromonas* produced quantitatively more toxin after each passage (fig. 2). This may suggest that *Aeromonas*, being ubiquitous in our aquatic environment, may have easy access to the human intestine in a community with suboptimal hygienic conditions, and therein develop increased enterotoxicity. The present study demonstrates that strains of all three species are potentially enterotoxigenic regardless of their source.

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### References


