

should be initiated immediately because β -lactam antimicrobial drugs are inefficient for the treatment of rickettsioses (9).

This research was funded by the Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), Marseille, France.

D.R. is a cofounder of the biotechnology company Inodiag (www.inodiag.com) and is the inventor of a serologic diagnostic method for which the patent is held by Aix-Marseille University; this method was not used in this study. All other authors have no conflicts of interest.

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DOI: <http://dx.doi.org/10.3201/eid2002.130575>

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Pandemic *Vibrio parahaemolyticus*, Maryland, USA, 2012

To the Editor: Since 1996, an increasing number of infections caused by *Vibrio parahaemolyticus* strains belonging to a pandemic clonal complex (CC), CC3, typically O3:K6, have been observed worldwide (1–3); most of these strains are sequence type (ST) 3. In the summer of 1998, outbreaks linked to O3:K6 occurred in Galveston Bay, Texas, and Oyster Bay, New York, USA; the illnesses were associated with oyster consumption (4). Strains belonging to CC36 are the leading cause of *V. parahaemolyticus* infections in the United States. These

strains are endemic to the West Coast (2) and have been historically linked to outbreak-associated *V. parahaemolyticus* infections caused by consumption of raw oysters harvested from the region (5).

In August 2012, a *V. parahaemolyticus* outbreak involving 6 persons occurred in Maryland, USA. The patients (members of 2 dining parties) had eaten in the same restaurant on the same day; raw and cooked seafood was served at the restaurant. Party A comprised 4 diners, of whom 2 had laboratory-confirmed illness and 2 were probable case-patients. Party B comprised 2 diners, of whom 1 had laboratory-confirmed illness and 1 was a probable case-patient. Probable case-patients were epidemiologically linked to confirmed case-patients, but *V. parahaemolyticus* was not detected in their stool samples. The epidemiologic investigation did not conclusively identify the specific food responsible for the outbreak. The affected diners had not eaten oysters, lobster, or mussels, but they had eaten cooked clams, fish, crab, and shrimp. Because the patients had not eaten oysters, a traceback investigation was not conducted. The outbreak possibly was caused by cross-contamination during food preparation. No other cases were reported from this restaurant or the surrounding area.

V. parahaemolyticus was isolated from stool samples of 3 of the patients. The isolates were characterized by real-time PCR for virulence-related genes (*tdh* and *trh*). All 3 isolates were *tdh* positive and lacked the *trh* gene. Pulsed-field gel electrophoresis (PFGE) was run, using *SfiI* and *NotI*; the resulting K16S12.0138 (*SfiI*) and K16N11.0143 (*NotI*) patterns were indistinguishable. The PFGE pattern combination was queried against combination entries made in PulseNet (www.cdc.gov/pulsenet/) during February 4, 2010–April 16, 2013, and found to be indistinguishable from other clinical entries (online Technical

Appendix 1 Table, wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp1.pdf). This PFGE pattern combination has been seen 25 times; all patterns were for strains from humans (N. Facundo, pers. comm.). In 2012, this PFGE pattern combination was observed in 3 US states—California (6 cases), Arizona (6 cases), and Texas (5 cases)—but those isolates were not further tested (S.G. Stroika, pers. comm.), suggesting that other cases of pandemic *V. parahaemolyticus* infections have occurred in the United States but were not identified as being caused by pandemic clones.

The whole genomes of the 3 Maryland strains were sequenced by using the Ion Torrent personal genome machine (Life Technologies, Grand Island, NY, USA); in silico multilocus sequence typing (MLST) (2) showed that the isolates were all ST3, the most common ST belonging to CC3. Bioinformatic analysis of the whole genomes was conducted with the Bacterial Isolate Genome Sequence Database (6) genome comparator tool available within the *V. parahaemolyticus* MLST database (<http://pubmlst.org/vparahaemolyticus>) (7,8). Results confirmed that these outbreak isolates were linked to the O3:K6 pandemic clone of *V. parahaemolyticus* (Figure). We identified 2,613 variable loci in this analysis by using as reference genome the prototype pandemic *V. parahaemolyticus* clonal strain RIMD221633 (available from GenBank, www.ncbi.nlm.nih.gov/genome/?term=vibrio+parahaemolyticus) (10). Differences in variable loci and the absence of certain genes indicated that, although indistinguishable by MLST and PFGE, these strains are easily differentiated from RIMD2210633 (online Technical Appendix 1). The draft genome sequences for the 3 strains are available at the *V. parahaemolyticus* MLST database (identification nos. 1187 [Vp16MD], 1188 [Vp17MD], and 1189 [Vp18MD]).

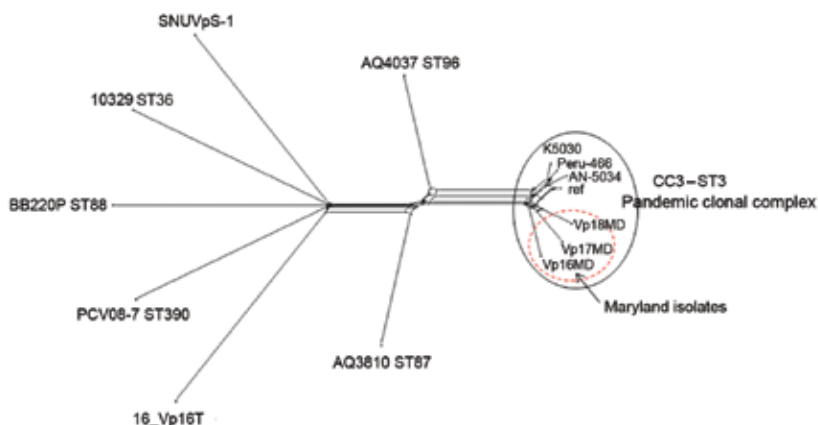


Figure. Neighbor-Net graph generated with the Bacterial Isolate Genome Sequence Database genome (BIGSdb) comparator tool implemented within the *Vibrio parahaemolyticus* MLST database (<http://pubmlst.org/vparahaemolyticus>) (7,8) using 2,613 variable loci. These loci were identified by using as a reference (ref) the *V. parahaemolyticus* strain RIMD2210633 chromosome I (3,080 genes) and conducting a whole-genome MLST (wgMLST) for *V. parahaemolyticus* genomes available through GenBank (AN-5034 O4:K68 ST3, Peru-466 ST3, K5030 ST3, 16_Vp16T, AQ3810 ST87, AQ4037 ST96, PCV08-7 ST390, BB220P ST88, and SNUVpS-1) and 3 Maryland outbreak strains (Vp16MD, Vp17MD, and Vp18MD). This typing showed that these 3 strains belonged to the pandemic CC3. A similar graph was obtained by using chromosome II of the same strain as reference (data not shown). In brief, the BIGSdb genome comparator tool performs wgMLST, which produces a color-coded wgMLST output (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp2.xlsx) that facilitates comparison among isolates. This loci output is further categorized into loci that are 1) variable among all isolates, 2) identical among all isolates, 3) missing in all isolates, and 4) incomplete because of being located at the ends of contigs. The variable loci among all isolates are the loci used for assessing relationships and producing a distance matrix based on the number of variable alleles; the strains are resolved into a network by using the NeighborNet algorithm (9). MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type.

V. parahaemolyticus strains belonging to the pandemic CC have caused thousands of infections and a *V. parahaemolyticus* pandemic (3). Foodborne illnesses caused by pandemic *V. parahaemolyticus* are uncommonly reported in the United States. In Maryland, 12 and 21 cases of *V. parahaemolyticus*-associated gastroenteritis were reported in 2012 and 2013, respectively. We report that the pandemic CC was still causing US outbreaks as recently as August 2012. It is possible that complete availability of PFGE patterns during the outbreaks (online Technical Appendix 1) could have provided additional insight into the scope of the outbreak and implicated food sources. The application of rapid, whole-genome sequencing

technology aided our discovery that the Maryland outbreak strains were part of the pandemic CC and likely related to *V. parahaemolyticus* strains that shared common PFGE patterns and that were reported as the cause of illnesses in several states around the same time as the Maryland outbreak.

The presence of this virulent *V. parahaemolyticus* strain in Maryland is an ongoing public health concern, requiring continued microbiological surveillance. This pandemic strain also indicates the need for establishing a *V. parahaemolyticus* genome database that is accessible worldwide. Such a database would enable improved tracking and faster responses to emergent and dangerous pandemic clonal strains.

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DOI: <http://dx.doi.org/10.3201/eid2004.130818>

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Serologic Evidence of Leptospirosis in Humans, Union of the Comoros, 2011

To the Editor: Leptospirosis is a worldwide bacterial zoonosis caused by infection with pathogenic *Leptospira* spp. (Spirochaetales, Leptospiraceae). Most mammals can be infected, but rats are considered the main reservoir, maintaining *Leptospira* spirochetes in the lumen of renal tubules and contaminating the environment with bacteria-infected urine. Transmission to humans is accidental, occurring through contact with animal secretions or with contaminated environmental materials.

In temperate countries, human leptospirosis is a sporadic disease; incidence is much higher in the tropics because climate and environmental conditions are conducive to the survival

of bacteria, resulting in increased exposure of humans to leptospirosis-causing pathogens (1). Among islands in the southwestern Indian Ocean, human leptospirosis is endemic to Mayotte, France, and La Réunion (2–4) and to the Seychelles, where the incidence of leptospirosis is one of the highest worldwide (5). Leptospirosis is poorly documented in other islands in the region, including Mauritius, Madagascar, and the Union of the Comoros (2,6–8). Whether the scant documentation indicates underdiagnosis or reflects local epidemiologic specificities is unknown. To improve knowledge of *Leptospira* infection in the region, we conducted a study in the Union of the Comoros to serologically assess the presence or absence of leptospirosis in humans. The Union of the Comoros consists of 3 islands: Grande-Comore, Mohéli, and Anjouan. Together with a fourth, southern island, Mayotte, these islands form the Comoros Archipelago.

For feasibility reasons, we used excess serum samples. Seventy-six samples were from healthy volunteers who gave informed consent; 318 clinical blood samples from patients had been obtained by private laboratories and by the surveillance laboratory of the National Malaria Control Programme (PNLP) during August 1–October 8, 2011. The Ministère de la Santé, de la Solidarité et de la Promotion du Genre of the Union of the Comoros, authorized the serologic investigation (authorization no. 1175/MSSPG/DNS).

We used the microscopic agglutination test (MAT) to test serum samples; the MAT was based on a panel of 15 *Leptospira* strains, enabling the screening of all recently reported serogroups for human and animal cases on neighboring Mayotte (2,4,9). A list of the tested strains follows, shown as *Genus species* Serogroup/Serovar (type strain): *L. borgpetersenii* Ballum/Castellonis (Castellon 3), *L. borgpetersenii* Sejroe/Hardjovovis (Sponselee), *L. borgpetersenii* Sejroe/Sejroe (M 84),