Clinical utility of laboratory detection of *Clostridium difficile* strain BI/NAP1/027

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Running Head: Clinical utility of detection of BI/NAP1/027

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Abstract

*C. difficile* strain BI/NAP1/027 is associated with increased *C. difficile* infection (CDI) rates and severity, and efficacy of some CDI therapies may be strain dependent. Although cultured *C. difficile* isolates can be reliably subtyped by various methods, long turn-around times, cost and availability of strain typing preclude their routine use. Nucleic acid amplification tests identify BI/NAP1/027 rapidly from stool, but the emergence of closely related strains compromises test specificity. Although detection of epidemiologically significant pathogens is generally useful for infection control programs, specific data supporting use of rapid detection of BI/NAP1/027 as an infection control tool are still awaited.

Abstract Word Count: 99
Clostridium difficile is an anaerobic, spore-forming, and toxin-producing bacterium that causes a wide spectrum of gastrointestinal illness, ranging from asymptomatic colonization to mild diarrhea to fulminant, life-threatening colitis. (1) C. difficile infection (CDI), is now the most common healthcare-associated infection in the U.S. (2) In the U.S. alone, nearly 500,000 infections and 30,000 deaths are attributable to C. difficile annually, and more than 20% of healthcare-associated CDIs recur. (3) CDI increases healthcare expenditures by at least $1 billion in the U.S. each year. (4) Because of the profound and widespread burden of CDI on the U.S. healthcare system, the U.S. Centers for Disease Control and Prevention (CDC) recently classified CDI among the most serious immediate antibiotic resistant infectious “public health threats that require urgent and aggressive action.” (4)

Changes in CDI Clinical and Molecular Epidemiology

Since 2001, CDI epidemiology has changed dramatically. (1) In the early part of the last decade, significantly increased frequency of CDI was reported in the U.S. and Canada. In addition, although CDI was traditionally considered an infection primarily among elderly patients receiving care in hospitals and nursing homes, CDI was increasingly recognized as a cause of diarrheal illness in the community and among young healthy adults and children. The clinical spectrum of CDI was also evolving with CDI severity and frequency of recurrences becoming more common. Because of reports of increased frequency and severity of CDI in healthcare facilities, molecular investigation of these changes in CDI clinical epidemiology in North America revealed the emergence of an epidemic C. difficile strain known as BI by
restriction endonuclease analysis (REA), NAP1 by pulsed-field gel electrophoresis (PFGE), and 027 by polymerase chain reaction (PCR) ribotyping. The proportion of CDI caused by BI/NAP1/027 was 51% among 8 healthcare facilities in the U.S. between 2000 and 2003 (7) and 84% among 12 Quebec hospitals in 2004 (8). This strain was quite rare prior to this outbreak in North America, accounting for only 14 CDI cases among a database that included 6000 isolates collected prior to 2001 (7). Unlike previous strains in this lineage, epidemic BI/NAP1/027 demonstrated high-level fluoroquinolone resistance. Widespread use of fluoroquinolones likely contributed to the predominance of this strain in healthcare settings (1).

Emergence of BI/NAP1/027 as a Global Threat

Over the past 15 years, BI/NAP1/027 has spread worldwide (9). However, the prevalence of BI/NAP1/027 varies significantly among geographical regions. For example, prevalence of BI/NAP1/027 was >40% among 186 U.K. hospitals in 2007-2008 (10). However, among 106 laboratories in 34 European countries in 2008, the prevalence of BI/NAP1/027 was the sixth most common strain, accounting for only 5% of CDIs (11). More recent data from the C. difficile Ribotyping Network indicate that BI/NAP1/027 prevalence has decreased in the U.K., from 55% in 2007 to 21% in 2010, potentially because of efforts to reduce fluoroquinolone and cephalosporin use (12). BI/NAP1/027 remains a frequent cause of CDI in the U.S. In a recent U.S. study describing CDI across 10 distinct geographic regions in 2011, BI/NAP1/027 was the most commonly identified strain, causing 31% of healthcare facility-associated (HCFA) CDI and 19% of community-associated (CA) CDI (3).
C. difficile is a novel pathogen in that new strains, some of which cause HCFA outbreaks, are constantly emerging, probably from a large pool of strains in the environment and introduced to healthcare facilities by newly admitted patients.\(^{(13)}\)

When followed over time, hospitals demonstrate a changing predominance of C. difficile strains, some causing major outbreaks whereas others cause only a few CDIs.\(^{(14)}\)

BI/NAP1/027 is one of the longest lasting and most widely distributed C. difficile strains. However, as with other epidemic strains, such as REA group J (PCR ribotype 001), it is likely that the prevalence of BI/NAP1/027 will decline worldwide as is already evident in the UK and parts of Europe. As CDI molecular epidemiology shifts, the importance of rapid identification of BI/NAP1/027 will be less important, but the need to rapidly identify other emerging strains will increase.

Little is known about the molecular epidemiology of CDI in children. In a pediatric cohort developed from population-based CDI surveillance in 2010-2011 among 10 diverse U.S. geographic locations, BI/NAP1/027 was the most commonly identified strain. Of the 132 C. difficile isolates available, BI/NAP1/027 was identified in 26% of HCFA-CDIs and 22% of CA-CDIs.\(^{(15)}\) However, more recent molecular epidemiologic data from a single urban pediatric academic medical center in a city with high BI/NAP1/027 prevalence in adults suggests that BI/NAP1/027 is much less common. Restriction endonuclease analysis of all laboratory-identified cases of CDI in 2013 identified BI/NAP1/027 in only 1 child in the 117-patient cohort, and this patient had recurrent, non-severe CDI.\(^{(16)}\) In Canada, active surveillance for CDI at a single children’s hospital in Ontario from 2007-2012 identified BI/NAP1/027 among only 2 of 20 (10%) isolates undergoing strain typing.\(^{(17)}\) Based on these limited data,
BI/NAP1/027 seems to be less frequent in children, although additional investigation of the molecular epidemiology of pediatric CDI is needed.

Clinical Implications of CDI Caused by BI/NAP1/027

Several risk factors for acquisition of BI/NAP1/027 have been identified, many of which are risk factors for CDI in general, including advanced age, hospitalization, and exposure to fluoroquinolone and cephalosporin antibiotics.(18) The evidence suggesting an association between BI/NAP1/027 and severe CDI in adult patients are conflicting.(19) However, differences in study setting, CDI and BI/NAP1/027 prevalence, small study sample size, and the specific CDI outcomes assessed likely contribute to the discrepancies among previous studies. A recent U.S. study of patients identified from population-based CDI surveillance in 8 states comprehensively assessed the relationship between strain type and CDI severity.(19) In this study of 2057 CDI cases, after controlling for several confounding variables for CDI severity, BI/NAP1/027 was associated with severe disease (i.e., leukocytosis, ileus, toxic megacolon, or pseudomembranous colitis; adjusted odds ratio [AOR], 1.74; 95% confidence interval [CI], 1.36–2.22), severe outcome (i.e., intensive care unit admission, colectomy for CDI, or death within 30 days of CDI; AOR, 1.66; 95% CI, 1.09–2.54), and death within 14 days (AOR, 2.12; 95% CI, 1.22–3.68). Therefore, population-based investigation of CDI in a large patient cohort suggests that BI/NAP1/027 is indeed associated with worse clinical outcomes compared to non-BI/NAP1/027 strains.

Pathogenesis of BI/NAP1/027
After identification of the clonal expansion of BI/NAP1/027 in North America in the early 2000s, much attention was focused on identifying the pathogenesis of this particular strain. Like historical toxigenic strains, BI/NAP1/027 also expresses toxins A and B (encoded by \textit{tcdA} and \textit{tcdB}, respectively), the major \textit{C. difficile} virulence factors. However, BI/NAP1/027 uniquely demonstrates high-level fluoroquinolone resistance, expression of a novel binary toxin (encoded by \textit{cdtA} and \textit{cdtB}), and an 18 bp deletion in \textit{tcdC}, a gene in the \textit{C. difficile} pathogenicity locus that encodes a negative regulator of \textit{tcdA} and \textit{tcdB}.\textsuperscript{(1)} This 18 bp deletion was previously postulated to impact function of \textit{tcdC}. However, subsequent genomic sequence analyses of BI/NAP1/027 strains demonstrated a single bp deletion in nucleotide position 117 (\textit{tcdC}Δ117) that results in a frameshift mutation and a truncated and non-functional \textit{tcdC} protein.\textsuperscript{(20)} Thus, \textit{tcdC}Δ117, rather than the 18 bp deletion in nucleotide positions 330-347, likely leads to loss-of-function of \textit{tcdC}. Because \textit{tcdC} encodes the negative regulator of \textit{tcdA} and \textit{tcbB}, \textit{tcdC}Δ117 could lead to increased production of toxins A and B. However, restoration of intact \textit{tcdC} did not affect toxin A and B levels in a BI/NAP1/027 strain.\textsuperscript{(21)} The pathogenesis of severe CDI caused by BI/NAP1/027 has not been definitively delineated. Potential factors contributing to BI/NAP1/027 pathogenicity and transmission include enhanced sporulation (subsequently refuted),\textsuperscript{(22)} increased toxin A and B production,\textsuperscript{(1)} and presence of binary toxin.\textsuperscript{(23)}

**Laboratory Detection of BI/NAP1/027**

For most typing methods, DNA extraction from a bacterial isolate is required.\textsuperscript{(6)} Therefore, anaerobic stool culture must first be performed on a clinical stool specimen,
followed by DNA extraction from the isolate. Because these initial steps require several days, and because they may also require batch processing of specimens to save costs, these typing methods are not feasible to guide real-time treatment decisions for individual patients or healthcare facility infection control investigation. PCR ribotyping and PFGE are most commonly used for population-based CDI surveillance in the U.K. and U.S., respectively. In addition, REA has been used extensively in the U.S. for typing of C. difficile isolates. PCR ribotyping and PFGE suffer from lower discriminatory power and portability of typing data is limited. REA offers better discriminatory power, but portability of data is also limited and labor requirements are high. Genomic methods of isolate characterization, such as multilocus sequence typing (MLST), multilocus variable-number tandem-repeat analysis (MLVA), and whole genome sequencing (WGS), offer improved data portability, although experience with these technologies for C. difficile typing is considerably less than PCR ribotyping, PFGE, and REA. An important advantage of MLVA (and WGS) is the ability to delineate phylogenetic relationships among strains. WGS provides superior discriminatory power, and as WGS become less expensive and more widely available, its use for C. difficile may expand.

Cepheid (Sunnyvale, CA) and Nanosphere (Northbrook, IL) both offer commercially available nucleic acid amplification tests (NAATs) that can presumptively identify BI/NAP1/027 directly from clinical stool specimens with very short turn-around times (i.e., several hours). Like most C. difficile NAATs, both the Cepheid Xpert C. difficile/Epi assay and the Nanosphere Verigene assay detect tcdB (and the Verigene assay additionally detects tcdA). To presumptively identify BI/NAP1/027 directly from
clinical stool specimens, these 2 assays also detect a nucleotide sequence unique to one of the 2 binary toxin (cdt) genes and the tcdCΔ117.

Despite the commercial availability of these assays, investigation of their specificity and sensitivity for detection of BI/NAP1/027 is relatively limited. Carroll and colleagues reported data from their investigation of the Verigene assay on 1,875 clinical stool specimens collected from patients with diarrhea.(25) Of the 58 specimens that were presumptively identified as BI/NAP1/027 by the Verigene assay and also underwent PCR ribotyping, 53 (91%) were confirmed to be BI/NAP1/027. Of the 189 specimens that were presumptively negative for BI/NAP1/027 by the Verigene assay and also underwent PCR ribotyping, 188 (99%) were confirmed to be a non-BI/NAP1/027 strain.

Pancholi and colleagues performed the Xpert C. difficile/Epi assay on 250 clinical stool specimens collected from adults with diarrhea.(26) BI/NAP1/027 was presumptively diagnosed by the Xpert C. difficile/Epi assay in 9 of the 43 (21%) tcdB-positive specimens. The authors report that those 9 specimens were confirmed to be BI/NAP1/027 by PFGE, although PFGE data are not presented for any other stool specimens. Babady, et al. performed PCR ribotyping and WGS on 45 clinical diarrheal stool specimens that had first been tested by the Xpert C. difficile/Epi assay.(27) Of the 45 specimens, 13 (29%) were positive and 32 (71%) were negative for BI/NAP1/027 by the Xpert C. difficile/Epi assay. Using WGS, the NAAT results were confirmed in 42/45 (93%) specimens, suggesting excellent concordance.

An important issue complicating the use of NAATs for the diagnosis of BI/NAP1/027 is the emergence of genetically similar non-BI/NAP1/027 strains. For
example, REA group AF (PCR ribotype 244) was recently identified as a strain closely
related to BI/NAP1/027 that is also associated with severe CDI. (28) Because AF/244 is
both cdt- and tcdCΔ117-positive, it is presumptively identified as BI/NAP1/027 by
commercially available NAATs. (28) With use of WGS, Zhou et al. additionally identified
3 unique non-BI/NAP1/027 sequence types (ST-41 and 2 novel sequence types) that
are tcdA-, tcdB-, cdtA-, cdtB-, and tcdCΔ117-positive. (29) Although the stool specimens
from which these isolates were derived were not tested with a NAAT designed to
identify BI/NAP1/027, specimens with these genotypes would presumptively be
identified as BI/NAP1/027 with the commercially available NAATs. As WGS becomes
more widely available for C. difficile characterization, additional identification of similar
strains is likely to occur. Thus, identification of non-BI/NAP1/027 strains that are both
cdt- and tcdCΔ117-positive somewhat limits the specificity of commercially available
assays that presumptively identify BI/NAP1/027.

A new ultrasensitive quantitative digital enzyme-linked immunosorbent assay
(ELISA) for toxins A and B using a novel single molecule array (Simoa) has been
evaluated for detection of epidemic C. difficile strains. Because of antigenic differences
in toxin B between BI/NAP1/027 (as well as REA group BK [PCR ribotype 078] and
possibly AF/244) and other C. difficile strains, differential detection of toxin B from
BI/NAP1/027 was demonstrated using this ultrasensitive ELISA. (30) Although this assay
demonstrates promise for the detection of BI/NAP1/027, additional studies including a
larger and more diverse population of C. difficile strains are needed to determine the
sensitivity and specificity of this assay for the detection of BI/NAP1/027. This assay is
currently in clinical development and is not commercially available for CDI diagnosis.
Recognition of a healthcare facility CDI outbreak typically requires regular assessment of CDI rates that are ascertained through active CDI surveillance by infection prevention and control programs. An outbreak may not be recognized until rising CDI rates are documented over a time period of several months. Strain typing of C. difficile is invaluable in tracking transmissions in the healthcare setting. Thus, subsequent investigation of a potential CDI outbreak requires coordination with the microbiology laboratory to save clinical stool specimens from patients with CDI and coordinate stool culture and typing of the isolates. Because of slow turn-around time for traditional C. difficile typing methods that require culture of the organism, such transmissions are often only recognized after substantial delays. Real-time recognition of increased incidence of a pathogenic strain, such as BI/NAP1/027, irrespective of any change in the overall CDI rate, is at least in theory highly advantageous in detecting and instituting early aggressive infection control measures to reduce transmission. Although rapid detection of epidemiologically significant pathogens can be particularly useful for infection control programs, data supporting rapid detection of BI/NAP1/027 as an infection control tool are limited. Interestingly, data from a single healthcare facility utilizing a NAAT that rapidly identifies BI/NAP1/027 suggest that providers more frequently changed antibiotic therapy from metronidazole alone to vancomycin plus intravenous metronidazole when BI/NAP1/027 was identified.31 This treatment combination is recommended for severe, complicated CDI (also called fulminant CDI), rather than for a specific strain type causing CDI. The authors could not determine if the
treatment change was made as a result of reporting of the BI/NAP1/027 strain or because of the CDI severity, although the latter should guide the decision to use dual antibiotic therapy. Caution is advised in reporting of BI/NAP1/027 as it may result in inappropriate changes in treatment of CDI.

**Association between Treatment Efficacy and CDI Strain Type**

At this time, antibiotic therapy with metronidazole and/or vancomycin remain the primary treatment modalities for CDI. Limitations of current standard antibiotic therapies include treatment failure for severe CDI (particularly with metronidazole) and further perturbation of the intestinal flora, leading to an unacceptable rate of CDI recurrence. Therefore, much attention has been focused on understanding subsets of patients, such as those with CDI caused by specific strain types, who are likely to benefit from emerging CDI therapies.

Fidaxomicin is a novel macrocyclic antibiotic approved by the U.S. Food and Drug Administration (FDA) for treatment of CDI in adult patients (and currently in phase 3 trials for CDI in children). Fidaxomicin has potent bactericidal activity against \textit{C. difficile}. Because, unlike metronidazole and vancomycin, fidaxomicin has very little \textit{in vitro} activity against components of the intestinal microbiota thought to confer colonization resistance against \textit{C. difficile}, fidaxomicin potentially protects against subsequent CDI recurrences. Analysis of pooled data from 2 phase 3 fidaxomicin clinical trials in North America and Europe assessed treatment efficacy in a subset of patients with CDI caused by BI/NAP1/027. Patients with BI/NAP1/027 CDI had lower cure rates (214/247 [87%]) than those infected with non-BI/NAP1/027 strains (445/472...
[94%]; \( p < .001 \) after treatment with either vancomycin (\( p = .02 \)) or fidaxomicin (\( p = .007 \)). In those with CDI caused by BI/NAP1/027, recurrence rates were not statistically different (30/96 [31.3%] patients receiving vancomycin and 21/90 [23.3%] patients receiving fidaxomicin; \( p = .23 \)).(32) Fidaxomicin provided no benefit over vancomycin (a less expensive option) in patients with CDI caused by BI/NAP1/027. However, neither antibiotic was as effective against BI/NAP1/027 as it was against other C. difficile strain types. Therefore, these data suggest that ruling out BI/NAP1/027 as the cause of CDI in the clinical setting may support use of fidaxomicin in those patients.

New treatment modalities under investigation (i.e., antibiotics, biotherapeutics, vaccines, and passive antibodies) may or may not be associated with differences in treatment efficacy between BI/NAP1/027 and non-BI/NAP1/027 strains.(33) For example, monoclonal antibodies against toxins A (actoxumab [ACT]) and B (bezlotoxumab [BEZ]) were investigated in 2 phase 3 clinical trials of CDI in 2413 adult patients. Compared to the placebo group, lower rates of CDI recurrence were demonstrated both among subjects receiving an intravenous infusion of ACT/BEZ (15% vs. 27%, \( p < 0.0001 \)) and among those receiving BEZ alone (17% vs. 27%, \( p < .0001 \)). Among the subgroup of patients with CDI caused by BI/NAP1/027, compared to placebo, lower rates of CDI recurrence were demonstrated both among subjects receiving ACT/BEZ (12% vs. 34%) or BEZ alone (24% vs. 34%), although probabilities were not presented for this subgroup analysis.

Colonization with non-toxigenic strains of C. difficile (NTCD) has been demonstrated to protect against toxigenic C. difficile colonization and CDI in both humans and hamsters, including against BI/NAP1/027 strains.(34) Spores of NTCD
strain M3 (also known as VP20621) were investigated in a phase 2 clinical trial of 173 adults with CDI. (35) NTCD-M3 was associated with a significantly lower risk of CDI recurrence (13/43 [30%] patients receiving placebo vs. 14/125 [11%] patients receiving NTCD-M3; OR 0.28; 95% confidence interval 0.11-0.69; \( p = 0.006 \)). REA was performed on 72 isolates in this study, and 25% were identified as BI/NAP1/027. NTCD-M3 colonization rates and CDI recurrence rates were similar in patients with and without BI/NAP1/027.

**Summary**

BI/NAP1/027 is associated with increased CDI frequency, severity and complications. Because of possible infection control benefits, and because efficacy of various CDI treatment modalities may be strain dependent, there is interest in identifying BI/NAP1/027 in the clinical setting. The prevalence of BI/NAP1/027 significantly varies among geographical regions and patient subsets. Although currently available NAATs identify BI/NAP1/027 with reasonably high specificity, test performance may be compromised by false-positive test results from emerging closely related strains of yet unclear clinical significance. Although rapid detection of epidemiologically significant pathogens can be particularly useful for infection control programs, data supporting rapid detection of BI/NAP1/027 as an infection control tool are still awaited. Laboratories should consider rapid detection methods for BI/NAP1/027 primarily for epidemiologic purposes when identifying increased CDI frequency and/or severity.
References


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Antibiotics: Integrated Results of 2 Phase 3 Studies (MODIFY I and MODIFY II).
In: IDWeek; 2015 Oct 7-11; San Diego, CA.


Biosketch

Larry Kociolek, MD is the Associate Medical Director of Infection Prevention and Control at Ann & Robert H. Lurie Children’s Hospital of Chicago and Instructor of Pediatrics at Northwestern University Feinberg School of Medicine. Dr. Kociolek received his medical degree from St. Louis University School of Medicine, completed pediatrics residency and chief residency at the University of Chicago, and completed pediatric infectious diseases fellowship at Lurie Children’s. Dr. Kociolek’s academic interests are in the field of healthcare epidemiology, particularly the clinical and molecular epidemiology of *C. difficile* infection (CDI) in children. At Lurie Children’s, Dr. Kociolek has led quality-improvement initiatives to optimize CDI testing, transmission, and treatment. Dr. Kociolek is a member of the Society for Healthcare Epidemiology of America (SHEA) Pediatric Leadership Council and received the SHEA Trainee Award in 2013 in recognition of his work in pediatric healthcare epidemiology.
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<td>Restriction endonuclease analysis (REA)</td>
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