References


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Multidrug-Resistant IncA/C Plasmid in Vibrio cholerae from Haiti

To the Editor: The agents of epidemic cholera are Vibrio cholerae toxigenic serogroups O1 and O139. Cholera symptoms include watery diarrhea and severe dehydration, which can rapidly result in death unless rehydration therapy is prompt (1). Antimicrobial agents may reduce the severity and duration of disease (1); commonly used are tetracyclines, fluoroquinolones, macrolides, and trimethoprim/sulfamethoxazole (1). However, V. cholerae resistance to antimicrobial drugs is increasing because of the accumulation of genetic mutations and acquisition of resistance genes, which are usually transferred on mobile genetic elements such as integrating conjugative elements (ICEs) (1).

As of March 12, 2014, the ongoing cholera outbreak that began in Haiti in October 2010 had caused 700,796 cases and 8,548 deaths (2). To characterize infections, the National Public Health Laboratory in Haiti and the US Centers for Disease Control and Prevention (CDC) collaborated to perform standard microbiological and antimicrobial-drug susceptibility testing on isolates from case-patients. Since October 2010, the National Public Health Laboratory has identified 465 isolates, which were then forwarded to CDC for determination of MICs for 15 antimicrobial agents by broth microdilution (Sensititer; Trek Diagnostics Systems, Cleveland, OH, USA) according to manufacturer’s recommendations (Table). Resistance was defined by the Clinical and Laboratory Standards Institute interpretive standards, when available (3). The typical outbreak strain (2010EL–1786) displayed resistance to streptomycin, sulfisoxazole, trimethoprim/sulfamethoxazole, and nalidixic acid, and decreased susceptibility to ciprofloxacin and chloramphenicol (4). Resistance was caused by mutations in the QRDR regions of the gyrA and parC genes and presence of ICEVchHai1 containing the dfrA1, floR, strAB, and sul2 resistance genes (4).

In April of 2012, the 2 agencies began sentinel laboratory-based surveillance for acute diarrheal disease at 4 hospitals in Haiti (5). As part of this surveillance, fecal specimens were sent to the National Public Health Laboratory for organism isolation, identification, antimicrobial-drug testing, and subsequently to CDC for expanded antimicrobial-drug testing and molecular characterization. One isolate, 2012EL–2176, showed the typical resistance phenotype of the outbreak strain but additional resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftriaxone, cefotiofur; the tetracycline MIC was intermediate (Table).

Analysis of this isolate by serotype, pulsed-field gel electrophoresis, multilocus variable number–tandem repeat analysis, and whole-genome sequencing confirmed that the isolate was similar to outbreak isolates (data not shown) (6). PCR and whole-genome sequencing analysis by use of ResFinder (http://www.genomicepidemiology.org/) identified the original outbreak resistance determinants and additional determinants (aac(3)-Ia, blaTEM, blaCMY-2, blaCTX-M-2, dfrA15, dfrA1).

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V. cholerae located in ICE, a second copy of the resistance genes has been observed previously, possibly and the lack of resistance in tetA and tetA

Since discovery of isolate 2012EL-2176, sentinel surveillance has not detected increased antimicrobial-drug resistance among V. cholerae in Haiti. The ability of IncA/C plasmids to acquire novel resistance cassettes from multiple sources makes it difficult to hypothesize as to the origins of plasmid p2012EL-2176. Although this plasmid was most closely related to a plasmid found in E. coli, it was also closely related to plasmids in Salmonella, Klebsiella, and Providencia. Enterobacteriaceae are found in the environment and/or in the host gut; therefore, the isolate could have acquired the plasmid in the environment or within the host. The latter scenario would limit the possible spread of this plasmid and could explain its rarity. The original Haiti outbreak isolate has been shown to be poorly naturally transformable, accounting for the lack of acquired chromosomal genes and nearly homologous genomic content among outbreak isolates (6). Therefore, the acquisition of plasmids, and their resistance genes, may represent the major source of future variability among V. cholerae involved in the Haiti outbreak.

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Table. Susceptibility, resistance genes, and plasmids associated with Haiti Vibrio cholerae outbreak isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Resistance profiles [MIC mg/L]*</th>
<th>Resistance genes/mutations</th>
<th>Plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. cholerae</td>
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<tr>
<td>2010EL-1786</td>
<td>STR &gt;64, FIS &gt;256, TMP/SXT &gt;4, CIP(l) &gt;0.5, NAL &gt;32</td>
<td>strAB sul2, dfrA1, floR, gyrA(S83I)parC(S85L)</td>
<td>None</td>
</tr>
<tr>
<td>V. cholerae</td>
<td></td>
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<tr>
<td>2012EL-2176</td>
<td>AMP &gt;32, AMC &gt;32, CRO &gt;64, FOX &gt;8, GEN &gt;8, STR &gt;64, FIS &gt;256, TMP/SXT &gt;4, TET(l) &gt;8, CIP(l) &gt;0.5, NAL &gt;32</td>
<td>blaCMY-2, blaCTX-M-2, blaTEM-1, floR, aac(3)-Ia, strAB, sul2, dfrA1, dfrA27, tetA, mphA, gyrA(S83I)parC(S85L)</td>
<td>IncA/C2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>AMP &gt;32, AMC &gt;32, CRO &gt;64, FOX &gt;8, AZM &gt;16, CHL &gt;32, GEN &gt;8, STR &gt;64, TMP/SXT &gt;4, TET &gt;32</td>
<td>blaCMY-2, blaCTX-M-2, blaTEM-1, mphA, floR, aac(3)-Ia, strAB, sul2, dfrA27, tetA</td>
<td>IncA/C2</td>
</tr>
</tbody>
</table>

*Drugs tested were AMP, ampicillin; AMC, amoxicillin/clavulanic acid; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; FIS, sulfisoxazole; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; TET, tetracycline; TIO, ceftiofur; and TMP/SXT, trimethoprim/sulfamethoxazole; S83I, serine-to-isoleucine change at amino acid position 83; S85L, serine-to-leucine change at amino acid position 85. Drugs that yielded intermediate results are followed by (I). Clinical Laboratory Standards Institute break points are not established for AZM. The AZM break points used in this study (>16 mg/L) were established by the National Antimicrobial Resistance Monitoring System and should not be used to predict clinical efficacy.

†E. coli DH10B is intrinsically resistant to STR.
Human Co-Infection with Avian and Seasonal Influenza Viruses, China

To the Editor: In April 2013, a case of co-infection with avian-origin influenza A(H7N9) virus and seasonal influenza A(H3N2) virus was reported in Jiangsu Province, China (1). This case raised concern over the possible occurrence of new reassortants with enhanced transmissibility among humans. Because of the nature of the dynamic reassortment of A(H7N9) virus with A(H9N2) virus in the environment and in poultry (2,3), close surveillance for possible new reassortment in human patients with A(H7N9) infection is needed.

We report co-infection in 2 patients in Hangzhou, the capital Zhejiang Province, China, in January 2014. The co-infections involved influenza A(H7N9) virus and a seasonal A(H1N1)pdm09 virus (1 patient) or a seasonal influenza B virus (1 patient).

Of 60 patients with laboratory-confirmed influenza A(H7N9) infections in Hangzhou in April 2013 and in January–February 2014, testing of pharyngeal swab samples indicated that 2 patients were also positive for seasonal influenza virus. The pharyngeal samples were tested by real-time reverse transcription PCR according to protocols provided by the Chinese National Influenza Center. Informed consent for this study was provided by each patient’s spouse.

On January 6, 2014, patient 1 (male, 58 years of age), a resident of Xiaoshan District, had a high fever (39.6°C) and a cough; at a hospital, he received a diagnosis of severe acute interstitial pneumonia. The patient had a history of chronic myelogenous leukemia; his history of exposure to live poultry was not clear. On January 13, infection with influenza A(H7N9) virus was laboratory confirmed; viral RNA from a pharyngeal swab sample collected before oseltamivir treatment was positive for the following: influenza A virus (cytomegalovirus (1) = 16.3), H7 (Ct = 27), N9 (Ct = 26), influenza A(H1N1)pdm09 virus H1 (Ct = 30), and N1 (Ct = 30). The 2 viruses were named A/Hangzhou/10–1/2014(H7N9) and A/Hangzhou/10–2/2014(H1N1)pdm09. The patient received oseltamivir while in the hospital but died on January 18.

On January 5, patient 2 (male, 54 years of age), also from Xiaoshan District, had fever and a cough; at a hospital, he received a diagnosis of severe acute pneumonia. He had a history of aplastic anemia and had been exposed to live poultry 1 week before symptom onset. On January 18, infection with influenza A(H7N9) virus was laboratory confirmed. Viral RNA from a pharyngeal swab sample collected before oseltamivir treatment was positive for the following: influenza A virus (Ct = 26), H7 (Ct = 23), N9 (Ct = 22), influenza A(H1N1)pdm09 virus H1 (Ct = 30), and N1 (Ct = 30). The 2 viruses were named A/Hangzhou/10–1/2014(H7N9) and A/Hangzhou/10–2/2014(H1N1)pdm09. The patient received oseltamivir while in the hospital but died on January 18.

The hemagglutinin (HA) and neuraminidase (NA) sequences of viruses from these 2 patients were determined by Sanger sequencing. The specific primers used are listed in Table 1 (http://www.ncbi.nlm.nih.gov/Articles/20/11/14-0897-Techapp1.pdf). The accession numbers of these sequences and the reference sequences for phylogenetic analyses are