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ORIGINAL ARTICLE

Molecular typing and epidemiology of *Clostridium difficile* in respiratory care wards of central Taiwan

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KEYWORDS
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Molecular typing;
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Prevalence

Background/purpose: In industrialized countries, *Clostridium difficile* is the major cause of nosocomial diarrhea. This study involved a broad overview of baseline epidemiology for *C. difficile* in Taiwan.

Materials and methods: Point prevalence was estimated from a prospective survey conducted in the respiratory care wards of six hospitals in central Taiwan. Polymerase chain reaction (PCR) ribotyping and multiple-locus variable-number tandem-repeat analysis (MLVA) were performed on all toxigenic *C. difficile* isolates, including asymptomatic and symptomatic strains.

Results: A total of 149 patients were screened for *C. difficile*; the point prevalence for *C. difficile* infection (CDI) and *C. difficile* colonization was 4% and 19%, respectively. CDI cases were significantly related to end-stage renal disease, and *C. difficile* colonization cases were significantly associated with previous admission to an acute-care facility. No hypervirulent PCR

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ribotype 027 strain was found. MLVA detected two clusters of CDI-related and three clusters of asymptomatic *C. difficile* strains circulating in wards.

Conclusion: Our results demonstrate a high prevalence of toxigenic *C. difficile* colonization in hospitals. Infection control personnel should pay attention to the increasing numbers of CDI cases, and molecular typing for *C. difficile* should be performed when necessary.

Introduction

Clostridium difficile is the major cause of nosocomial diarrhea and colitis in industrialized countries.[1,2] The spectrum of *C. difficile* infection (CDI) varies from asymptomatic carriage or mild diarrhea to severe colitis, and it may lead to megacolon, perforation, sepsis, and death.[3] Since 2002, several outbreaks of severe colitis, caused by the hypervirulent *C. difficile* strain NAP1/027, have been recognized in health-care facilities in Canada, the United States, and Europe[4,5]; the disease is highly associated with advanced age, antacid use, antibiotic use, and increased length of hospital stay.[6]

A few epidemiologic studies on *C. difficile* have been conducted in Taiwan. In 2003, a 4-month prospective study of intensive care unit (ICU) and infection wards found that the average incidence of CDI was 1/1000 patient days.[7] During 2007 and 2008, a 14-month retrospective survey showed that the overall incidence in a southern medical center was 4.3/10,000 patient days.[8] Recently, a prospective study showed the average incidence in a northern medical center to be 4.5/10,000 patient days, and indicated a downward trend in CDI incidence.[9] However, these results are limited to single hospitals and may not reflect the overall epidemiology in Taiwan. In addition, no suitable molecular typing method was applied to detect the endemic and/or epidemic strains.

To determine the burden of *C. difficile* on multiple long-term respiratory care wards (RCWs), the baseline prevalence of both colonizing and infectious *C. difficile* strains was estimated. Furthermore, polymerase chain reaction (PCR) ribotyping, a classical method used to represent the virulent strain of *C. difficile*,[10] was performed on all *C. difficile* isolates to detect the possible existence of the hypervirulent *C. difficile* strain NAP1/027. Multiple-locus variable-number tandem-repeat analysis (MLVA) typing, one of the most discriminatory methods used for the molecular analysis of *C. difficile* strains,[11] was used to detect *C. difficile* clusters in wards. In conclusion, we present the epidemiology of *C. difficile* in Taiwan, and provide evidence for the presence of several asymptomatic and CDI-related endemic strains in hospitals.

Materials and methods

Patients, specimens, and facility

This research, including the collection of demographic and clinical patient data and stool specimens, was approved by the Institutional Review Boards at each investigative site. Only those patients who were admitted to wards for more than 48 hours were considered to be hospital-associated patients, as recommended by the *Ad Hoc Clostridium difficile* Surveillance Working Group.[12] Information was collected on patient age, sex, date of birth, date of admission, ward of acquisition, clinical symptoms, underlying disease, and clinical treatment. We gathered this information through consultation with the physician or nurses in charge.

RCWs of six hospitals in central Taiwan were involved in this project. Stool specimens from each RCW were collected over 3 days between April 21, 2009 and October 23, 2009. All stool specimens were transported using anaerobic transport swabs and were delivered within 24 hours to the central-region laboratory at the Centers for Disease Control in Taiwan for isolation of *C. difficile*. Facility data (such as location, type, and number of licensed beds) were obtained from the website of the Bureau of National Insurance, Taiwan Department of Health (http://www.nhi.gov.tw/AmountInfoWeb/).

Definitions

A *C. difficile* strain was confirmed to be toxigenic by a positive toxin test and/or toxigenic type determined by PCR. Diarrhea was defined as $\geq$3 unformed stools per 24-hour period.[12] CDI was defined as the presence of diarrhea, in combination with a positive report for toxigenic *C. difficile* and a negative culture for *Salmonella* species, *Shigella* species, and *Staphylococcus aureus*. *C. difficile* colonization was defined as a positive toxin test for *C. difficile*, regardless of the presence of diarrhea. Previous acute-care admission was defined as hospitalization in an ICU for 2 weeks within 1 year. Antibiotic use was defined as any antibiotic treatment for 1 week within the past 3 months.

DNA preparation

Genomic *C. difficile* DNA was purified using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Genomic DNA isolated from *C. difficile* was then used for PCR amplification of variable-number tandem-repeat sequences and PCR ribotyping.

Isolation and determination of *C. difficile*

All stool specimens were cultured on cycloserine cefoxitin fructose agar (Oxoid Ltd, Hampshire, UK) and were