Concomitant Infection of Enterotoxigenic *Escherichia coli* in an Outbreak of Cholera Caused by *Vibrio cholerae* O1 and O139 in Ahmedabad, India

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In Ahmedabad, a major city in the state of Gujarat, an outbreak of acute secretory diarrhea caused by *Vibrio cholerae* O1 Ogawa El Tor, *V. cholerae* O139, and multiple serotypes of enterotoxigenic *Escherichia coli* (ETEC) occurred in January 2000. All of the representative *V. cholerae* O1 and O139 isolates examined harbored the *ctxA* gene (encoding the A subunit of cholera toxin) and the El Tor variant of the tcpA gene (encoding toxin-coregulated pilus). ETEC isolates of different serotypes were positive for the *elt* gene, encoding heat-labile enterotoxin. To further understand the molecular characteristics of the pathogens, representative isolates were examined by ribotyping and pulsed-field gel electrophoresis (PFGE). Ribotyping showed that the isolates of *V. cholerae* O1 Ogawa exhibited a pattern identical to that of the prevailing clone of O1 in areas where cholera is endemic in India, and all of the O139 isolates were identical to the BII clone of *V. cholerae* O139. PFGE of the representative O1 Ogawa isolates exhibited an identical pattern, comparable to the H pattern of the new clone of O1 reported in Calcutta, India. PFGE analysis of the *V. cholerae* O139 isolates showed identical patterns, but these differed from the PFGE patterns of O139 isolates reported during 1992 to 1997 in Calcutta. ETEC isolates showed genetic heterogeneity among isolates belonging to the same serotype, although the identical PFGE pattern was also observed among ETEC isolates of different serotypes. Antibiotics of the isolates were unused, because all of the O139 isolates were resistant to nalidixic acid. Likewise, all of the *E. coli* isolates showed resistance to ciprofloxacin, norfloxacin, and nalidixic acid. This is a unique outbreak, and we believe that it is the first in which *V. cholerae* and ETEC were concomitantly involved.

Acute diarrheal diseases have been recognized as one of the major causes of morbidity and mortality in developing and underdeveloped countries. The common pathogens associated with diarrhea in developing countries are diarrheagenic *Escherichia coli*, *Vibrio cholerae*, *Salmonella* spp., and *Shigella* spp., etc. Cholera is caused by toxigenic strains of *V. cholerae* belonging to the O1 or O139 serogroup, which have the potential to cause epidemics (4, 25). It is estimated that tens of thousands of people in the world are affected every year due to cholera outbreaks and epidemics. Outbreaks of cholera are generally due to lack of sanitation or contamination of drinking water (28, 30). The etiologic agent, enterotoxigenic *E. coli* (ETEC), causes nearly 400 million diarrheal episodes and 700,000 deaths annually among children less than 5 years old (15). The present investigation highlights an association of three pathogens associated with a large outbreak of diarrhea in a metropolitan city of Gujarat state, India.

**MATERIALS AND METHODS**

Description of the outbreak. From 1 to 17 January 2000, a total of 809 patients reported to three different hospitals, namely, I. D. Hospital, V. S. General Hospital, and L. G. Hospital, in Ahmedabad, India, with acute watery diarrhea.

Cases of diarrhea were reported from at least 40 wards. The total population served by these three hospitals is in the range of 800,000 to 900,000. Bacteriological culture was performed on 734 of the 809 hospitalized patients. Pathogens were not isolated from all patients. Only the patients with acute illness accompanied by severe dehydration were hospitalized. Of the 734 stool specimens tested, 72 were positive for *V. cholerae* O1, 31 were positive for *V. cholerae* O139, and 24 were positive for *E. coli*. The attack rate of this outbreak was about 0.2%. Data on the background prevalence of these pathogens during this outbreak are not available, since routine surveillance for diarrheal etiologies is not maintained. However, in Ahmedabad, the seasonal peak of cholera is generally recorded between summer and early monsoon season, i.e., from April to August (J. S. Deokule, unpublished observation).

Identification of bacterial isolates. One hundred three isolates of *V. cholerae* and 24 isolates of *E. coli* from this outbreak were received at the National Institute of Cholera and Enteric Diseases (NICED). For confirmation of identity, the *V. cholerae* isolates were plated on thiosulfate-citrate-bile salts-sucrose agar (Eiken, Tokyo, Japan) and the *E. coli* isolates were plated on MacConkey agar (Difco, Detroit, Mich.). The identities of these isolates were confirmed by different biochemical, physiological, and serological tests according to standard methods (33). The serotyping of *E. coli* was done using a commercially available kit (Denka Seiken Co., Ltd., Tokyo, Japan). Monoclonal antibodies against *V. cholerae* O1 and O139 serogroups generated at NICED were used for serotyping the *V. cholerae* isolates. Representative isolates were selected at random to exclude any bias for the detection of different virulence genes by PCR, molecular typing, and antibiotic susceptibility testing.

PCR assay for virulence genes. A multiplex PCR-based assay was used to determine the presence of the A-subunit cholera toxin gene (*ctxA*) and to biotype the *V. cholerae* isolates by targeting tcpA (encoding the major structural subunit of the toxin-coregulated pilus), which is specific for El Tor and classical isolates (19), by a method described earlier (16). All of the *E. coli* isolates were screened for the presence of a variety of virulence factors using a PCR assay; these factors included *elt* (gene encoding heat-labile toxin) and *est* (gene encoding heat-stable
cation buffer (500 mM KCl, 100 mM Tris HCl, 15 mM MgCl₂ [pH 8.3]),

amplification was done with appropriate volumes of 10×

prepared from the whole organism by boiling in a water bath for 10 min and instantly 
stx (gene encoding Shiga toxin 2) (22) for enterohemorrhagic

E. coli (EPEC); 

eaadherence factor (12) for EPEC; 

eae toxin) (29) for ETEC; 

V. cholerae 25923 were used as quality control strains. Representative isolates of 

of ETEC isolates were inoculated on CFA agar plates with and without bile salts 

(isolates as susceptible, intermediately resistant, or resistant was based on the size 

for ETEC isolates was performed by preparing agarose plugs as described previously (17, 26) and EAST1 (gene for enteroaggregative stable toxin) (34) for enteroaggregative E. coli. 

nucleic acid polymerase (Takara Shuzo, Otsu, Japan), and 5 μl of template. 

reaction volume was adjusted to 25 μl using sterile triple-distilled water. Uniplex 

30 cycles using the conditions described in Table 1. 

isolate included in this study were examined for susceptibility to 

amikacin (30 μg), gentamicin (30 μg), norfloxacin (10 μg) 

Staphylococcus aureus ATCC 25923 were used as quality control strains. Representative isolates of V. cholerae 

ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (25 μg), 

ciprofloxacin (5 μg), furazolidone (5 μg), norfloxacin (10 μg) 

antimicrobial susceptibility. 

inoculated on CFA agar plates with and without bile salts and incubated at 37°C overnight. The colonies from CFA agar plates were tested for the expression of CFA/I, CS1 to CS7, CS12 (PCF0159), CS14 (PCF0166), 

hybridization with rRNA probes were performed as described previously (1), 

PFGE. Pulsed-field gel electrophoresis (PFGE) of V. cholerae and E. coli 

isolates was performed by preparing agarose plugs as described previously (17, 35). NotI (Takara)-digested inserts of V. cholerae and XbaI (Takara)-digested 

and incubated at 37°C 1.5 min; 

E. coliencoding region Primer sequences (5’-3’) Amplicon size (bp) PCR conditions Reference 

29

E. coli group Target gene or 

Simplex ETEC etl GGGCAGACATTATACCGTGCCGTCGCCTATATGCCGGTGT 450 94°C, 1.0 min; 55°C, 1.5 min; 72°C, 1.5 min 29

est ATTTTATTTCTGTATAGTCTTTCAACCGGTACAA/GGCAGGATT 190 94°C, 1.0 min; 55°C, 1.5 min; 72°C, 1.5 min 29

EPEC eae AACAGGTTAAACTGTGCCCCTCAGGAGATATACCCCTCTGGC 454 94°C, 1.0 min; 55°C, 1.5 min; 72°C, 1.5 min 36

bfpA AATGGTGTGGCGTTGTGCGGCGTTTTATCAACCGTGGTA 324 94°C, 1.0 min; 56°C, 1.5 min; 72°C, 1.5 min 14

EAF CAGGGTAAAGAGGATGAATGATGATCGGAGCATGATGATCCTGAC 397 94°C, 1.0 min; 60°C, 1.5 min; 72°C, 1.5 min 12

Multiplex EHEC stx1 CAACACTGGATGATCTCAGCCCCCTCAACTGCTAATA 350 94°C, 1.0 min; 55°C, 1.0 min; 72°C, 1.0 min 22

stx2 ATCAGTCGTCATCTCAGTCTGCTGCAGATGCACAAA 110

EAgg CTGCGAAAGACGTATCATCAATGTTATAAATCCGCTGTT 630 94°C, 1.0 min; 53°C, 1.0 min; 72°C, 1.0 min 26

EAggEC EAST1 CACAGTATATCCGAAGGC CGAAGTGCAGGCTTGTAG 94 This study

V. cholerae tcpA (classical) CAGGATAAGAAGACCGTCAGAG ACCAAATGCAACGCCGAATGGAG 617 94°C, 1.0 min; 60°C, 1.5 min; 72°C, 1.5 min 16

tcpA (El Tor) GAAGAGGGTTGAAAGAAGAACAC CAATGTATAGAAATCCGCTGTT 471

* NOTE: E. coli, enterobacterial E. coli; EHEC, enterohemorrhagic E. coli; EAggEC, enteroaggregative E. coli.

** Thirty cycles consisting of denaturation, annealing, and extension.

** Table 1. PCR primer sequences and conditions used for the detection of genes specific for diarrheagenic E. coli and V. cholerae isolates

** This study

** Antimicrobial susceptibility

** Antimicrobial susceptibility testing was done using standard methods (3). E. coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used as quality control strains. Representative isolates of V. cholerae were tested for susceptibility using commercially available discs (HiMedia, Mumbai, India) of ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), furazolidone (5 μg), norfloxacin (10 μg) gentamicin (10 μg), neomycin (30 μg), streptomycin (10 μg), and tetracycline (30 μg). All of the E. coli isolates included in this study were examined for susceptibility to cephalothin (30 μg), amikacin (30 μg), ceftriaxone (30 μg), and nalidixic acid (30 μg) in addition to the antibiotics used for V. cholerae isolates, with the exception of furazolidone. Characterization of isolates as susceptible, intermediately resistant, or resistant was based on the size of the inhibition zones according to the manufacturer’s instructions, which matched the interpretative criteria recommended by the NCCLS (21). In addition, we analyzed three representative quinolone-resistant ETEC isolates for norfloxacin and ciprofloxacin MICs using the E-test method (AB Biodisk, Solna, Sweden).

** Ribotyping of V. cholerae. The 7.5-kb BamHI fragment of plasmid pKK3535 containing 16S and 23S rRNA genes of E. coli was used as the rRNA probe (6). The modified method of Murray and Thompson (20) was used for genomic DNA extraction. For ribotyping, the transfer of digested DNA from gels to Hybond N+ membranes (Amersham International PLC, Buckinghamshire, England) and hybridization with rRNA probes were performed as described previously (1), using the ECL nucleic acid detection system (Amersham). The membranes were washed, exposed to Biomax film (Eastman Kodak Co., Rochester, N.Y.), and developed according to the manufacturer’s instructions.

** PFGE. Pulsed-field gel electrophoresis (PFGE) of V. cholerae and E. coli isolates was performed by preparing agarose plugs as described previously (17, 35). NotI (Takara)-digested inserts of V. cholerae and XbaI (Takara)-digested
RESULTS

Examination of the 103 isolates of *V. cholerae* revealed that 72 (70%) were *V. cholerae* O1 Ogawa serotype, El Tor biotype, while 31 (30%) were identified as belonging to the *V. cholerae* O139 serogroup. All of the 25 representative *V. cholerae* isolates, including 17 serogroup O1 and 8 serogroup O139 isolates, were positive in multiplex PCR for *ctxA* and *tcpA* of the El Tor variant. Six different serotypes of *E. coli* were seen (Table 2), with the O1 serotype being dominant (41.6%), followed by O146 (16.6%); 16.6% of the isolates were untypeable. In the PCR assay, 18 (75%) of the *E. coli* isolates harbored the *elt* gene, of which 9 (50%) belonged to serotype O1 (Table 2). None of the *E. coli* isolates tested harbored the *est*, *stx1*, or *stx2* gene, and all were negative in the EAgg PCR assay. None of the ETEC isolates possessed any of the 12 commonly prevalent CFAs that were examined in this study.

Antibiotic susceptibility results for 23 O1 isolates and 9 O139 isolates revealed that all of these isolates were resistant to ampicillin, furazolidone, and nalidixic acid (Table 3). In addition, *V. cholerae* O1 isolates were resistant to co-trimoxazole and streptomycin, and 21.7% of them were resistant to chloramphenicol, whereas *V. cholerae* O139 isolates were susceptible to these antibiotics. The majority of the *E. coli* isolates showed high resistance to several antibiotics, including members of the quinolone group of antimicrobial drugs (Table 3). MICS for three ETEC isolates (E2, E14, and E15) were found to be ≥32 μg/ml for ciprofloxacin and ≥256 μg/ml for norfloxacin.

Recent findings have shown that species other than *V. cholerae* might act as an extraordinary reservoir for both CTXφ and VPIφ and might play an important role in the emergence of new toxigeneic strains (5, 10). In view of this, we tested the *E. coli* isolates for the *ctxA* and *tcpA* genes, which are specifically found in *V. cholerae*, to detect any lateral gene transfer event. The multiplex PCR showed that none of the *E. coli* isolates harbored *ctxA* or *tcpA*. Ribotyping of eight representative isolates of O1 Ogawa revealed that seven of them (Fig. 1, lanes 2, 5, 7, and 8 [only representative isolates are shown]) showed the previously documented RIII type (27), while one isolate AH094 (Fig. 1, lane 4) showed a pattern slightly different from the RIII type, which is the currently prevailing type (27), by the presence of an additional band at approximately 5.6 kb. The ribotype patterns of five representative *V. cholerae* O139 isolates (Fig. 1, lanes 1, 3, and 6 [only three isolates are shown]) were identical to the most commonly found BII ribotype pattern, which is the ribotype currently prevailing among *V. cholerae* O139 isolates in Calcutta and Bangladesh (11).

PFGE of all six representative O1 Ogawa isolates (Fig. 2A, lanes 1 to 6) exhibited identical patterns which were comparable to the H pattern of the new clone of O1 reported in Calcutta (35) (Fig. 2B, lane 1). PFGE analysis of the three *V. cholerae* O139 isolates (Fig. 2A, lanes 7 to 9) showed identical patterns which differed from the PFGE patterns of O139 isolates reported during 1992 to 1997 in Calcutta (2).

PFGE analysis was done on eight representative *E. coli* isolates. Of five of these isolates belonging to *E. coli* serotype

![Table 2](https://example.com/table2.png)

**Table 2. Serotyping, PCR, and CFA results for the *E. coli* isolates from the Ahmedabad outbreak**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>PCR results</th>
<th>CFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>elt</em></td>
<td><em>eae</em></td>
</tr>
<tr>
<td>E-2</td>
<td>O146</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-3</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-4</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-5</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-6</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-7</td>
<td>O146</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-8</td>
<td>O146</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-9</td>
<td>O146</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-10</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-11</td>
<td>O158</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>E-12</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-13</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-14</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-15</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-16</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E-17</td>
<td>O146</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AV-155</td>
<td>O114</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>AV-159</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AV-170</td>
<td>O8</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AV-185</td>
<td>O1</td>
<td>+</td>
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<tr>
<td>AV-188</td>
<td>O114</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>AV-189</td>
<td>O25</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AV-193</td>
<td>ONT</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AV-195</td>
<td>O1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>A-89</td>
<td>ONT</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>
|<sup>a</sup> None of the *E. coli* isolates yielded positive results with *est*, *stx1*, and *stx2*.
|<sup>b</sup> EAP, EPEC adherence factor.
|<sup>c</sup> ONT, not typeable.
|<sup>d</sup> ND, not done.

![Table 3](https://example.com/table3.png)

**Table 3. Antimicrobial resistance of the Ahmedabad outbreak isolates of *V. cholerae* and *E. coli***

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th><em>V. cholerae</em> O1 (23)</th>
<th><em>V. cholerae</em> O139 (9)</th>
<th><em>E. coli</em> (24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>4.16</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>100</td>
<td>95.8</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>ND</td>
<td>ND</td>
<td>54.2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>ND</td>
<td>ND</td>
<td>66.7</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>21.7</td>
<td>0</td>
<td>66.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0</td>
<td>79.2</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>100</td>
<td>0</td>
<td>91.7</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>100</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>ND</td>
<td>ND</td>
<td>83.3</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>100</td>
<td>100</td>
<td>95.8</td>
</tr>
<tr>
<td>Neomycin</td>
<td>65.2</td>
<td>55.2</td>
<td>91.6</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0</td>
<td>0</td>
<td>79.2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>95</td>
<td>95</td>
<td>95.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0</td>
<td>0</td>
<td>91.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not done.
O1 (Fig. 3, lanes 1 to 5), two isolates, E14 and AV185 (Fig. 3, lanes 4 and 5), exhibited identical patterns, while the remaining three (Fig. 3, lanes 1 to 3) were different from each other despite belonging to the same serotype. Two isolates of *E. coli* serotype O146 (Fig. 3, lanes 6 and 7) showed different PFGE profiles. Surprisingly, the PFGE pattern of isolate E10, belonging to the O1 serotype (Fig. 3, lane 3), was identical to that of isolate E2, belonging to the O146 serotype (Fig. 3, lane 7). The PFGE pattern of one representative *E. coli* O-untypable isolate (Fig. 3, lane 8) was different from those of *E. coli* isolates belonging to either the O1 or O146 serotype.

**DISCUSSION**

The outbreak of acute diarrhea in Ahmedabad was unusual in that two major enterotoxic enteropathogens, namely, *V. cholerae* and ETEC were involved. To our knowledge, this is the first report describing the involvement of more than one enteric pathogen in an outbreak setting in India. Involvement of more than one pathogen during outbreaks has been reported elsewhere and was usually attributed to gross contamination of food or drinking water (9, 18). However in this study, the concurrent incidence of two different enteric pathogens, *V. cholerae* and ETEC, in a single patient was not encountered. This outbreak was predominantly due to *V. cholerae* O1, Ogawa serotype, biotype El Tor, the serotype and biotype currently prevailing in India. Uniquely, however, *V. cholerae* serogroup O139 and multiple serotypes of ETEC were also involved. When the 18 ETEC isolates were analyzed in detail, we were unable to detect any of the 12 commonly occurring CFAs (24). The probable reasons for this result might be either...
the loss of CFAs due to repeated subculture in vitro or the prevalence of a hitherto-unrecognized CFA different from the 12 CFAs assayed in this study. *V. cholerae* O1 Ogawa isolates had antibiotic resistance patterns similar to those of the prevailing O1 Ogawa strains in the rest of the country (13). The other significant observation was that all of the isolates of *V. cholerae* O139 examined were resistant to nalidixic acid, and such a high percentage of resistance to nalidixic acid has not been previously reported for this serogroup (13). Interestingly, all of the ETEC and other *E. coli* isolates were resistant to almost all of the antimicrobial drugs tested (Table 3) and showed alarmingly high levels of resistance to ciprofloxacin, norfloxacin, and nalidixic acid. As far as *E. coli*-mediated diarrhoea is concerned, a prevalence of ETEC strains resistant to fluoroquinolones has rarely been reported (31).

PCR results indicated that all *V. cholerae* O1 and O139 isolates tested harbored ctxA and that 75% of the ETEC isolates harbored elt only (Table 2). Ribotyping of the 13 representative isolates of *V. cholerae* was done using the BglII restriction endonuclease, which is known to produce good discriminatory patterns for *V. cholerae* (23). Ribotyping of representative isolates of *V. cholerae* O1 Ogawa (Fig. 2) showed that 87.5% had an identical ribotype, which was similar to the reported ribotype of the new clone of O1 (27). Ribotyping analysis of representative isolates of *V. cholerae* O139 (Fig. 2) indicated that all of the isolates were identical to the BII clone (11), which is the prevailing ribotype in many parts of India. Overall, based on the ribotyping results, it appears that the Ahmedabad outbreak was caused by the prevailing clones of *V. cholerae* O1 and O139 found in Calcutta and rest of the country.

In the PFGE analysis, all of the *V. cholerae* O1 isolates exhibited the H pattern of the new clone of O1 (35). PFGE analysis of three representative O139 isolates (Fig. 2) clearly showed that the pattern was very different from that of the prevailing O139 clone in Calcutta (2). PFGE of *E. coli* isolates revealed very interesting results. Two *E. coli* isolates, E14 and AV185, belonging to serotype O1 had identical patterns (Fig. 3) although their antibiograms were very different: E14 was sensitive to all of these drugs. AV185, belonging to serotype O1 had identical patterns (Fig. 3) although their antibiograms were very different: E14 was sensitive to all of these drugs. AV185 was sensitive to all of these drugs.

Generally, the clonal diversity among *E. coli* is high even though the strains are phenotypically identical but genetically dissimilar. The existence of such genetic heterogeneity among *E. coli* strains belonging to the same serotype has been recorded previously (7, 32). Surprisingly, two *E. coli* isolates (E10 and E2) were identical in both PFGE and antibiotic susceptibility testing although they belonged to serotypes O1 and O146, respectively. Such a phenomenon has been observed among pandemic *Vibrio parahaemolyticus* isolates (8). The outbreak reported in the present study was due to the contamination of drinking water with sewage. What is intriguing in this outbreak is why instead of having *V. cholerae* infection some patients were infected with ETEC, even though the same population was exposed to the common source of infection. It could be possible that such patients had protective levels of antibody due to previous exposures to toxigenic *V. cholerae*, and thus ETEC prevailed in these individuals. More detailed analysis of patients in such concomitant outbreaks would provide a wealth of information which would be useful from the perspective of development of vaccines for enteric infections.

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