Changing Epidemiology of *Clostridium difficile* and Emergence of New Virulent Strains

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The epidemiology of *Clostridium difficile* infection (CDI) is constantly changing and is influenced by antibiotic usage patterns, healthcare patient environment, and emergence of new strains. Between 1982 and 1991, 4 outbreak periods were noted in 1 hospital in Minnesota, each marked by clusters of specific strains, designated by restriction endonuclease typing (REA) as groups B and K (both type 053 by polymerase chain reaction [PCR] ribotyping) [1]. In addition to these outbreaks, there was a background incidence of CDI due to a wide variety of *C. difficile* strains including 6 isolations of a REA group BI (ribotype 027) strain, a strain that would dramatically impact North America and Europe 2 decades later. In the 1990s, there were hospital CDI outbreaks in the United States due to a highly clindamycin-resistant strain (REA group J, ribotype 001) that was specifically associated with clindamycin use and which responded in several instances to restriction of that drug [2]. The strain with the most profound impact on CDI epidemiology, strain BI/027 (also designated NAP1 by pulsed-field gel electrophoresis typing), emerged in North America in 2000 and in Europe a few years later [3–5]. Whole-genome sequencing and phylogenetic analysis has shown that BI/027 strains compose 2 distinct lineages that emerged in North America in the early to mid-1990s shortly after acquisition of a fluoroquinolone resistance–conferring mutation and a related conjugative transposon [6]. Hospital and institutional outbreaks due to fluoroquinolone-resistant BI/027 strains were notable for unprecedented incidence and severity of the associated CDI cases.

In this issue of *Clinical Infectious Diseases*, Lim et al have documented a new outbreak strain in a multihospital setting in Melbourne, Australia, that, although not associated with a larger number of cases, was associated with increased severity and higher mortality among those infected compared with other circulating strains in these hospitals [7]. This strain was initially thought to be the BI/027 strain based on results of the Xpert *C. difficile* PCR assay (Cepheid, Sunnyvale, California) used as a diagnostic test on submitted stool specimens by the clinical laboratory. The Xpert *C. difficile* assay identifies presumptive 027 strains based on detection of the genes for toxin B (*tcdB*), binary toxin CDT (*cdt*), and a single base-pair (bp) deletion at position 117 in the gene coding for the anti-sigma factor TcdC (*tcdC*) [8]. When these stool specimens were cultured, and the recovered *C. difficile* isolates subjected to formal ribotype analysis, they turned out to be ribotype 244, not 027. Whole-genome sequencing analysis of strain 244 showed >10 000 single-nucleotide polymorphisms between this strain and a 027 strain, but placed 244 in the same clade as 027 and in agreement with results obtained earlier by microarray analysis [9].

Although the Xpert *C. difficile* PCR assay incorrectly identified this strain, it did identify a related strain and was associated with severe disease. All 3 of the targets for the Xpert assay are postulated to be virulence factors for the BI/027 strain, although none is conclusively demonstrated to be the factor responsible for the increased virulence associated with this strain. The single bp deletion at position 117 in *tcdC* results in a nonsense mutation and a premature stop codon and ultimately a truncated, nonfunctional TcdC protein. TcdC is a negative regulator of toxin A and toxin B production, and the lack of a functional TcdC in the BI/027 strains was postulated to be responsible for the increased production
of toxin A and B in these strains [10]. Toxin A and, in particular, toxin B are potent cytotoxins in vitro and are intimately involved in the pathogenesis of CDI [11, 12]. Regulation of toxin A and B production, however, is complex and involves other factors than TcdC. Despite the characteristic tcdC deletion, in vitro toxin B production by strain 244 was not elevated compared with the 027 comparison strain (Figure 4 in Lim et al) [7]. The authors further demonstrated that the toxin B produced by strain 244 was a variant toxin causing a different cytotoxic effect on Vero cells, and they speculated that in vitro toxin production might not mimic that which occurs in vivo.

The third target of the Xpert assay, cdt, indicates the potential for binary toxin CDT production. Binary toxin is unrelated to the large clostridial toxins A and B but is consistently present in the BI/027 strain and the BK/078 strain, another emerging strain with clinical evidence supporting increased disease severity [13, 14]. Ribotype 078 belongs to a distant clade, unrelated to the 027/244 clade, but was shown to be associated with similar disease severity to 027 cases in the Netherlands [13]. Ribotype 078 was, however, more likely to be community-associated and was genetically related to 078 strains recovered from pigs. Walker et al demonstrated in a large, population-based study in Oxfordshire that 14-day mortality was highest for CDI due to clade 5 (ribotype 078) and clade 2 (ribotype 027) strains [14]. Although the role of binary toxin CDT in C. difficile has been harder to demonstrate experimentally [15], new data suggest this toxin may play an important adjunctive role in the pathogenesis of CDI [16, 17].

What is the current status of the epidemiology of BI/027/NAP1? Although BI/027 outbreaks with marked disease severity occurred in various European countries in the early 2000s, recent data suggest that the prevalence of BI/027 has decreased significantly and that it is no longer a leading cause of CDI there [18]. Despite the overall decreased presence of BI/027 in Europe, clusters of severe disease due to 027 are still reported [19]. Data from the C. difficile Ribotyping Network enhanced surveillance system for CDI in England showed a decrease in the prevalence of ribotype 027 from 55% to 21% from 2007 to 2010 [20]. This trend also paralleled marked decreases in CDI rates and CDI mortality and a national initiative by the National Health Service targeting CDI. In the United States, BI/027 has remained the most common strain identified over a decade since outbreaks were first identified [3]. In 2009, BI/027 strains accounted for 61% of infections in 25 acute-care health facilities in the Chicago area [21]. Active, population-based CDI surveillance from the Centers for Disease Control and Prevention’s Emerging Infections Program indicate that between 2009 and 2011, NAP1 (BI/027) was the most common strain type, accounting for 28.4% of the cases; the next most common strain type accounted for <10% [22]. In multivariate analysis controlling for patient factors, healthcare exposure, and antibiotics, NAP1 was associated with greater odds of severe disease, severe outcome, and death within 14 days compared with other strains. The 14-day mortality of NAP1-associated CDI cases was 5.0% compared with 1.8% associated with other strains.

BI/027/NAP1 is still rarely identified in Australia, but ribotype 244 was also identified in New Zealand around the time of the outbreak in Melbourne [23]. CDI cases associated with 244 in New Zealand were also associated with severe disease, but 50% of the cases were community associated. The source and factors influencing emergence of ribotype 244 have been subject to speculation. Unlike BI/027, ribotype 244 strains are not marked by increased fluoroquinolone resistance, but there was no analysis of the specific antibiotic usage patterns precipitating CDI in these cases. The relatively high proportion of cases linked to nonhospital/institutional settings may suggest other environmental sources, but currently there is no “smoking gun.”

Ongoing surveillance will continue to be important to track the ever-changing epidemiology of this remarkably adaptable pathogen. A better understanding of C. difficile virulence determinants may also lead to more universal diagnostic markers of strains with increased virulence or transmission potential.

Notes

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