Short Communication

Clostridium difficile PCR Ribotype 027 Emerges in Taiwan

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SUMMARY: *Clostridium difficile* PCR ribotype 027 is a hypervirulent strain that has caused significant nosocomial diarrhea in many countries but has not yet been reported or isolated in Taiwan previously. Here, we present the characteristics of a case of *C. difficile* PCR ribotype 027 identified in Taiwan. Taiwan is located in a key transportation center of Asia. This report is important for alerting hospitals and public health departments in Asia about the emergence of this hypervirulent strain so that close monitoring may be enacted to prevent potential outbreaks.

C. difficile is the most important etiological agent of nosocomial diarrhea. *C. difficile* infection (CDI) varies in its manifestation, ranging from asymptomatic colonization to mild diarrhea to severe life-threatening pseudomembranous colitis and death, mostly in elderly patients who have been exposed to antibiotics. In the early 2000s, morbidity and mortality resulting from *C. difficile*-associated diseases increased significantly in North America and Europe, particularly those associated with the hypervirulent ribotype 027 strain (1).

On August 18, 2013, an 83-year-old man presenting with severe watery diarrhea and septic shock was admitted to Taichung Veterans Hospital (TCVGH). He had a history of rheumatoid arthritis and had been treated with steroids. In 2012, he had suffered chronic obstructive pulmonary disease with recurrent bilateral pneumonia. After that, he was admitted to the respiratory care ward (RCW) of a local hospital for 14 months. On July 31, 2013, he developed fever without cough and accompanied by yellowish sputum, and ampicillin–sulbactam was administered. On August 5, he presented with severe watery diarrhea and abdominal distension. On August 18, he was then transferred to TCVGH because of persistent diarrhea, low blood pressure, and an elevated heart rate. He was admitted to the intensive care unit for further management.

After admission, antibiotic therapy was initiated with ceftriaxone. Piperacillin–tazobactam, erythromycin, and ciprofloxacin were also administered; however, the patient's fever and diarrhea persisted. Abdominal computed tomography scan revealed diffuse bowel wall swelling with mild loculated pancolitis. A sigmoidoscopic examination suggested pseudomembrane colitis. Bacterial cultures from a stool specimen were negative

for major enteropathogens, including *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Staphylococcus aureus*. Enzyme immunoassays were performed, and they confirmed the presence of *C. difficile* toxins A and B in the stool specimen. Stool samples were also plated on cycloserine-cefoxitin-fructose agar for *C. difficile* isolation. After 48 h, *C. difficile* colonies were identified using conventional biochemistry tests. The isolate was non-susceptible to moxifloxacin, and the minimum inhibitory concentration determined by E-test was more than 32 µg/ml. On the basis of the above test results, metronidazole was prescribed and the patient stabilized after treatment. By September 23, he was in a relatively stable condition and was transferred to the RCW of a local hospital for further care.

In order to investigate the genotypic characteristics of this isolate (CDI/Taiwan/TCVGH001), we performed a multiplex PCR assay, toxinotyping, and PCR-ribotyping according to the literature (2–4). The PCR-ribotyping amplified products were analyzed by capillary gel electrophoresis. Two *C. difficile* strains were used as a comparison control: the NAP1/027 reference strain, which was kindly provided by Dr. Brandi Limbago from the United States Centers for Disease Control and Prevention, and the CA08222 strain. The toxigenic type results demonstrated that this isolate was positive for both *tcdA* and *tcdB* (Fig. 1A). In addition, it also contained binary toxin genes (*cdtA* and *cdtB*) and was thus subjected to *tcdC* gene sequencing analysis. Sequencing revealed an 18-bp deletion and a single-nucleotide deletion at position 117, indicating an inactivating frameshift mutation in the *tcdC* gene of this isolate compared with the reference strain (ATCC43594) (Fig. 1C). The toxinotype of this strain was identified as type III (Fig. 1B). Finally, the capillary gel electrophoresis pattern of PCR-ribotyping identified it as the ribotype 027 reference strain NAP1/027 (Fig. 1D). We also imported the capillary gel electrophoresis results to the WEBRIBO database of AGES (<https://webribo.ages.at/>), which confirmed it was the ribotype 027 strain. To the best of our knowledge, this is the first *C. difficile* ribotype 027 strain isolated and identified in Taiwan.

CDI occurs by exposure to *C. difficile* or its spores via

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Fig. 1. Molecular typing of the *C. difficile* PCR ribotype 027 isolate in Taiwan. (A) The toxin genes profile. M: 100-bp DNA marker. (B) The RFLP of toxinotyping. Lane 1, 3, 5: CDI/Taiwan/TCVGH001; Lane 2, 4, 6: NAP1/027; M: DNA size marker. (C) The alignment of *tcdC* sequence revealed an 18-bp deletion and a single-nucleotide deletion in the isolate. (D) The PCR ribotyping. Dendrogram is based on UPGMA analysis of capillary electrophoresis-based PCR ribotyping.

the fecal-oral route. Asymptomatic carriage of *C. difficile* is considered the major source of transmission (5). The genotypic characteristic of the isolate in this study was identified with the typical ribotype 027 strain (1), including binary toxin genes (*cdtA* and *cdtB*), an 18-bp deletion in the *tcdC* gene, and fluoroquinolone (e.g., moxifloxacin) resistance. In this case, no travel history was related to the patient's infection; however, high risk factors for CDI, including hospitalization, antibiotic therapy, advanced age, and immunosuppression, were all evident in his medical history. Before CDI disease onset, the patient was hospitalized in another local hospital for more than 3 months. We suspected that the patient was infected with the ribotype 027 strain in this setting via asymptomatic carriers; however, evidence was lacking.

Initially, *C. difficile* ribotype 027 only caused severe outbreaks in North America and Europe during the early 2000s (1). Since 2005, this epidemic strain has been

detected in different Asian countries, including Japan, Korea, Hong Kong, Singapore, and Mainland China (in this order) (6–11). Notably, all these countries have the demographic characteristic of an aging population a well-known risk factor for CDI. In addition, most of the above countries are developed countries that have high-quality healthcare systems for delivering antibiotic treatment. Reporting of CDI to public health departments in Asia is not mandatory; however, routine diagnosis and surveillance of CDI are also lacking in most Asian countries. Consequently, the real epidemiological data are not yet available. A recent international study suggested that the prevalence of CDI is underestimated in Asia because of unregulated antibiotic use and unawareness of CDI (11). Taiwan, an island with well-developed public transportation, lies off the southeastern coast of Mainland Asia. Previous studies have indicated the ribotype 027 strain is associated with high virulence and dissemination; therefore, it is important for public

health departments and hospitals to be vigilant. In addition, surveillance of this emerging hypervirulent strain in Asia is imperative to avoid potential outbreaks.

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Conflict of interest None to declare.

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