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C.difficile (including epidemiology)

Impact of the NAP-1 strain on disease severity, mortality, and recurrence of healthcare-associated *Clostridium difficile* infection

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ABSTRACT

Objectives: Studies are conflicting regarding the association of the North American pulsed-field gel electrophoresis type 1 (NAP1) strain in *Clostridium difficile* infection (CDI) and outcomes. We evaluated the association of NAP1 with healthcare-associated CDI disease severity, mortality, and recurrence at our academic medical center.

Methods: Healthcare-associated CDI cases were identified from November 1, 2011 through January 31, 2013. Multivariable regression models were used to evaluate the associations of NAP1 with severe disease (based on the Hines VA severity score index), mortality, and recurrence.

Results: Among 5424 stool specimens submitted to the Clinical Microbiology Laboratory, 292 (5.4%) were positive for *C. difficile* by polymerase chain reaction (PCR) on or after hospital day 4; 70 (24%) of these specimens also tested positive for NAP1. During the study period, 247 (85%) patients had non-severe disease and 45 (15%) patients had severe disease. Among patients with non-severe disease, 65 (26%) had NAP1 and among patients with severe disease, 5 (11%) had NAP1. After controlling for potential confounders, NAP1 was not associated with an increased likelihood of severe disease (adjusted odds ratio [aOR] = 0.35; 95% confidence interval [CI], 0.13–0.93), in-hospital mortality (aOR = 1.02; 95% CI, 0.53–1.96), or recurrence (aOR = 1.16, 95% CI, 0.36–3.77).

Conclusions: The NAP1 strain did not increase disease severity, mortality, or recurrence in this study, although the incidence of NAP1-positive healthcare associated-CDI was low. The role of strain typing in outcomes and treatment selection in patients with healthcare-associated CDI remains uncertain.

1. Introduction

Clostridium difficile has become one of the most common causes of healthcare-associated infection in the United States, with attributable excess costs totaling as much as $4.8 billion for acute care facilities alone [1]. Importantly, increases in the incidence and severity of *Clostridium difficile* infection (CDI) have been reported in the past decade and have been attributed to the emergence of a "hypervirulent" strain, identified as North American pulsed-field gel electrophoresis type 1 (NAP1/BI/027 (NAP1)) [2–5]. Currently, there is lack of consensus on the importance of NAP1 on patient outcomes because of inconsistent association between NAP1, disease severity, and recurrence. Some studies suggest that CDI caused by NAP1 is associated with severe disease, mortality, and recurrence [3–5]; however, other studies have reported no associations [6–8]. These studies used a variety of different measures to define disease severity and/or involved small sample sizes. The lack of consistent results has presented a significant challenge for antimicrobial stewardship programs (ASPs) when evaluating new rapid diagnostic technologies for CDI and applying them to "real world"

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patient care. The purpose of this study was to determine if NAP1 as detected by the Xpert® *C. difficile* Epi assay correlates with severe healthcare-associated CDI. Secondary objectives were to determine whether NAP1 is associated with [1] in-hospital mortality or [2] CDI recurrence.

2. Methods

The Xpert® *C. difficile* Epi (Cepheid, Sunnyvale, CA, USA) assay for the detection of *C. difficile* was implemented in our institution in January 2011. This assay is a qualitative *in vitro* diagnostic test for the rapid detection of toxin B gene sequences and for the presumptive identification of 027/NAP1/BI strains of toxigenic *C. difficile* [9]. The presumptive identification generated by the system was used to classify patients into NAP1 and non-NAP1 subgroups as further strain typing of isolates was not performed. We conducted a retrospective cohort study of all hospitalized adult patients with healthcare-associated CDI (on or after hospital day 4) defined by $\geq$ 3 unformed stools in a 24 h period and a positive polymerase chain reaction (PCR) for *C. difficile* from November 1, 2011 through January 31, 2013 at The Ohio State University Wexner Medical Center (OSUWMC), a 1367-bed-tertiary-care academic medical center located in Columbus, OH. Healthcare-associated CDI was independently reviewed and diagnosed by an infectious diseases physician blinded to NAP1 results. The study was approved by the Office of Responsible Research Practices Institutional Review Board, with a waiver of consent granted.

Patients with healthcare-associated CDI were stratified based on the presence of NAP1 strain and non-severe or severe disease. Disease severity was determined based on the Hines VA severity score [10] given its predictive ability for more severe forms of CDI [11]. Patients with $\geq$3 points were considered to have severe CDI. One point each was given for fever (oral temperature $\geq$ 100.4 °F), presence of ileus (diagnosed by clinical or radiographic findings), systolic blood pressure (SBP) < 100 mmHg, white blood cell (WBC) $\geq$ 15,000/mm^3, or a single finding on computerized tomography (CT) scan (thickened colonic wall, colonic dilatation, or ascites). Two points were given for WBC $\geq$30,000/mm^3 or $\geq$2 findings on CT scan. Peak values for temperature and WBC count and minimum systolic blood pressure were evaluated within 48 h of the positive stool PCR. Comorbidities were collected based on the International Statistical Classification of Diseases and Related Health Problems version 9 (ICD-9) if they were noted any time prior to the positive PCR and included: cardiovascular disease, chronic respiratory disease, diabetes mellitus, renal failure, and malignancy [12,13]. Additional data collected included: age, sex, antibiotic use 90 days prior CDI, prior history of CDI within 8 weeks of index culture, length of hospitalization from admission to positive PCR for *C. difficile*, length of stay (LOS), intensive care unit (ICU) admission, infectious diseases (ID) consult, surgery consult, in-hospital mortality, recurrence within 90 days, and readmission within 90 days. Patients were excluded if they were <18 or >89 years of age, did not receive anti-CDI antibiotic therapy, were diagnosed with CDI as an outpatient, or were incarcerated.

2.1. Statistical analysis

Data are presented as number (%) or median [minimum-maximum]. P-values describing demographics and clinical characteristics were determined by Pearson's chi-square test or Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Individual multivariable models were built to explore predictors of severe disease, in-hospital mortality, and recurrence using logistic regression via a backwards-stepwise approach. Consideration for multivariable model inclusion was based on a two-tailed *P* value $\leq$ 0.20 on univariate analysis. *P* value of $\leq$0.05 was considered statistically significant in the final model. As presence of the NAP1 strain was the focus of this study, NAP1 status was forced into all multivariable models regardless of significance on univariate analysis. All multivariate models were performed with and without the inclusion of NAP1 in order to assess its effect on other independent predictors. Model goodness of fit was assessed via the area under the curve of the receiver operating characteristic (AUC-ROC). Collinearity was assessed via tolerance and variance and variance inflation factor. All analyses were performed using SPSS Version 22 (IBM Corporation, Armonk, NY).

3. Results

Of 5424 stool specimens, 292 (5.4%) were positive for healthcare-associated *C. difficile* by polymerase chain reaction (PCR) on or after hospital day 4; 70 (24%) of these specimens were also reported as positive for NAP1. Demographics and clinical characteristics are presented in Table 1. Patients with NAP1 CDI were more likely to have chronic respiratory disease or WBC count <15,000/mm^3 and less likely to have malignancy or an abdominal computed tomography scan (Table 1). There were no differences in age, sex, prior antibiotic use, prior CDI, or length of hospitalization from admission to positive PCR between NAP1 CDI and non-NAP1 CDI patients. Overall, 68 (23%) required ICU admission, 3 (1%) underwent a colectomy, and 23 (8%) died while in the hospital. More patients with NAP1 CDI received oral metronidazole while intravenous metronidazole and oral vancomycin use were more common in patients with non-NAP1 CDI. There were no differences in clinical outcomes between patients with CDI associated with NAP1 CDI compared to patients with non-NAP1 CDI.

3.1. Disease severity

Demographics and clinical characteristics for patients with NAP1 and non-NAP1 CDI stratified by disease severity are presented in Table 2. During the study period, 247 (85%) patients had non-severe disease and 45 (15%) patients had severe disease. Among patients with non-severe disease, 65 (26%) had NAP1 and among patients with severe disease, 5 (11%) had NAP1. In patients with non-severe, non-NAP1 CDI, the incidence of malignancy was higher while chronic respiratory disease was lower. More patients with NAP1 CDI received oral metronidazole treatment and fewer received oral vancomycin. For those with non-severe disease, the length of stay was 2 days shorter in patients with non-NAP1 CDI (17 (2–71) vs. 19 (5–108) days, *P*=0.04). Among patients with severe disease, the use of penicillin antibiotics in the 90 days prior to admission was significantly higher in patients with NAP1 CDI. Length of stay for patients with severe, non NAP1 CDI was more than double that of patients with severe NAP1 CDI. Candidate variables associated with severe CDI upon univariate analysis are shown in Table 3. After multivariable analysis, only the presence of a cardiovascular comorbidity and NAP1 strain were retained in the model, with only NAP1 strain being significantly associated with a decreased odds of severe CDI (aOR 0.35, 95% CI 0.13–0.93, *P*=0.036). The AUC-ROC for this model was 0.631.

3.2. Mortality

Sixty-two deaths occurred while patients were hospitalized. NAP1 was not significantly more common among those who died than those who survived (21.4% vs 21.2%, *P*=1.00) (Table 1). When stratified by disease severity, there were no significant differences in mortality between NAP1 and non-NAP1 strains (Table 2). There