Genetic Changes of Reemerged Influenza A(H7N9) Viruses, China

To the Editor: From March 30, 2013, through April 8, 2014, a total of 401 human infections with novel avian influenza A (H7N9) virus were reported in China (1). In the initial wave from February through May 2013, cases were laboratory confirmed for 133 patients (45 died), mainly in eastern China. From June through early October 2013, only 2 laboratory-confirmed cases were reported in China. One of these, identified on August 10, 2013, was the first case of influenza A(H7N9) virus infection in Guangdong Province (strain A/Guangdong/HZ-01/2013). However, a second wave of influenza A(H7N9) virus infection began on October 14, 2013 (2). As of April 8, 2014, a total of 266 laboratory-confirmed cases had been reported, mainly in Zhejiang Province in eastern China (92 cases, 37 deaths) and Guangdong Province in southern China (99 cases, 30 deaths).

Previous sequencing studies suggested that 6 of the 8 influenza A(H7N9) virus RNA segments were acquired from influenza A(H9N2) virus. This acquisition process involved at least 2 steps of sequential reassortment; the most recent event most likely occurred in the Yangtze River Delta area of eastern China (3–5). To date, nearly all analyses have been performed by using sequences obtained from viruses isolated during the first wave of infection; changes associated with viruses isolated during the second wave are largely unknown (6). We therefore conducted phylogenetic analyses of whole-genome sequence data for 15 influenza A(H7N9) viruses isolated from human patients in Guangdong from November 4, 2013, through January 15, 2014.

We estimated maximum-likelihood trees for all 8 RNA segments by using MEGA version 5.2 and the general time-reversible model (7). RNA segments encoding the hemagglutinin, neuraminidase, and matrix genes of A/Guangdong/H7N9 viruses isolated after November 2013 were genetically similar to those of A/Guangdong/HZ-01/2013 and H7N9 strains from the first wave of influenza (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/9/14-0250-Techapp1.pdf). An additional 4 RNA segments (nonstructural protein [NS], nucleocapsid protein [NP], polymerase basic proteins [PB] 1 and 2) of A/Guangdong/H7N9 influenza viruses isolated after November 2013 were clustered with A/Guangdong/HZ-01/2013 and H7N9 strains from the first wave of influenza (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/9/14-0250-Techapp1.pdf).

Notably, 2 separate clusters were observed for the phylogenetic tree of the RNA segment encoding the polymerase acidic gene (online Technical Appendix Figure, panel H). A/Guangdong/HZ-01/2013-like viruses clustered with subtype H7N9 viruses from the first wave of influenza. However, A/Guangdong/DG-02/2013-like viruses were clustered with subtype H9N2 influenza viruses circulating in Guangdong, suggesting that recent reassortment with circulating subtype H9N2 viruses occurred after the first case of infection with influenza A(H7N9) virus reported in Guangdong (online Technical Appendix Figure, panel H).

This study provides evidence that influenza A(H7N9) viruses isolated during the second wave of influenza in Guangdong differ genetically (in 5 of the 8 RNA segments) from that of influenza A(H7N9) viruses isolated during the first wave. High similarity of these 5 segments with those of locally circulating subtype H9N2 viruses suggests that rapid and continued reassortment with circulating subtype H9N2 viruses occurred during the second wave of the influenza A(H7N9) virus epidemic. Because reassortment and genetic changes can contribute to host fitness and infection capacity of reemerged influenza A(H7N9) viruses, studies of pathogenicity and transmission, to reveal the exact role of each genetic alteration, are needed.

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To the Editor: Carbapenem-resistant Enterobacter cloacae Isolates Producing KPC-3, North Dakota, USA

Carbapenem-resistant Enterobacter cloacae (CRE) continue to emerge as a serious public health threat throughout the world (1). CRE infections in the United States are often mediated by acquisition of Klebsiella pneumoniae carbapenemase (KPC) expressed by Klebsiella spp., although KPC is also found in other genera (2). The spread of KPC-producing, gram-negative bacteria in hospitals has been linked to severity of illness, co-existing medical conditions, exposure to antimicrobial drugs, and need for chronic care (3).

After reporting of CRE infections to the North Dakota Department of Health became mandatory in 2011, a total of 20 CRE cases were noted in 12 of 53 counties (2.9 cases/100,000 population [4]). Most cases involved infection with Enterobacter cloacae and occurred in Cass County, where the state’s largest city, Fargo, is located. We describe an outbreak of clonal carbapenem-resistant E. cloacae in a health care system in Fargo.

Sanford Health is a 583-bed, acute-care facility, representing >70% of acute-care beds in Fargo. The hospital handles >27,000 admissions/year and serves as a referral center for a large area of the state, and the only long-term acute-care (LTAC) facility in the eastern half of the state operates on its campus. During December 2011–December 2012, all isolates of Enterobacteriaceae with reduced susceptibility to ertapenem (MIC $\geq$1 $\mu$g/mL) identified at the hospital’s clinical microbiology laboratory were screened for carbapenemase production by using the modified Hodge test (mHT), according to Clinical and Laboratory Standards Institute recommendations (5). Identification and susceptibility testing were done with the MicroScan system (Siemens Healthcare Diagnostics, Tarrytown, NY, USA); MICs of carbapenems were confirmed with Etest (bioMérieux, Durham, NC, USA). Three carbapenem-resistant E. cloacae isolates from documented cases of CRE infection at the hospital during 2010 were analyzed for comparison.

To characterize carbapenem-resistant and mHT-positive isolates, we used PCR to amplify and sequence the carbapenemase genes bla_{IMP}, bla_{NDM}, bla_{VIM}, and bla_{KPC} by using established methods (6). The upstream sequence of bla_{KPC}-positive strains was analyzed to determine the isoform of the transposon Tn4401 that harbored bla_{KPC} (7). We investigated genetic similarity among isolates by repetitive-sequence-based PCR; isolates with >95% similarity were considered clonal (6). We also sequenced the highly conserved hsp60 gene (8) and attempted conjugative transfer of the bla_{KPC} gene by growing KPC-producing E. cloacae along with sodium azide–resistant Escherichia coli J-53. As part of the study, we examined records of patients from whom carbapenem-resistant E. cloacae was isolated. The study was approved by the Institutional Review Board at Sanford Health.

During December 2011–December 2012, a total of 19 single-patient E. cloacae isolates and 1 E. aerogenes isolate had positive mHT results. bla_{KPC} was detected in 17 of the 19 E. cloacae isolates and in the 3 carbapenem-resistant E. cloacae isolates from 2010. For all 20 of those isolates, sequencing revealed bla_{KPC-3} in association with isoform d of the transposon Tn4401, and all isolates were clonally related (Figure). All 20 isolates also had an identical hsp60 sequence belonging to cluster VI in the Hoffman and Roggenkamp scheme (8). Conjugation of a bla_{KPC}-containing plasmid into E. coli J-53 was successful for 1 strain.
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Technical Appendix
Technical Appendix Figure. Phylogenetic tree of the 8 RNA segments from influenza A(H7N9) virus isolates. Nucleotide sequences were analyzed by using the maximum-likelihood method (A–G). Supporting bootstrap values >70 are shown. Scale bars indicate nucleotide substitutions per site. The collection times (year, month) and cities for influenza A(H7N9) virus Guangdong strains are indicated. Green, influenza A(H7N9) viruses isolated during the first influenza wave; red, influenza A(H7N9) virus isolated during the second wave; triangles, A/Guangdong/HZ-01/2013 isolated between the first and second waves on July 28, 2013; circles, influenza A(H9N2) viruses circulating in Guangdong; GZ, Guangzhou city; DG, Dongguan city; YJ, Yangjiang city; FS, Foshan city; SZ, Shenzhen city. A) hemagglutinin, B) neuraminidase, C) matrix protein, D) nonstructural protein, E) nucleocapsid protein, F) polymerase basic protein 1, G) polymerase basic protein 2; H) polymerase acidic protein.