Vibrio fluvialis in Patients with Diarrhea, Kolkata, India


We identified 131 strains of Vibrio fluvialis among 400 nonagglutinating Vibrio spp. isolated from patients with diarrhea in Kolkata, India. For 43 patients, V. fluvialis was the sole pathogen identified. Most strains harbored genes encoding hemolysin and metalloprotease; this finding may contribute to understanding of the pathogenicity of V. fluvialis.

Many members of the family Vibrionaceae cause diarrheal disease; among these, Vibrio cholerae O1/O139 and V. parahaemolyticus are responsible for several epidemics and pandemics (1,2). In Indonesia, >20% of diarrheal infections are caused by pathogenic Vibrio spp. (3). Some of these Vibrio spp. can grow in thiosulfate–citrate–bile salts–sucrose agar as yellow colonies and do not agglutinate with V. cholerae O1 antisera. These species are broadly defined as nonagglutinating (NAG) vibrios.

The emerging etiologic agent V. fluvialis has caused sporadic cases and outbreaks of diarrhea in several countries (4–6). Species-specific minimal biochemical tests, e.g., lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, and L-arabinose, are used to identify V. fluvialis; without these tests, it may be confused with NAG vibrios, V. cholerae, and even Aeromonas spp. In most resource-poor countries, these tests are not performed, which may lead to labeling of V. fluvialis as a NAG vibrio.

Although V. fluvialis is known to cause diarrhea, the mechanisms involved in its pathogenicity are not well established. To evaluate the prevalence of V. fluvialis in India and possible mischaracterization as an NAG vibrio, we examined cases in which isolates from hospitalized patients with diarrhea were identified as NAG vibrios and characterized the strains using phenotypic and genetic methods.

The Study

We examined 400 isolates identified as NAG vibrios that were collected during 2002–2009 from 11,904 stool specimens from patients with diarrhea admitted to the Infectious Diseases and Beliaghata General Hospital, Kolkata, India. Specimens were screened for common enteric pathogens, according to standard protocols (7). Oxidase, string test, and arginine dihydrolase–positive strains that did not agglutinate with V. cholerae O1 polyvalent or O139 monovalent antiserum were further confirmed as V. fluvialis by using a multiplex PCR targeting the toxR gene of V. fluvialis and the ompW gene of V. cholerae (8,9). Isolates were also subjected to PCRs targeting different virulence-associated genes encoding the repeat in toxin (rtxA, rtxC), heat-stable enterotoxin (stn), type 3 secretion system (vcsC2, vcsV2, vcsN2 and vspD), cholera toxin (ctxA), toxin co-regulated pilus (tcpA), thermostable direct-hemolysin (tdh), TDH-related hemolysin (trh), V. fluvialis hemolysin (VHF), and metalloproteases, according to published methods (10–12).

Expression of VFH was determined in vitro by using erythrocytes from rabbit and sheep. Cytotoxin assay was performed with HeLa and Chinese hamster ovary cell lines by using sterile culture filters of the V. fluvialis strains that were isolated as a sole pathogen. Antimicrobial drug susceptibility testing was performed by using the disk diffusion method with commercially available disks (Becton Dickinson, Sparks Glencoe, MD, USA), according to Clinical and Laboratory Standards Institute criteria (13). Because these guidelines do not include interpretive criteria for V. fluvialis, breakpoints for Enterobacteriaceae were adopted. Escherichia coli ATCC 25922 was used as a quality control strain.

Pulsed-field gel electrophoresis was performed according to the PulseNet standardized protocol for V. cholerae (14). Gel Compare II software (Applied Maths NV, Sint-Martens-Latem, Belgium) was used for electrophoresis pattern comparison that runs on Dice similarity index and unweighted pairgroup with arithmetic mean method.
Among the 400 isolates presumptively identified NAG vibrios, multiplex PCR confirmed 131 and 269 strains (each strain representing a case) as *V. fluvialis* and *V. cholerae*, respectively. The overall prevalence rate of *V. fluvialis* among 11,904 hospitalized patients with diarrhea was 1.1%. Abrupt appearance of *V. fluvialis* was identified in 2002, although the surveillance of diarrheal infection was initiated at the Infectious Diseases and Beliaghata General Hospital in 1996 (www.niced.org.in/annual_reports.htm). The isolation rate of *V. fluvialis* gradually increased from 0.7% in 2002 to 2.2% in 2009 (Table 1). Of the 131 strains of *V. fluvialis*, 43 (33%) were identified as the sole pathogen; the remaining 88 (67%) were isolated as a copathogen with either *V. cholerae*, *V. parahaemolyticus*, *E. coli*, *Shigella* spp., parasites, or enteric viruses (data not shown). Among the mixed infections, *V. fluvialis* with *V. cholerae* was isolated most often (17%), followed by *V. fluvialis* and *V. parahaemolyticus* (8%). The presence of *Vibrio* spp. as mixed pathogens indicates that these patients likely acquired the infection from contaminated water or food. We analyzed the date of admission and place from where the patients resided and found no evidence for clusters of infection or small outbreaks caused by *V. fluvialis*.

*V. fluvialis* infection was much more often detected in adults (73%) than in children <5 years of age (27%). Clinical symptoms of sole infection caused by *V. fluvialis* were similar to that of cholera: watery diarrhea (86%), severe dehydration status (28%), and abdominal pain (12%) (Table 2). Several previous investigations have identified cholera-like diarrheal outbreaks caused by *V. fluvialis* (4,5).

All the *V. fluvialis* strains were negative for the virulence genes commonly reported in *V. cholerae* and *V. parahaemolyticus*, but >90% were positive for genes encoding VFH and metalloproteases. More than 80% of the strains expressed hemolysin against rabbit and sheep red blood cells. Hemolysin is a widely distributed virulence factor in most pathogenic *Vibrio* spp. Metalloprotease produced by *V. fluvialis* is related to hemagglutination proteases of *V. vulnificus*, which enhances permeability and hemorrhagic activities (12). These factors may increase the virulence of *V. fluvialis* and contribute to diarrhea.

*Vibrio fluvialis* in Patients with Diarrhea

Table 1. Prevalence of *Vibrio fluvialis* among patients with diarrhea, Kolkata, India, 2002–2009

<table>
<thead>
<tr>
<th>Year</th>
<th>No. samples</th>
<th>No. (%) <em>V. fluvialis</em> isolates</th>
<th>No. (%) patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sole infection</td>
<td>Mixed infection</td>
</tr>
<tr>
<td>2002</td>
<td>2,285</td>
<td>16 (0.7)</td>
<td>5 (0.2)</td>
</tr>
<tr>
<td>2003</td>
<td>1,673</td>
<td>8 (0.5)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>2004</td>
<td>2,430</td>
<td>19 (0.8)</td>
<td>6 (0.2)</td>
</tr>
<tr>
<td>2005</td>
<td>1,472</td>
<td>17 (1.1)</td>
<td>7 (0.5)</td>
</tr>
<tr>
<td>2006</td>
<td>930</td>
<td>12 (1.3)</td>
<td>4 (0.4)</td>
</tr>
<tr>
<td>2007</td>
<td>842</td>
<td>9 (1.1)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>2008</td>
<td>1,124</td>
<td>24 (2.1)</td>
<td>8 (0.7)</td>
</tr>
<tr>
<td>2009</td>
<td>1,153</td>
<td>26 (2.2)</td>
<td>10 (0.9)</td>
</tr>
<tr>
<td>Total</td>
<td>11,909</td>
<td>131 (1.1)</td>
<td>43 (0.4)</td>
</tr>
</tbody>
</table>

When the culture filtrates were tested, cytotoxic effect was readily noticed in the Chinese hamster ovary and HeLa cell lines, i.e., cytoplasmic vacuolation, cell rounding, and destruction of the monolayer. In most strains isolated as a sole pathogen, the cytotoxic endpoint titer was 2–256 (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/12-0520-Techapp.pdf). The cell vacuolation phenomenon has been reported as a virulence factor in several enteric pathogens (online Technical Appendix References).

In this study, *V. fluvialis* strains were highly resistant to ampicillin (92%), streptomycin (85%), furazolidone (85%), and sulfamethoxazole/trimethoprim (70%) (online Technical Appendix Table 2). About half the number of strains were resistant to ciprofloxacin and 45% to nalidixic acid; the lower resistance rate for nalidixic acid compared with fluoroquinolones is unexpected and warrants further investigation to confirm the additional mechanisms. In a previous study, we found that some *V. fluvialis* strains carried the plasmid-mediated quinolone resistance gene allele qnrA1 and a gene encoding the aminoglycoside acetyltransferase (aac(6′)-Ib-cr), which reduces ciprofloxacin activity (15). Fluoroquinolone resistance and intermediate susceptibility to erythromycin (92%) are the unique features of the *V. fluvialis* isolated in this study;
least 4 major clades were identified. Although the 
V. cholerae
strains exhibited distinct

this trend was not recorded in other 

Vibrio spp., e.g., 

V. cholerae
and 

V. parahaemolyticus.

Although the 
V. fluvialis
strains exhibited distinct

Figure. Dendrogram of 

NotI-digested pulsed-field gel electrophoresis (PFGE) profiles with representative 

Vibrio fluvialis
isolates. Clustering identified 4 clades (A–D). AM, ampicillin; S, amoxicillin; NA, nalidixic acid; CIP, ciprofloxacin; C, chloramphenicol; E, erythromycin; SXT, sulfamethoxazole-trimethoprim; FZ, furazolidone; NOR, norfloxacin. Scale bar indicates degree of similarity.

Conclusions

Our results demonstrate an emerging trend of prevalence of 

V. fluvialis
among patients with acute diarrhea patients in Kolkata. The expression of cytotoxic activity and hemolysin may contribute to understanding the pathogenicity of 

V. fluvialis.

Further epidemiologic studies are necessary to elucidate the public health importance of 

V. fluvialis–mediated diarrhea.

This work was supported in part by the Ministry of Health, Labor and Family Welfare of Japan (Project H17-Shinkou-3); Initiative for Global Research Network on Infectious Diseases, Ministry of Education, Culture, Sports, Science and Technology, Japan; and intramural grants of the Indian Council of Medical Research, New Delhi, India.

Mr Chowdhury is a doctoral candidate at the National Institute of Cholera and Enteric Diseases, Kolkata, India. His main research interest is the pathogenesis and molecular biology of enteric 

Vibrio spp.

References

2. Nair GB, Ramamurthy T, Bhattacharya SK, Dutta B, Takeda Y, Sack DA. Global dissemination of 

Vibrio parahaemolyticus
3. Lesmana M, Subekti DS, Tjaniadi P, Simanjuntak CH, Punjabi NH, Campbell JR, et al. Spectrum of 

Vibrio species
5. Thekdi R, Lakhani AG, Vachha SM, Chandrakapure MR. 

Vibrio fluvialis
(group F 

Vibrio)

Vibrio cholerae

Vibrio cholerae
11. Han JH, Lee JH, Choi YH, Park JH, Choi TJ, Kong IS. Purification, characterization and molecular cloning of 

Vibrio fluvialis

Vibrio fluvialis
Vibrio fluvialis in Patients with Diarrhea


Address for correspondence: Thandavarayan Ramamurthy, National Institute of Cholera and Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Kolkata 700010, India; email: tramu@vsnl.net

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

etymologia

Coxsackievirus [kok-sak’e-ve’rəs]

Named for Coxsackie, the small town on the Hudson River where they were first isolated, human coxsackieviruses are nonenveloped, positive-sense, single-stranded RNA viruses in the family Picornaviridae, genus Enterovirus. They were first described by Gilbert Dalldorf, who was investigating suspected poliomyelitis outbreaks in upstate New York in the summer of 1947. Coxsackieviruses are divided into 2 groups, A and B. In suckling mice, group A viruses cause generalized myositis and flaccid paralysis, and group B viruses cause focal myositis and spastic paralysis. With the discovery of coxsackieviruses, Dalldorf also helped popularize the suckling mouse as an inexpensive laboratory animal model.

Sources


Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30333, USA; email: boq3@cdc.gov

DOI: http://dx.doi.org/10.3201/eid1811.ET1811

Medscape CME Sign up to receive email announcements when a new article is available.

Get an online subscription at wwwnc.cdc.gov/eid/subscribe.htm

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 18, No. 11, November 2012 1871
**Vibrio fluvialis** in Patients with Diarrhea, Kolkata, India

**Technical Appendix**

Technical Appendix Table 1. Results of assays of clinical *Vibrio fluvialis* strains to determine ability to lyse rabbit erythrocytes and cytotoxic effect on CHO and HeLa cells.

<table>
<thead>
<tr>
<th>Strain identification</th>
<th><em>V. fluvialis</em> hemolysin</th>
<th><em>V. fluvialis</em> metalloprotease</th>
<th>Hemolytic titer</th>
<th>Cytotoxic titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chinese hamster</td>
</tr>
<tr>
<td>IDH00612</td>
<td>+</td>
<td>+</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>IDH00629</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>IDH00653</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>IDH01036</td>
<td>+</td>
<td>+</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>IDH01577</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>H8942</td>
<td>+</td>
<td>+</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>H17768</td>
<td>+</td>
<td>+</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>L15318</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>K24681</td>
<td>+</td>
<td>+</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>J11969</td>
<td>+</td>
<td>+</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

Technical Appendix Table 2. Antimicrobial drug resistance of *Vibrio fluvialis*.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistant, %</th>
<th>Intermediate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>70</td>
<td>14</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>Neomycin</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

**References**

