Emergence of a Novel Binary Toxin–Positive Strain of \textit{Clostridium difficile} Associated With Severe Diarrhea That Was Not Ribotype 027 and 078 in China

To the Editor—\textit{Clostridium difficile} is a frequent cause of antibiotic-associated diarrhea and healthcare-associated infections.\textsuperscript{1,2} Epidemics of \textit{C. difficile} infection (CDI) have occurred in North America and Europe over recent decades. In particular, epidemic \textit{C. difficile} (sequence type [ST] 1/027/NAP1) has rapidly emerged in the past decade as the leading cause of \textit{C. difficile}–associated diarrhea worldwide, resulting in high morbidity and mortality in hospitalized patients. Very little is known about the epidemiology of \textit{C. difficile}–associated diarrhea outside of North America and Europe. However, the hypertoxigenic strain \textit{C. difficile} RT027 has rarely been detected in Asia to date, specifically not in China before 2013.\textsuperscript{3} In addition, cases of \textit{C. difficile} RT078 have not been reported in China. We report a novel binary toxin–positive non-027, non-078 \textit{C. difficile} associated with severe diarrhea recently identified in China.

A 65-year-old man was admitted to Xiangya Hospital in Changsha, China, with fever, headache, diarrhea, and impaired consciousness. He had been diagnosed with a central nervous system infection 9 days earlier in a local hospital, presumptively diagnosed as suppurative or tubercular meningitis. Antimicrobial therapy was initiated with ceftriaxone over the next 4 days in that local hospital. The patient’s condition did not improve; he developed a pulmonary infection, with \textit{Acinetobacter baumannii} isolated from the sputum, for which he was intravenously treated with cefepime for 5 days. The consciousness level of the patient deteriorated, and he developed severe diarrhea and experienced bouts of vomiting. Therefore, on February 8, 2014, he was transferred to Xiangya Hospital. Stool specimens collected during hospitalization tested positive for \textit{C. difficile} toxin B by polymerase chain reaction assay, and \textit{C. difficile} was confirmed by culture and biochemical characteristics. Enteral vancomycin was prescribed for the patient, but symptoms further deteriorated and he died on day 4 of hospitalization.

Stool specimens from the patient were cultured for anaerobic bacteria, and \textit{C. difficile} was isolated and identified. The toxin genes \textit{tcdA}, \textit{tcdB}, \textit{cldA}, and \textit{cldB} were detected by polymerase chain reaction assay.\textsuperscript{4} The isolate (LC693) was positive for toxin A, toxin B, and binary toxin. DNA sequence analysis of the toxin gene to the genome sequence of CD630 (GenBank accession No. AM180355.1) revealed an 18–base pair deletion (nucleotides 330–347) located in \textit{tcdC} (682 base pairs, GenBank accession No. KM609431.1) of LC693. It did not express a variation at nucleotide position (nt) 117 (associated with RT027). However, there was a point mutation at nt 184, resulting in the introduction of a premature stop codon (TAA) in the putative \textit{TcdC} protein (Figure 1). Multilocus sequence typing indicated the isolate was ST201. Alleles for the profile were \textit{adk-1}, \textit{atpA-6}, \textit{dx-4}, \textit{glyA-7}, \textit{recA-2}, \textit{sod-8}, and \textit{tpi-31}, making the Xiangya hospital clinical isolate LC693 unique from the RT027 (ST1, R20291, GenBank accession No. FN545816.1) and RT078 (ST11, M120, GenBank accession No. FN665653.1) clones. Whole genome sequencing of \textit{C. difficile} LC693 was performed using a MiSeq (Illumina) by PE300 strategy. Approximately 424 Mb clean data were obtained, with a mean read length of 300 base pairs, 100 times coverage of the approximately 4.07 Mb genome. Hylogenomic analysis showed that the LC693 was more closely related to the RT027 (CD196, R20291) cluster than to the RT078 (M120) (Figure 2). Between LC693 and R20291, the single-nucleotide polymorphisms were 35,505; between LC693 and M120, the single-nucleotide polymorphisms were 87,672.

The incidence and associated mortality of CDI have been increasing. This changing epidemiology has coincided with the emergence and rapid spread of \textit{C. difficile} RT027/078, involved in several large outbreaks of severe CDI in North America and Europe. Furthermore, hypervirulent RT027 and RT078 have been associated with more severe disease because of the production of higher amounts of toxin A and B due to \textit{tcdC} deletion.\textsuperscript{5,6} Sequence analysis of \textit{tcdC} of the isolate LC693 showed an 18–base pair deletion and a premature stop codon (TAA), compatible with the genotype of a hypertoxigenic strain. However, it also differed from RT027 and RT078, indicating possible existence of a new hypervirulent strain in China. Our findings were similar to those of Lim et al.,\textsuperscript{7} who identified and characterized a \textit{C. difficile} strain associated with a severe clinical phenotype that genetic analysis showed to be ST41/RT244 that was also different from RT027 and RT078 in Melbourne, Australia. There were 10,803 single-nucleotide polymorphisms between ST41/RT244 and RT027. Further tests are required to determine the ribotype and toxin production of the isolate LC693, and study of the epidemiology of the
strain will be helpful in monitoring its spread in future. However, laboratory confirmation for *C. difficile* is not routinely performed in hospitals in China, so there is not a current CDI surveillance system or plan in China. Many *C. difficile*–associated diarrhea cases are suspected by a subjective judgment and clinical assessment of patient characteristics in most hospitals. Such cases are administered empirical treatment. Estimating the incidence of CDI in China is difficult because there are no national surveillance data. In order to enable a more accurate diagnosis of CDI and to prevent outbreaks of such infection, implementing standardized laboratory-confirmed diagnosis and treatment procedures and developing healthcare-associated infection surveillance programs of *C. difficile* should be encouraged in China.

**figure 1.** Comparison of the tcdC nucleotide sequences of the *Clostridium difficile* reference strain CD630 with the isolated strain from a patient with diarrhea that contains both the 18–base pair deletion (from nucleotide position [nt] 330–347) and a premature stop codon TAA (from nt 184–186); Query, tcdC gene (from nt 1-681) of the isolated strain LC693; Subject (Subjct), tcdC gene of the reference strain CD630 (from nt 805000–804310).

**figure 2.** Whole-genome phylogenomic tree of strains LC693 (sequence type [ST] 201) compared with 31 publicly available *Clostridium difficile* genomes. Bootstrap values are labeled along main branches. Bar = 0.05.

**NUCLEOTIDE SEQUENCE ACCESSION NUMBER**

The tcdC nucleotide sequences of the isolate LC693 was submitted to GenBank. The sequence is available under accession No. KM609431.1.

**ACKNOWLEDGMENTS**

We thank Carol Y. Rao, John D. Klena, Yuan Li, Yang Huai, Yuzhi Zhang, and Hongbing Jia for technical support and language proofreading.

**Financial support.** US Centers for Disease Control and Prevention (Cooperative Agreement No. 5U2GGH000018).

**Potential conflicts of interest.** All authors report no conflicts of interest relevant to this article.

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https://doi.org/10.1017/ice.2015.120 Published online by Cambridge University Press
Methicillin-Resistant Staphylococcus aureus: More Attention Should Be Paid in Mainland China

To the Editor—Methicillin-resistant Staphylococcus aureus (MRSA) represents a major problem for public health systems with resistance to methicillin and other antibiotics with high prevalence in hospital and community settings. Remarkably, in the United States the estimated number of deaths due to MRSA infections exceeds that due to human immunodeficiency virus (HIV)/AIDS.1 MRSA colonization plays a key role in the epidemiology and pathogenesis of staphylococcal infections in HIV-infected patients. Recently, Zervou and colleagues2 conducted a meta-analysis and showed that individuals with HIV infection are frequently colonized with MRSA. The overall estimated prevalence was 6.9% (95% CI, 4.8%–9.3%) and varied between geographic regions, from 1.0% in Europe to 8.8% in North America. The pooled prevalence rate was 5.8% (95% CI, 2.8%–9.8%) in the Asian HIV-infected population.2 Information about MRSA colonization amongst HIV-infected populations may be useful for implementation of effective strategies to prevent staphylococcal infections.

The prevalence of AIDS cases in mainland China has grown steadily in the period of 2004 to 2013, with a 10-fold increase.3 Unfortunately, the study by Zervou et al2 did not provide prevalence data specifically about MRSA colonization rates among HIV-infected individuals in China.

Currently, strains of MRSA are the most prevalent nosocomial pathogen in China, and several reports have shown that this is an increasing trend.1,5 One surveillance study performed in China showed that 63% of S. aureus isolates were MRSA, including 77% of nosocomial isolates and 43% of community isolates.5 Dissemination of virulent MRSA clones among healthy persons in China may contribute to the presence of clinically significant MRSA infections in some locales,6 but few studies have described the epidemiology and prevalence of MRSA colonization in healthy Chinese individuals. Most studies have been conducted in inpatients, especially in intensive care units.7 Only 3 published studies4,6,8 were found when we searched for the prevalence of MRSA colonization among healthy Chinese individuals using both English and Chinese databases (Table 1). We found that in mainland China the prevalence of MRSA colonization among healthy persons was higher than in other countries, including the United States9 and European countries.10 Such high MRSA colonization rates pose a significant challenge for MRSA prevention programs in China. Although there is no study investigating the prevalence of MRSA colonization in Chinese HIV-infected populations thus far, it seems reasonable to speculate that the prevalence will be high. There is a clearly a critical need to characterize the epidemiology of MRSA colonization/infection more fully in mainland China so that effective prevention programs can be implemented.

ACKNOWLEDGMENTS

Financial support. The National Natural Science Foundation of China (grant 81072367); and the Key University Science Research Project of Anhui Province (grant KJ2014ZD32).

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article.

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