AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

MINIREVIEW

Clinical Utility of Laboratory Detection of *Clostridium difficile* Strain BI/NAP1/027

Larry K. Kociolek,[a,b] Dale N. Gerding[c,d]

Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA[a]; Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA[b]; Edward Hines, Jr., Veterans Affairs Hospital, Hines, Illinois, USA[c]; and Loyola University Chicago Stritch School of Medicine, Maywood, Illinois, USA[d]

***Clostridium difficile* strain BI/NAP1/027 is associated with increased *C. difficile* infection (CDI) rates and severity, and the efficacy of some CDI therapies may be strain dependent. Although cultured *C. difficile* isolates can be reliably subtyped by various methods, the long turnaround times, high cost, and limited 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.**

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 health care-associated infection in the United States (2). In the United States alone, nearly 500,000 infections and 30,000 deaths are attributable to *C. difficile* annually, and more than 20% of health care-associated CDIs recur (3). CDI increases health care expenditures by at least $1 billion in the United States each year (4). Because of the profound and widespread burden of CDI on the U.S. health care 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 United States and Canada. In addition, although CDI was traditionally considered an infection occurring 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 increased CDI severity and frequency of recurrences becoming more common.

Because of reports of increased frequency and severity of CDI in health care facilities, molecular investigation of these changes in CDI clinical epidemiology in North America revealed the emergence of an epidemic *C. difficile* strain identified as BI by restriction endonuclease analysis (REA), NAP1 by pulsed-field gel electrophoresis (PFGE), and 027 by PCR ribotyping (1, 5, 6). The proportions of CDI caused by BI/NAP1/027 were 51% among 8 health care facilities in the United States 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 those in a database that included 6,000 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 health care 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, the prevalence of BI/NAP1/027 was >40% among 186 United Kingdom hospitals in 2007 to 2008 (10). However, among 106 laboratories in 34 European countries in 2008, 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 United Kingdom, 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 United States. 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 health care facility-associated CDIs (HCFA-CDIs) and 19% of community-associated CDIs (CA-CDIs) (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 health care facilities by newly admitted patients (13). When the incidence of infection was followed over time, hospitals demonstrated a changing predominance of *C. difficile* strains, some of which have caused major outbreaks whereas others have caused 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 strains (PCR ribotype

Accepted manuscript posted online 28 October 2015

Citation Kociolek LK, Gerding DN. 2016. Clinical utility of laboratory detection of *Clostridium difficile* strain BI/NAP1/027. J Clin Microbiol 54:19–24. doi:10.1128/JCM.02340-15.

Editor: C. S. Kraft

Address correspondence to Larry K. Kociolek, lkociolek@luriechildrens.org.

001), it is likely that the prevalence of BI/NAP1/027 will decline worldwide, as is already evident in the United Kingdom and parts of Europe. As CDI molecular epidemiology shifts, the importance of rapid identification of BI/NAP1/027 will become 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 to 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 suggest that BI/NAP1/027 is much less common among children. 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 that patient had recurrent, nonsevere CDI (16). In Canada, active surveillance for CDI at a single children's hospital in Ontario from 2007 to 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 these are risk factors for CDI in general, including advanced age, hospitalization, and exposure to fluoroquinolone and cephalosporin antibiotics (18). The data suggesting an association between BI/NAP1/027 and severe CDI in adult patients are conflicting (19). However, differences in study settings, CDI and BI/NAP1/027 prevalences, small study sample sizes, 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 that study of 2,057 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 to 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 to 2.54), and death within 14 days (AOR, 2.12; 95% CI, 1.22 to 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 than 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 expresses toxins A and B (encoded by *tcdA* and *tcdB*, respectively), the major *C. difficile* virulence factors. However, BI/NAP1/027 uniquely demonstrates high-level fluoroquinolone resistance, expression of a novel binary toxin (encoded by *cdtA* and *cdtB*), and an 18-bp deletion in *tcdC*, a gene in the *C. difficile* pathogenicity locus that encodes a negative regulator of *tcdA* and *tcdB* (1). This 18-bp deletion was previously postulated to impact the function of *tcdC*. However, subsequent genomic sequence analyses of BI/NAP1/027 strains demonstrated deletion of a single base pair in nucleotide position 117 (*tcdCΔ117*) that results in a frameshift mutation and a truncated and nonfunctional *tcdC* protein (20). Thus, *tcdCΔ117*, rather than the 18-bp deletion in nucleotide positions 330 to 347, likely leads to loss of function of *tcdC*. Because *tcdC* encodes the negative regulator of *tcdA* and *tcbB*, *tcdCΔ117* could lead to increased production of toxins A and B. However, restoration of the intact *tcdC* gene did not affect toxin A and B levels in a BI/NAP1/027 strain (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) (22), increased toxin A and B production (1), and the presence of the binary toxin (23).

LABORATORY DETECTION OF BI/NAP1/027

For most typing methods, DNA extraction from a bacterial isolate is required (Table 1) (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 for guiding real-time treatment decisions for individual patients or health care facility infection control investigation. PCR ribotyping and PFGE are the methods most commonly used for population-based CDI surveillance in the United Kingdom and the United States, respectively. In addition, REA has been used extensively in the United States for typing of *C. difficile* isolates. PCR ribotyping and PFGE suffer from lower discriminatory power, and the portability of typing data is limited. REA offers better discriminatory power, but the portability of data is likewise limited and intensive labor is required. 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 researcher experience with these technologies for *C. difficile* typing is considerably less extensive than experience with PCR ribotyping, PFGE, and REA. An important advantage of MLVA (and WGS) is the ability to delineate phylogenetic relationships among strains (24). WGS provides superior discriminatory power, and as WGS become less expensive and more widely available, its use for detection of *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 turnaround times (i.e., several hours) (25–27). 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 1 of the 2 binary toxin (*cdt*) genes and the mutation at nucleotide 117 in the *tcdC* gene.

Despite the commercial availability of these assays, investigation of their specificity and sensitivity for detection of BI/NAP1/027 has been relatively limited. Carroll and colleagues reported data from their investigation of the Verigene assay

TABLE 1 Laboratory methods for identification of BI/NAP1/027[a]

Laboratory method(s)	Requirement for *C. difficile* isolation by culture?	Advantage(s)	Disadvantage(s)
Nucleic acid amplification tests	No	Rapid results; DNA primers for BI/NAP1/027 incorporated into the clinical CDI assay (i.e., minimal additional cost/labor)	False-positive results because of emerging closely related strains of as-yet-unknown clinical significance; do not identify emergence or expansion of new unrelated strains
REA	Yes	Highly discriminatory; widely published molecular epidemiology data for CDI	Laborious; limited portability of typing data
PFGE	Yes	Highly discriminatory; widely published molecular epidemiology data for CDI	Laborious; limited portability of typing data; less discriminatory than REA
PCR ribotyping	Yes	Less labor-intensive than REA and PFGE; widely published molecular epidemiology data for CDI	Less discriminatory than REA
MLVA	Yes	Highly discriminatory; can delineate phylogenetic relationships among strains	Laborious; molecular epidemiology data for CDI relatively limited
Multilocus sequence typing	Yes	Data portability	Less discriminatory than REA, PCR ribotyping, PFGE, and MLVA; molecular epidemiology data for CDI relatively limited
Whole-genome sequencing	Yes	Highly discriminatory; data portability	Requires specialized equipment and bioinformatics expertise; molecular epidemiology data for CDI relatively limited

[a] Data are from references 6, 24–26, and 27. CDI, *C. difficile* infection; REA, restriction endonuclease analysis; PFGE, pulsed-field gel electrophoresis; MLVA, multilocus variable-number tandem-repeat analysis.

using 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 non-BI/NAP1/027 strains.

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 (21%) of the 43 *tcdB*-positive specimens. The authors reported that those 9 specimens were confirmed to be BI/NAP1/027 by PFGE, although PFGE data were not presented for any other stool specimens. Babady et al. performed PCR ribotyping and WGS of 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* gene positive and *tcdCΔ117* positive, it has been 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 positive for *tcdA*, *tcdB*, *cdtA*, *cdtB*, and *tcdCΔ117* (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 positive for both *cdt* and *tcdCΔ117* 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 identification of toxins A and B that uses 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 that include larger and more-diverse populations 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.

PREVENTION AND CONTROL OF CDI IN HEALTH CARE FACILITIES

Recognition of a health care 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 health care setting. Thus, subsequent investigation of a potential CDI outbreak requires coordination with the microbiology laboratory to save clinical stool specimens collected from patients with CDI and coordination of stool culture and typing of the

isolates. Because of the long turnaround times for traditional *C. difficile* typing methods that require culture of the organism, such transmissions are often recognized only after substantial delays. Real-time recognition of an 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 infections 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 health care 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 CDI caused by a specific strain type. 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 therapies 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 is currently in phase 3 trials for CDI in children). Fidaxomicin has potent bactericidal activity against *C. difficile*. Because fidaxomicin, unlike metronidazole and vancomycin, has very little *in vitro* activity against components of the intestinal microbiota thought to confer resistance to colonization by *C. difficile*, fidaxomicin potentially protects against subsequent CDI recurrences. Analysis of pooled data from two 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%]; $P < 0.001$) after treatment with either vancomycin ($P = 0.02$) or fidaxomicin ($P = 0.007$). 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; $P = 0.23$) (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 the 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 between BI/NAP1/027 and non-BI/NAP1/027 strains in treatment efficacy (33). For example, monoclonal antibodies against toxins A (actoxumab [ACT]) and B (bezlotoxumab [BEZ]) (ACT/BEZ) were investigated in two phase 3 clinical trials of CDI in 2,413 adult patients. Compared to the results seen with the placebo group, lower rates of CDI recurrence were demonstrated both among subjects receiving an intravenous infusion of ACT/BEZ (15% versus 27%; $P < 0.0001$) and among those receiving BEZ alone (17% versus 27%; $P < 0.0001$). Among the members of 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% versus 34%) and among subjects receiving BEZ alone (24% versus 34%), although the probabilities were not presented for that subgroup analysis.

Colonization with nontoxigenic strains of *C. difficile* (NTCD) has been demonstrated to protect against toxigenic *C. difficile* colonization and CDI in both humans and hamsters, including protection against BI/NAP1/027 strains (34). Spores of NTCD strain M3 (NTCD-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 versus 14/125 [11%] patients receiving NTCD-M3) (OR, 0.28; 95% confidence interval, 0.11 to 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 the efficacy of various CDI treatment modalities may be strain dependent, there is interest in identifying BI/NAP1/027 in the clinical setting. The prevalences of BI/NAP1/027 significantly differ 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 as-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.

ACKNOWLEDGMENTS

L.K.K. has received research funding from Merck and Cubist as well as research supplies from Alere. D.N.G. holds patents for the prevention and treatment of CDI licensed to Viropharma/Shire and has served as an advisory board member for Actelion, Merck, Summit, and Rebiotix and as a consultant for Pfizer, MedImmune, DaVoltera, Sanofi Pasteur, and Viropharma/Shire.

REFERENCES

1. **Kelly CP, Lamont JT.** 2008. Clostridium difficile—more difficult than ever. N Engl J Med **359:**1932–1940. http://dx.doi.org/10.1056/NEJMra0707500.
2. **Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team.** 2014. Multistate point-prevalence survey of health care-associated infections. N Engl J Med **370:**1198–1208. http://dx.doi.org/10.1056/NEJMoa1306801.
3. **Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC.** 2015. Burden of Clostridium difficile infection in the United States. N Engl J Med **372:**825–834. http://dx.doi.org/10.1056/NEJMoa1408913.
4. **Centers for Disease Control and Prevention.** 2013. Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
5. **Killgore G, Thompson A, Johnson S, Brazier J, Kuijper E, Pepin J, Frost EH, Savelkoul P, Nicholson B, van den Berg RJ, Kato H, Sambol SP, Zukowski W, Woods C, Limbago B, Gerding DN, McDonald LC.** 2008. Comparison of seven techniques for typing international epidemic strains of Clostridium difficile: restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. J Clin Microbiol **46:**431–437. http://dx.doi.org/10.1128/JCM.01484-07.
6. **Huber CA, Foster NF, Riley TV, Paterson DL.** 2013. Challenges for standardization of Clostridium difficile typing methods. J Clin Microbiol **51:**2810–2814. http://dx.doi.org/10.1128/JCM.00143-13.
7. **McDonald LC, Killgore GE, Thompson A, Owens RC, Kazakova SV, Sambol SP, Johnson S, Gerding DN.** 2005. An epidemic, toxin-gene variant strain of Clostridium difficile. N Engl J Med **353:**2433–2441. http://dx.doi.org/10.1056/NEJMoa051590.
8. **Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, Rene P, Monczak Y, Dascal A.** 2005. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med **353:**2442–2449. http://dx.doi.org/10.1056/NEJMoa051639.
9. **He M, Miyajima F, Roberts P, Ellison L, Pickard DJ, Martin MJ, Connor TR, Harris SR, Fairley D, Bamford KB, D'Arc S, Brazier J, Brown D, Coia JE, Douce G, Gerding D, Kim HJ, Koh TH, Kato H, Senoh M, Louie T, Michell S, Butt E, Peacock SJ, Brown NM, Riley T, Songer G, Wilcox M, Pirmohamed M, Kuijper E, Hawkey P, Wren BW, Dougan G, Parkhill J, Lawley TD.** 2013. Emergence and global spread of epidemic healthcare-associated Clostridium difficile. Nat Genet **45:**109–113.
10. **Brazier JS, Raybould R, Patel B, Duckworth G, Pearson A, Charlett A, Duerden BI; HPA Regional Microbiology Network.** 2008. Distribution and antimicrobial susceptibility patterns of Clostridium difficile PCR ribotypes in English hospitals, 2007–08. Euro Surveill **13:**19000. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19000.
11. **Bauer MP, Notermans DW, van Benthem BHB, Brazier JS, Wilcox MH, Rupnik M, Monnet DL, van Dissel JT, Kuijper EJ.** 2011. Clostridium difficile infection in Europe: a hospital-based survey. Lancet **377:**63–73. http://dx.doi.org/10.1016/S0140-6736(10)61266-4.
12. **Wilcox MH, Shetty N, Fawley WN, Shemko M, Coen P, Birtles A, Cairns M, Curran MD, Dodgson KJ, Green SM, Hardy KJ, Hawkey PM, Magee JG, Sails AD, Wren MWD.** 2012. Changing epidemiology of Clostridium difficile infection following the introduction of a national ribotyping-based surveillance scheme in England. Clin Infect Dis **55:**1056–1063. http://dx.doi.org/10.1093/cid/cis614.
13. **Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CL, Golubchik T, Batty EM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH, Peto TE, Walker AS.** 2013. Diverse sources of C. difficile infection identified on whole-genome sequencing. N Engl J Med **369:**1195–1205. http://dx.doi.org/10.1056/NEJMoa1216064.
14. **Belmares J, Johnson S, Parada JP, Olson MM, Clabots CR, Bettin KM, Peterson LR, Gerding DN.** 2009. Molecular epidemiology of Clostridium difficile over the course of 10 years in a tertiary care hospital. Clin Infect Dis **49:**1141–1147. http://dx.doi.org/10.1086/605638.
15. **Wendt JM, Cohen JA, Mu Y, Dumyati GK, Dunn JR, Holzbauer SM, Winston LG, Johnston HL, Meek JI, Farley MM, Wilson LE, Phipps EC, Beldavs ZG, Gerding DN, McDonald LC, Gould CV, Lessa FC.** 2014. Clostridium difficile infection among children across diverse US geographic locations. Pediatrics **133:**651–658. http://dx.doi.org/10.1542/peds.2013-3049.
16. **Kociolek LK, Patel SJ, Shulman ST, Gerding D.** 2015. Molecular epidemiology of Clostridium difficile infections in children: a retrospective cohort study. Infect Control Hosp Epidemiol **36:**445–451. http://dx.doi.org/10.1017/ice.2014.89.
17. **Le Saux N, Gravel D, Mulvey MR, Dennis J, Yasseen AS, Barrowman N, Bowes J.** 2014. Pediatric Clostridium difficile infection: 6-year active surveillance in a defined patient population. Infect Control Hosp Epidemiol **35:**904–906. http://dx.doi.org/10.1086/676875.
18. **O'Connor JR, Johnson S, Gerding DN.** 2009. Clostridium difficile infection caused by the epidemic BI/NAP1/027 strain. Gastroenterology **136:**1913–1924. http://dx.doi.org/10.1053/j.gastro.2009.02.073.
19. **See I, Mu Y, Cohen J, Beldavs ZG, Winston LG, Dumyati G, Holzbauer S, Dunn J, Farley MM, Lyons C, Johnston H, Phipps E, Perlmutter R, Anderson L, Gerding DN, Lessa FC.** 2014. NAP1 strain type predicts outcomes from Clostridium difficile infection. Clin Infect Dis **58:**1394–1400. http://dx.doi.org/10.1093/cid/ciu125.
20. **Curry SR, Marsh JW, Muto CA, O'Leary MM, Pasculle AW, Harrison LH.** 2007. tcdC genotypes associated with severe TcdC truncation in an epidemic clone and other strains of Clostridium difficile. J Clin Microbiol **45:**215–221. http://dx.doi.org/10.1128/JCM.01599-06.
21. **Cartman ST, Kelly ML, Heeg D, Heap JT, Minton NP.** 2012. Precise manipulation of the Clostridium difficile chromosome reveals a lack of association between the tcdC genotype and toxin production. Appl Environ Microbiol **78:**4683–4690. http://dx.doi.org/10.1128/AEM.00249-12.
22. **Burns DA, Heeg D, Cartman ST, Minton NP.** 2011. Reconsidering the sporulation characteristics of hypervirulent Clostridium difficile BI/NAP1/027. PLoS One **6:**e24894. http://dx.doi.org/10.1371/journal.pone.0024894.
23. **Gerding DN, Johnson S, Rupnik M, Aktories K.** 2014. Clostridium difficile binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut Microbes **5:**15–27. http://dx.doi.org/10.4161/gmic.26854.
24. **Marsh JW, O'Leary MM, Shutt KA, Sambol SP, Johnson S, Gerding DN, Harrison LH.** 2010. Multilocus variable-number tandem-repeat analysis and multilocus sequence typing reveal genetic relationships among Clostridium difficile isolates genotyped by restriction endonuclease analysis. J Clin Microbiol **48:**412–418. http://dx.doi.org/10.1128/JCM.01315-09.
25. **Carroll KC, Buchan BW, Tan S, Stamper PD, Riebe KM, Pancholi P, Kelly C, Rao A, Fader R, Cavagnolo R, Watson W, Goering RV, Trevino EA, Weissfeld AS, Ledeboer NA.** 2013. Multicenter evaluation of the Verigene Clostridium difficile nucleic acid assay. J Clin Microbiol **51:**4120–4125. http://dx.doi.org/10.1128/JCM.01690-13.
26. **Pancholi P, Kelly C, Raczkowski M, Balada-Llasat JM.** 2012. Detection of toxigenic Clostridium difficile: comparison of the cell culture neutralization, Xpert C difficile, Xpert C difficile/Epi, and Illumigene C difficile assays. J Clin Microbiol **50:**1331–1335. http://dx.doi.org/10.1128/JCM.06597-11.
27. **Babady NE, Stiles J, Ruggiero P, Khosa P, Huang D, Shuptar S, Kamboj M, Kiehn TE.** 2010. Evaluation of the Cepheid Xpert Clostridium difficile Epi assay for diagnosis of Clostridium difficile infection and typing of the NAP1 strain at a cancer hospital. J Clin Microbiol **48:**4519–4524. http://dx.doi.org/10.1128/JCM.01648-10.
28. **Lim SK, Stuart RL, Mackin KE, Carter GP, Kotsanas D, Francis MJ, Easton M, Dimovski K, Elliott B, Riley TV, Hogg G, Paul E, Korman TM, Seemann T, Stinear TP, Lyras D, Jenkin GA.** 2014. Emergence of a ribotype 244 strain of Clostridium difficile associated With severe disease and related to the epidemic ribotype 027 strain. Clin Infect Dis **58:**1723–1730. http://dx.doi.org/10.1093/cid/ciu203.
29. **Zhou Y, Burnham CA, Hink T, Chen L, Shaikh N, Wollam A, Sodergren E, Weinstock GM, Tarr PI, Dubberke ER.** 1 October 2014. Phenotypic and genotypic analysis of Clostridium difficile isolates: a single-center study. J Clin Microbiol http://dx.doi.org/10.1128/JCM.02115-14.
30. **Pollock NR, Song L, Zhao M, Duffy DC, Chen X, Sambol SP, Gerding DN, Kelly CP.** 2015. Differential immunodetection of toxin B from highly

virulent Clostridium difficile BI/NAP-1/027. J Clin Microbiol **53:**1705–1708. http://dx.doi.org/10.1128/JCM.03419-14.

31. **Scardina T, Labuszewski L, Pacheco SM, Adams W, Schreckenberger P, Johnson S.** 2015. Clostridium difficile infection (CDI) severity and outcome among patients infected with the NAP1/BI/027 strain in a non-epidemic setting. Infect Control Hosp Epidemiol **36:**280–286. http://dx.doi.org/10.1017/ice.2014.45.
32. **Petrella LA, Sambol SP, Cheknis A, Nagaro K, Kean Y, Sears PS, Johnson S, Gerding DN.** 2012. Decreased cure and increased recurrence rates for Clostridium difficile infection caused by the epidemic C. difficile BI strain. Clin Infect Dis **55:**351–357. http://dx.doi.org/10.1093/cid/cis430.
33. **Wilcox M, Gerding D, Poxton I, Kelly C, Nathan R, Cornely O, Rahav G, Lee C, Eves K, Pedley A, Tipping R, Guris D, Kartsonis N, Dorr MB.** 2015. Bezlotoxumab (BEZ) alone and with actoxumab (ACT) for prevention of recurrent C. difficile infection (rCDI) in patients on standard of care (SoC) antibiotics: integrated results of 2 phase 3 studies (MODIFY I and MODIFY II), abstr 67. Abstr IDWeek, 7 to 11 October, San Diego, CA.
34. **Nagaro KJ, Phillips ST, Cheknis AK, Sambol SP, Zukowski WE, Johnson S, Gerding DN.** 2013. Nontoxigenic Clostridium difficile protects hamsters against challenge with historic and epidemic strains of toxigenic BI/NAP1/027 C. difficile. Antimicrob Agents Chemother **57:**5266–5270. http://dx.doi.org/10.1128/AAC.00580-13.
35. **Gerding DN, Meyer T, Lee C, Cohen SH, Murthy UK, Poirier A, Van Schooneveld TC, Pardi DS, Ramos A, Barron MA, Chen H, Villano S.** 2015. Administration of spores of nontoxigenic Clostridium difficile strain M3 for prevention of recurrent C. difficile infection: a randomized clinical trial. JAMA **313:**1719–1727. http://dx.doi.org/10.1001/jama.2015.3725.

Larry Kociolek 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 his pediatrics residency and chief residency at the University of Chicago, and completed a 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 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.