**Vibrio furnissii isolated from humans in Peru: a possible human pathogen?**


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**SUMMARY**

During a cholera surveillance programme, *Vibrio furnissii* was isolated in late January and early February 1994 from stool samples collected from 14 persons of whom six had diarrhoea. The remaining eight persons were healthy family members or neighbours to cholera cases. No common source of infection was found. Strains isolated from stool samples each showed typical biochemical reactions of *V. furnissii* including gas production. Each isolate, except one, agglutinated O-antisera yielding a total of eight different serotypes. Most isolates were sensitive to 10 antibiotics tested, except to ampicillin and the vibriostatic agent O/129 (10 µg). Eight of 14 (57%) strains carried plasmids in the size range 2.6–88 kb, however, no correlation was found between antibiotic susceptibility patterns and plasmid content. Altogether, seven closely related *Hin*III ribotypes were observed among the 14 *V. furnissii* isolates studied. *V. furnissii* strains isolated from family members and other persons living close together often showed different ribotypes suggesting that the isolation was not associated with neighbourhood. Serotyping, plasmid profiling and ribotyping revealed a high strain diversity within *V. furnissii*, however, the importance of *V. furnissii* as an enteric pathogen remains to be elucidated.

**INTRODUCTION**

*Vibrio* spp. are natural inhabitants of aquatic environments, and most people acquire infections by exposure to such environments or to foods derived from or contaminated by them [3]. Of several established *Vibrio* spp., an increasing number are recognized to be pathogenic to human, with *Vibrio cholerae* being the most important [3].

The species name *Vibrio furnissii* was proposed in 1983 by Brenner and colleagues [1] for the biogroup 2 strains of *Vibrio fluvialis* that produced gas from the fermentation of carbohydrates [2]. The separation of *V. furnissii* from *V. fluvialis* was supported by studies of DNA relatedness [1].

*V. fluvialis* has been associated with sporadic cases [2, 4, 5], and it appears to have the potential to cause outbreaks of diarrhoea [3, 6]. Diarrhoea associated with *V. fluvialis* has often been connected with the consumption of seafood, especially raw shellfish [5]. *V. furnissii* has frequently been isolated from the estuarine environment but only from few human cases of diarrhoea, and the role of the organism as an enteric pathogen remains to be establish [1–3].

This paper presents the clinical status of six diarrhoea cases and eight healthy persons in Lima, Peru from whom *V. furnissii* was isolated from stool.
samples. Bacterial specimens were characterized phenotypically and by plasmid profiling and ribotyping to elucidate whether _V. furnissii_ was associated with diarrhoea and to determine any possible epidemiological relationship.

**METHODS**

**Bacterial specimens**

A cholera surveillance was carried out from February 1994 to July 1995 in Lima, Peru to evaluate the efficacy of a _V. cholerae_ vaccine [7]. Stool specimens were obtained from all diarrhoeal cases detected by an active (household visits) and/or passive (hospital referrals) surveillance programme. When a cholera case was detected, stool samples were collected from family members and neighbours. All stool specimens were screened for pathogenic _Vibrio_ spp. at the Microbiology Laboratory, US Naval Medical Research Institute Detachment (NAMRID), Lima, Peru using standard procedures including enrichment for 6 h in alkaline peptone water (pH 8.6) followed by plating onto thiosulfate citrate bile salt sucrose (TCBS) agar (Difco, Detroit, MI) [8].

During the surveillance period a number of non-O1 _V. cholerae_ were isolated from patients with diarrhoea. We have previously reported an outbreak of diarrhoea caused by _V. cholerae_ non-O1 serotypes [9]. _V. fluvialis_ was frequently isolated from cases of diarrhoea throughout the surveillance period (Fig. 1). However, from 28 January to 17 February 1994, stool samples obtained from 14 persons produced growth of yellow colonies on TCBS agar which resembled _V. fluvialis_ in their biochemical reactions except they were aero-
genic. The isolates were characterized and identified as _V. furnissii_ [3]. Haemolysis was determined on tryptic soy agar (Difco) supplemented with 5% calf blood. All media used to identify _V. furnissii_ contained 1% NaCl unless specified otherwise. In addition, the API 20E assay (bioMérieux, France) was used to identify isolates suspected to be _V. furnissii_ following the manufacturers recommendation for the identification of _Vibrio_ species. No other pathogenic _Vibrio_ spp. were recovered from stool samples of the 14 persons and none was treated with antibiotics.

_V. furnissii_ type strain ATCC 35016 [1] and _V. fluvialis_ type strain ATCC 33809 [2] previously isolated from human faeces and the marine environment, respectively, were included as reference strains in subsequent studies of _V. furnissii_ isolates.

**Serotyping**

Isolates were tested for agglutination of O serogroup antisera at the National Institute of Infectious Diseases, Tokyo, Japan, according to a combined scheme for serotyping of _V. fluvialis_ and _V. furnissii_ originally established by Shimada and Sakazaki in 1983 [4]. The scheme was extended by Shimada and colleagues [10] and presently covers 52 O-antigens, however, O36–O52 are unpublished (personal communication, Dr Shimada, National Institute of Infectious Diseases, Japan).

**Antibiotic susceptibility testing**

The 14 _V. furnissii_ isolates were tested for antibiotic susceptibilities to 10 antibacterial agents by the disk diffusion method on Mueller-Hinton II Agar (Difco) containing 1% NaCl with disks (BBL, Sensi-disc, Becton, Dickinson, MD) containing (µg/disk): nalidixic acid 30, ampicillin 10, carbenicillin 100, cephalothin 30, chloramphenicol 30, doxycycline 30, gentamicin 10, norfloxacin 10, tetracycline 30 and trimethoprim/sulphamethoxazole 1:25/23:75. In addition, strains were tested for susceptibility to the vibriostatic agent (O/129) with disks containing 10 and 150 µg. Antibiotic susceptibility testing was carried out by the Bauer–Kirby method [11] and strains were recorded as either sensitive or resistant.

**Isolation of plasmid DNA**

Plasmid preparation was carried out using the method of Kado and Liu [12], modified by incubating the cells...
at elevated pH (12.75) for 30 min at 56 °C during the lysis step [13]. Following electrophoresis, the plasmids were visualized essentially as described previously [14]. V. cholerae O1 strain V1075/25 containing an approximately 150 kb plasmid was used as control strain [15]. Plasmid sizes were estimated from the migration in the agarose gels relative to the migration of reference plasmids in E. coli strains V517 and 39R861 [16, 17] by the method of Rochelle and colleagues [18]. Repeated extraction of plasmid DNA was carried out for all isolates.

**Ribotyping**

Total bacterial DNA was extracted by the method of Murray and Thompson [19]. On the basis of previous studies [20, 21] and preliminary chromosomal digestion experiments using HindIII, EcoRI and BglII (Promega, Madison, WI), HindIII provided the best discrimination among V. furnissii isolates and was therefore used to digest chromosomal DNA from all strains. Ribotyping was performed by the procedure described by Dalsgaard and colleagues [13] with digoxigenin-labelled 16S and 23S rRNA probes. A 1-kb molecular weight standard (GIBCO BRL, Gaithersburg, MD) was used as a weight marker. Nylon membranes with immobilized DNA restriction fragments were hybridized, and fragments were detected colourimetrically as previously described [22].

**RESULTS**

The distribution of persons from whom V. furnissii and V. fluvialis were isolated from faecal specimens in Pampas de San Juan, Peru from January to June 1994 is shown in Figure 1. The clinical status of the 14 persons from whom V. furnissii was isolated is shown in Table 1. Five cases of diarrhoea occurred in adults and one child of either sex from at least two locations in Lima. One patient (patient no. 3) suffered profuse diarrhoea with traces of blood. However, eight persons did not show signs of diarrhoea. Unfortunately, stool samples were analysed for pathogenic Vibrio spp. and enterotoxigenic E. coli only, hence, we do not know whether a second pathogen could have been the primary cause of the diarrhoea.

All 14 V. furnissii strains produced yellow colonies on TCBS agar, were motile at 37 °C, and showed identical characteristics including the production of arginine dihydrolase, gas and indole. The strains showed growth in broths containing 3, 6 and 8% NaCl, but not in broths containing 0 and 10% NaCl. All strains were sensitive to O/129 (150 μg) but showed resistance to O/129 (10 μg). The reference strains of V. furnissii and V. fluvialis showed similar phenotypic characteristics compared with the clinical V. furnissii isolates, except that V. fluvialis showed a positive cellulose test and did not produce gas. All isolates produced opaque colonies on tryptic soy agar (Difco).

In the API 20E system three 7-digit profiles, 3246126, 3046126 and 3006126, were shown for the 14 V. furnissii strains isolated in Peru (Table 2). Variation was shown in the citrate test and the production of indole with three and nine strains showing positive reactions, respectively. All isolates showed a positive ONPG test, hydrolysed gelatin, produced acid from mannitol and arabinose but not from rhamnose. The reference strain of V. fluvialis ATCC 33809 showed profile 3004127. However, V. furnissii and V. fluvialis are not among the Vibrio species included in the matrix of the API 20E version 3.1 software recommended by the manufacturer for bacterial identification (bioMérieux), thus identification scores were not determined. In contrary to the API 20E assay where three strains showed a positive reaction in the citrate test, all strains showed a positive reaction when tested for citrate utilization by traditional biochemical testing. False negative results of the citrate test in the API 20E assay have been reported previously for Vibrio vulnificus [23]. In addition, gas production may not be detected in the API 20E assay. The results of our study suggest that the reactions in the API 20E assay are inadequate for the identification of V. furnissii.

The results of the serogrouping are shown in Table 2. Each of the V. furnissii isolates, except strain VIG 2171, agglutinated with antiserum demonstrating a total of eight different serotypes [10]. Serotype O11 was predominant and shown by four isolates.

Twelve (86%) of the clinical isolates showed resistance to ampicillin and three isolates exhibited resistance to cephalothin (Table 2). Strain VIG 1143 was the only strain showing resistance to trimethoprim/sulphamethoxazole whereas strain VIG 2171 was the only strain exhibiting resistance to nalidixic acid. No strains were multiply resistant (≥ 3 antibiotics). All isolates showed resistance to low dose vibriostaticum (10 μg) but all were sensitive to high dose vibriostaticum (150 μg).
Table 1. *Clinical status of persons from whom Vibrio furnissii was isolated from stool samples*

<table>
<thead>
<tr>
<th>Strain no./patient no.</th>
<th>Age/sex*</th>
<th>Date of isolation (1994)</th>
<th>Type of illness</th>
<th>Days of diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT 69/1</td>
<td>18/M</td>
<td>3 Feb</td>
<td>Diarrhoea</td>
<td>Unknown</td>
</tr>
<tr>
<td>CIT 143/2</td>
<td>19/M</td>
<td>3 Feb</td>
<td>Diarrhoea</td>
<td>Unknown</td>
</tr>
<tr>
<td>VIG 1052/3</td>
<td>20/M</td>
<td>28 Jan</td>
<td>Diarrhoea with blood</td>
<td>3</td>
</tr>
<tr>
<td>VIG 1143/4</td>
<td>36/F</td>
<td>31 Jan</td>
<td>Diarrhoea with dehydration</td>
<td>5</td>
</tr>
<tr>
<td>VIG 1174/5</td>
<td>34/F</td>
<td>31 Jan</td>
<td>Diarrhoea</td>
<td>1</td>
</tr>
<tr>
<td>VIG 1184/6</td>
<td>35/M</td>
<td>31 Jan</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 1301/7</td>
<td>2/F</td>
<td>2 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 1844/8</td>
<td>13/F</td>
<td>11 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 1869/9</td>
<td>4/M</td>
<td>11 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 2042/10</td>
<td>40/F</td>
<td>14 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 2048/11</td>
<td>35/M</td>
<td>14 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 2171/12</td>
<td>5/M</td>
<td>16 Feb</td>
<td>Diarrhoea</td>
<td>7</td>
</tr>
<tr>
<td>VIG 2318/13</td>
<td>34/M</td>
<td>17 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
<tr>
<td>VIG 2265/14</td>
<td>29/F</td>
<td>17 Feb</td>
<td>Well</td>
<td>0</td>
</tr>
</tbody>
</table>

* Age is indicated as number of years. Sex: M, male; F, female.

Table 2. *Phenotypic and genotypic characterization of 14 Vibrio furnissii strains isolated in Lima, Peru in 1994*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date of isolation (1994)</th>
<th>O serogroup*</th>
<th>API20E profile</th>
<th>Antibiogram†</th>
<th>Plasmid size (kb)</th>
<th>Ribotype‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT 69</td>
<td>3 Feb</td>
<td>O50</td>
<td>3246126</td>
<td>Am; Carb; Ceph; O/129</td>
<td>70</td>
<td>VFU1</td>
</tr>
<tr>
<td>CIT 143</td>
<td>3 Feb</td>
<td>O50</td>
<td>3246126</td>
<td>Am; Carb; Ceph; O/129</td>
<td>70</td>
<td>VFU1</td>
</tr>
<tr>
<td>VIG 1052</td>
<td>28 Jan</td>
<td>O11</td>
<td>3046126</td>
<td>O/129</td>
<td>—</td>
<td>VFU6</td>
</tr>
<tr>
<td>VIG 1143</td>
<td>31 Jan</td>
<td>O12</td>
<td>3006126</td>
<td>Am; TR; O/129</td>
<td>62</td>
<td>VFU2</td>
</tr>
<tr>
<td>VIG 1174</td>
<td>31 Jan</td>
<td>O21</td>
<td>3046126</td>
<td>Am; Ceph; O/129</td>
<td>—</td>
<td>VFU5</td>
</tr>
<tr>
<td>VIG 1184</td>
<td>31 Jan</td>
<td>O10</td>
<td>3006126</td>
<td>Am; O/129</td>
<td>—</td>
<td>VFU2</td>
</tr>
<tr>
<td>VIG 1301</td>
<td>2 Feb</td>
<td>O46</td>
<td>3246126</td>
<td>Am; O/129</td>
<td>88</td>
<td>VFU2</td>
</tr>
<tr>
<td>VIG 1844</td>
<td>11 Feb</td>
<td>O46</td>
<td>3046126</td>
<td>O/129</td>
<td>—</td>
<td>VFU4</td>
</tr>
<tr>
<td>VIG 1869</td>
<td>11 Feb</td>
<td>O21</td>
<td>3006126</td>
<td>Am; O/129</td>
<td>88; 70; 26; 46</td>
<td>VFU7</td>
</tr>
<tr>
<td>VIG 2042</td>
<td>14 Feb</td>
<td>O11</td>
<td>3006126</td>
<td>Am; O/129</td>
<td>26</td>
<td>VFU3</td>
</tr>
<tr>
<td>VIG 2048</td>
<td>14 Feb</td>
<td>O11</td>
<td>3006126</td>
<td>Am; O/129</td>
<td>26</td>
<td>VFU3</td>
</tr>
<tr>
<td>VIG 2171</td>
<td>16 Feb</td>
<td>Unknown</td>
<td>3046126</td>
<td>Am; O/129</td>
<td>—</td>
<td>VFU2</td>
</tr>
<tr>
<td>VIG 2318</td>
<td>17 Feb</td>
<td>O5</td>
<td>3046126</td>
<td>Am; O/129</td>
<td>—</td>
<td>VFU4</td>
</tr>
<tr>
<td>VIG 2265</td>
<td>17 Feb</td>
<td>O11</td>
<td>3046126</td>
<td>Am; O/129</td>
<td>34</td>
<td>VFU2</td>
</tr>
<tr>
<td>ATCC 35016</td>
<td></td>
<td>(V. furnissii)</td>
<td>O6</td>
<td>3006126</td>
<td>O/129</td>
<td>88</td>
</tr>
<tr>
<td>ATCC 33809**</td>
<td>(V. fluvialis)</td>
<td>O7</td>
<td>3004127</td>
<td>O/129</td>
<td>—</td>
<td>VFL1</td>
</tr>
</tbody>
</table>

* O serogroup designation according to the scheme original published by Shimada and Sakazaki in 1983 [4].
† Am, ampicillin; Carb, carbenicillin; Nal, nalidixic acid; Ceph, cephalothin; TR, trimethoprim/sulphamethoxazole; O/129, vibriostatic agent low (10 µg).
‡ Ribotypes were established using the enzyme HindIII. VFU: V. furnissii; VFL: V. fluvialis.
§ No plasmids were found.

ATCC, American Type Culture Collection [1]; ** NCIMB, National Culture of Industrial and Marine Bacteria, Aberdeen, Scotland.

Analysis of the plasmid content of the *V. furnissii* strains revealed that 8/14 (57%) strains carried plasmids including strain VIG 1869 which carried four plasmids (Fig. 2, Table 2). Strains CIT 69, CIT 143 and VIG 1869 carried an approximately 70 kb plasmid whereas strains VIG 1301, VIG 1869 and
Diarrhoea associated with *Vibrio furnissii*

Fig. 2. Plasmid profiles of *Vibrio furnissii*. Lanes: A, *Escherichia coli* 39R 861 (four plasmids ranging from 147 kb to 6·9 kb); B, *E. coli* V517 (eight plasmids ranging from 54 kb to 20 kb); C, *Vibrio cholerae* O1 strain V1075/25; D, VIG 1869; E, CIT 143; F, CIT 69; G, VIG 1301; H, ATCC 35016; I, VIG 1143; J, VIG 2042; K, VIG 2048; L, VIG 2265.

ATCC 35016 harboured a 88 kb size plasmid. The control strain V1075/25 contained an approximately 150 kb plasmid. There did not appear to exist any correlation between antibiotic susceptibility patterns and plasmid content.

Altogether, seven *HindIII* ribotypes were observed among the 14 *V. furnissii* clinical isolates studied (Fig. 3). Patterns were considered to be different when there was a difference of one band between isolates. The ribotypes appeared closely related as all isolates presented eight common fragments within the size ranges of 0·5 kb; 1·7–2·9 kb and 4 kb. Fragments within the size range 4·5–7·0 kb showed the highest degree of variability. Ribotypes were designated VFU (*V. furnissii*) followed by an arbitrary number (Table 2). Five isolates showed an identical ribotype VFU2 whereas each of the ribotypes VFU1 and VFU3 were demonstrated by two isolates. Isolates showing VFU2 agglutinated three different antisera (Table 2). Only limited correlation was demonstrated between O-serogroup designations and ribotypes.

The *V. furnissii* type strain ATCC 35016 showed an unique ribotype VFU8 closely related to the ribotypes demonstrated by the other *V. furnissii* isolates. A unique ribotype VFL1 shown by *V. fluvialis* type strain ATCC 33809 presented several identical

Fig. 3. *HindIII* ribotypes of *Vibrio furnissii* isolates recovered from 14 patients with diarrhoea in Lima, Peru. Explanation of lanes include strain designation and ribotype. Lanes: A, 1 kb molecular weight standard; B, CIT 69, type VFU1; C, CIT 143, type VFU1; D, VIG 1143, type VFU2; E, VIG 1184, type VFU2; F, VIG 1301, type VFU2; G, VIG 2171, type VFU2; H, VIG 2265, type VFU2; I, VIG 2042, type VFU3; J, VIG 2048, type VFU4; K, VIG 1844, type VFU4; L, VIG 2318, type VFU4; M, VIG 1174, type VFU5; N, VIG 1052, type VFU6; O, VIG 1869, type VFU7; P, ATCC 35016 (*V. furnissii*), type VFU8; Q, ATCC 33809 (*V. fluvialis*), type VFL1; R, 1 kb molecular weight standard.
DISCUSSION

In this study we present data on the isolation of *V. furnissii* from stool samples collected from 14 persons of whom only six had diarrhoea. The remaining eight persons were healthy family members or neighbours to cholera cases. The *V. furnissii* strains were isolated primarily in January and February 1994 with a few sporadic isolations during the rest of the year. January and February are the warm summer months in Peru and a time when cholera as well as other bacterial causes of diarrhoea are on the increase. The characterization of the *V. furnissii* strains showed a high degree of diversity and did not reveal any epidemiological relationship between the persons from whom *V. furnissii* was isolated. If *V. furnissii* was the cause of the diarrhoea the low case to infection ratio suggests a moderate virulence, however, the exact role of *V. furnissii* as an enteric pathogen remains to be elucidated. Since previous studies have shown production of enterotoxin or enterotoxin-like molecules by *V. fluvialis* similar studies could be performed with *V. furnissii* [24, 25].

From the patient data we were not able to determine the transmission of the *V. furnissii* and no evidence of a common source of infection was found. However, since *V. furnissii* is often found in water and seafood, such samples may have been vehicles of transmission [26].

All isolates were found susceptible to the majority of antibiotics tested. It was not surprising that most isolates showed resistance to ampicillin as the production of β-lactamases is common among *Vibrio* species [3]. In the present study, all isolates showed resistance to low dose vibriostaticum (10 µg) but all were sensitive to high dose vibriostaticum (150 µg). A high prevalence of resistance to the vibriostatic agent O/129 (150 µg) has been reported previously for *V. furnissii* [2, 3].

Although several isolates carried relatively large plasmids they did not seem to encode antibiotic resistance, neither did there appear to be any association between plasmid content and association with diarrhoea. Plasmid content in *V. furnissii* has not been reported previously and their importance remains to be determined [1].

There appeared to be only limited correlation between certain ribotypes and their association with diarrhoea as five strains showing ribotype VFU2 were recovered from patients with diarrhoea and healthy controls. *V. furnissii* strains isolated from family members and other persons living close together often showed different ribotypes suggesting that the isolation was not associated with neighbourhood. However, two *V. furnissii* strains isolated from patients 1 and 2 both with diarrhoea showed an identical ribotype VFU1. In addition, the two strains showed identical O serogroup, antibiogram and plasmid profile.

The association of *V. furnissii* with diarrhoea remains to be established, thus, we are continuing our studies to determine the role of *V. furnissii* as an enteric pathogen.

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