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Clostridium difficile carriage in healthy pregnant women in China

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ABSTRACT

Infection with *Clostridium difficile* has been shown to have particularly poor outcomes for pregnant women, including an increased risk of death. The purpose of this study was to investigate the prevalence, genotypic distribution, and characterization of *C. difficile* strains isolated from pregnant women without diarrhea in China. As part of this study, 3.7% (37 out of 1009) of samples acquired from pregnant females tested positive for *C. difficile*. Of these positive samples, 27.0% (10) were toxigenic isolates containing both toxin A and toxin B genes (A+B+), 13.5% (5) of the variant strains contained the toxin B gene (A−B+) only, while the rest were non-toxigenic isolates (59.5%, 22 isolates). Among the non-pregnant women without diarrhea tested, 1.4% (9 of 651) contained toxigenic isolates (all of which were A+B+). Sixteen different sequence types (STs) were isolated during the course of this study. ST-37 (ribotype 017) and ST-54 (ribotype 012) were the most frequent toxigenic types observed in pregnant women. All strains showed susceptibility to the antibiotics metronidazole and vancomycin. The resistance rates of toxigenic *C. difficile* strains isolated from pregnant females to clindamycin, erythromycin, moxifloxacin, levofloxacin, and rifampicin were 20%, 46.7%, 13.6%, 46.7% and 13.3%, respectively. There was no significant difference between resistance rates of toxigenic and non-toxigenic strains with respect to their susceptibility to these antibiotics. However, when compared with the same data from non-pregnant women, toxigenic strains from pregnant women showed lower resistance rates to clindamycin ($P < 0.05$).

1. Introduction

Clostridium difficile (CD) is a common cause of infectious diarrhea in hospitalized patients, and increased numbers of *C. difficile* infections (CDI) have become a growing concern in healthcare [1]. Previous studies have suggested that elderly patients (older than 65 years of age) with recent antibiotic exposure and recent hospitalization (within 8 weeks) were at greater risk of infection than their healthy counterparts [2]. However, severe cases of CDI have also been reported in young adults, such as pregnant women, who have historically been deemed low risk with respect to the development of CDI [3]. Indeed, in 2005, acute cases of CDI in pregnant women were reported in the US, re-enforcing the necessity for surveillance in order to better understand the changing epidemiology of CDI [4]. The factors underlying the shift in the epidemiology of CDI in pregnant women are unknown. Traditionally, symptomatic CDI patients were considered the primary reservoir for *C. difficile* transmission because they shed greater numbers of *C. difficile* bacterial cells in their stool compared to asymptomatic carriers [5]. However, published studies indicate that only one third or fewer of new CDI cases in hospital environments can be attributed to transmission from other symptomatic CDI patients [6]. Therefore, it is possible that asymptomatic carriers are an important source of *C. difficile* transmission [7]. In previous reports, intestinal *C. difficile* carriage rates in healthy adults ranged from 0 to 17.5% [8]. However, there is a paucity of research regarding *C. difficile* carriage in pregnant women.

Given the potential role for asymptomatic carriers in *C. difficile* transmission and CDI, it is important to understand the

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epidemiology of asymptomatic carriage. The purpose of this study is to survey the carriage rate of toxigenic *C. difficile* among women hospitalized for delivery in a maternity hospital in China. Epidemiological surveillance by *C. difficile*-specific cultivation methods was followed by detection of toxin genes, antibiotic susceptibility testing, and multilocus sequence typing (MLST) of the strains that were encountered upon routine analysis of fecal samples from pregnant women. We have also studied the prevalence of asymptomatic fecal carriage of *C. difficile* in healthy non-pregnant women and compared the data with that obtained for pregnant women.

Our survey was categorized as an "active surveillance study" and therefore did not require ethical approval. None of the test results were used to alter individual care. Each of the participants submitted demographic data anonymously.

2. Methods

2.1. Clinical setting and patient inclusion

The study was conducted at the Women's Hospital School of Zhejiang University (Hangzhou, China), which is a teaching hospital with 1000 beds spread across 30 wards. In this study, 1009 pregnant women (with an age range of 25–35 years of age, median 29 years) were examined for intestinal carriage of CD following culture of stool samples. None of the pregnant women were exposed to antimicrobial agents for at least eight weeks prior to initiating the study. The study was performed between September 1st 2012 and December 31st 2013. Stool samples were collected when patients first entered the maternity wards prior to delivery. Stool samples were also collected from a control group composed of 651 healthy non-pregnant women. Exclusion criteria for the study included: chronic constipation or diarrhea; a history of CDI; recent or anticipated hospitalization or surgery; and employment involving direct patient contact in a health care facility.

Our survey was categorized as an "active surveillance study" and therefore did not require ethical approval. None of the test results were used to alter individual care. Each of the participants submitted demographic data anonymously.

In order to characterize the pregnant women who took part in this study, medical charts were reviewed for epidemiologic and clinical features such as age, previous hospitalizations, antibiotic exposure, white blood cell counts, serum albumin, and creatinine.

2.2. Collection of C. difficile isolates

Stool specimens were diluted with alcohol to a final concentration of 75%. The diluted samples underwent spore selection before anaerobic isolation of *C. difficile* was performed using cycloserine–cefoxitin–taurocholate agar (Oxoid Ltd., Cambridge, UK), supplemented with 7% sheep's blood. Cultures were incubated at 35 °C for 48 h. Strains were identified by analyzing colony morphology features (flat, yellow, ground-glass appearance) and were confirmed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using the Microflex LT system (Bruker Daltonik GmbH, Bremen, Germany).

2.3. Characterization of Clostridium difficile

The protocol used for DNA isolation has been described in detail before [9]. All strains were tested for the presence of the *tcd*A and *tcd*B genes by PCR as described by Kato et al. [10]. The presence of both binary toxin genes, *cdt*A and *cdt*B, was detected as described by Stubbs et al. [11].

MLST was performed on seven housekeeping genes (*adk*, *atpA*, *dxr*, *glyA*, *recA*, *sodA* and *tpi*) for each isolate as described previously by Griffiths et al. [12]. Briefly, the amplification conditions were 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 40 s, and 72 °C for 70 s, with a final extension at 72 °C for 5 min and storage at 15 °C. The DNA sequences attained were submitted to the MLST database homepage (http://pubmlst.org/cdifficile/) to obtain the sequence type (ST).

Isolates were subcultured on sheep blood agar prior to susceptibility testing. Bacteria were suspended in tryptic soy broth to a McFarland standard of 1–1.5 for susceptibility testing by Etest on Brucella agar (Oxoid Ltd., Cambridge, UK) supplemented with 5% sheep blood according to the manufacturer's instructions. *C. difficile* ATCC 700057 was used as a control. The following agents were assessed as part of the E test: metronidazole, vancomycin, clindamycin, erythromycin, linezolid, moxifloxacin, levofloxacin, and rifampicin. The breakpoints used were 8 mg/L for erythromycin, clindamycin, and the fluoroquinolones, and 32 mg/L for metronidazole, in accordance with the Clinical and Laboratory Standards Institute (CLSI) interpretative categories approved for anaerobic bacteria [13]. The breakpoints used for rifampicin, linezolid, and vancomycin were >0.004 mg/L, 4 mg/L and >2 mg/L, respectively, according to the European Committee on Antimicrobial Susceptibility Testing [14].

2.4. Statistical analysis

The data were processed and univariate analyses were performed using SPSS v. 20 software (IBM Corp., Armonk, NY, USA). Odds ratios and their 95% confidence intervals are presented for categorical variables. The Student's *t*-test was used to compare differences between mean values, and χ^2 analysis was used to compare proportions. $P < 0.05$ was considered statistically significant.

3. Results

In total, 1009 healthy pregnant women (age range, 25–35 years; median, 29 years) and 651 healthy non-pregnant women (age range, 21–42 years; median, 31 years) were examined for intestinal carriage by *C. difficile* following stool culture. None of the subjects had a history of diarrhea or had been administered antimicrobial agents for at least four weeks prior to examination.

All 1009 stool samples submitted were considered formed and none were liquid (type 6 or 7 according to the Bristol Stool Chart). From the pregnant female stool samples, 37 strains of *C. difficile* were isolated and identified, including 15 toxigenic strains. The overall carriage rate for *C. difficile* was 3.7%, with a 1.5% carriage rate for toxigenic strains. Among the toxigenic strains, five contained the *tcd*B gene (A–B+) only, while 10 were positive for both toxin A and B (A+B+) based on PCR results. In non-pregnant females, nine toxigenic strains (1.4%) were identified, whilst no non-toxigenic strains were observed. Each of the nine toxigenic strains was positive for both toxins A and B genes (A+B+). Among these isolated strains, no strain was detected with binary toxins.

Factors associated with *C. difficile* carriage in pregnant women were evaluated by comparing carrying residents with those who did not carry the bacterium. There was no apparent association between carriage and; age, WBC count, platelet count, albumin level, hemoglobin, and renal function.

Each *C. difficile* strain was analyzed by MLST and the strains were divided into 16 different STs. According to Stabler et al., each of the ST types were converted to ribotypes where ribotyping information was available [15]. Toxigenic strains isolated from pregnant women were categorized into eight different STs, including ST-2 (ribotype 014/020) (2), ST-3 (ribotype 001/009) (2), ST-8 (ribotype 002) (1), ST-35 (ribotype 002/046) (1), ST-37 (ribotype 017) (4), ST-54