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**Multidrug-Resistant IncA/C Plasmid in V. 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 (2). However, *V. cholerae* resistance to antimicrobial drugs is increasing because of the accumulation of genetic mutations and the acquisition of resistance genes, which are usually transferred on mobile genetic elements such as integrating conjugative elements (ICEs) (3). 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, cefotaxime; 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)-IId*, *bla*_{CMY-2*}, *bla*_{CTX-M-2*}, *bla*_{TEM-P}, *dfrA15*). 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 (Sensititre; Trek Diagnostics Systems, Cleveland, OH, USA) according to manufacturer’s recommendations (Table).
mpA, sul1, and tetA) (7). Plasmid transfer by electroporation into *Escherichia coli* (DH10B) confirmed that the resistance determinants were plasmid encoded. PCR-based replicon testing identified an IncA/C2 plasmid, and PCR and whole-genome sequencing confirmed that the plasmid encoded a unique set of resistance determinants (*aac(3)-Ila, blaCMY-2, blaCTX-M-2, blaTEM-1, dfrA15, mpA, sul1, and tetA*) and a second copy of the resistance genes *floR*, *strAB*, and *sul2* identical to those located in ICEVchHai1 (8). Antimicrobial-drug susceptibility testing of the transformant demonstrated transfer of the resistance profile and additional resistance to chloramphenicol, tetracycline, and decreased susceptibility to azithromycin (Table). The lack of association between the presence of the resistance determinants *floR* and *tetA* and the lack of resistance in *V. cholerae* has been observed previously, possibly because of lower gene expression (9). The plasmid was mobilizable by conjugation (conjugation efficiency = 1.3–1.4 × 10⁻³) when *E. coli* J53 was used as the recipient.

IncA/C plasmids are widespread in *Enterobacteriaceae* and commonly confer multidrug resistance. BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) comparison of the completed plasmid p2012EL-2176 sequence with the National Center for Biotechnology Information nucleotide collection showed similarities to other IncA/C-*blaCMY* plasmids; most similarity (total score = 3.009 × 10⁶) was to pAR060302, found in an *E. coli* isolate from a dairy calf (10). Most IncA/C-*blaCMY* plasmids have 3 resistance regions: *sul2* region (*floR-tetA-strAB-sul2*), *cmy-2* insertion region, and Tn21-like region (*aad-aac*). Plasmid p2012EL-2176 contains the *sul2* and *cmy-2* insertion regions and a putative *arr3-dfrA27-aadA16-sul1* resistance gene cassette at the Tn21 location. This plasmid has additional resistance gene insertions: a putative cassette containing a *blaTEM1* and *aac(3)-Ila* gene upstream of the *sul2* region and insertions of *blaCTX-M-2, sul1*, and *mpA* genes downstream of the *arr3-dfrA27-aadA16-sul1* cassette (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/11/14-0889-Techapp1.pdf).

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 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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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 reasortants 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, following a single-source introduction to Haiti, 2010. Emerg Infect Dis. 2011;17:2151–4.


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 (cycle threshold $[C_t] = 26$, $H_7 (C_t = 27)$, $N_9 (C_t = 26)$, influenza A(H1N1)pdm09 virus $H_1 (C_t = 30)$, and $N_1 (C_t = 30)$. 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 (cycle threshold $[C_t] = 26$, $H_7 (C_t = 23)$, $N_9 (C_t = 22)$, influenza B virus ($C_t = 22$). The viruses were named A/Hangzhou/17–1/2014(H7N9) and B/Hangzhou/17–2/2014(H1N1)pdm09. This patient received oseltamivir but died on January 22.

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 online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/20/11/14-0897-Techapp1.pdf). The accession numbers of these sequences and the reference sequences for phylogenetic analyses are