Host-Pathogen Interactions in *Clostridium difficile* Infection: It Takes Two to Tango

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(See the Major Article by See et al on pages 1394–400.)

Clostridium difficile infection (CDI) has reemerged as a major infectious disease in the 21st Century. The millennium's first decade witnessed an approximate doubling of hospital discharge diagnoses for CDI and nearly a 10-fold increase in mortality [1]. The burden of this infection on our health and welfare has been significant, with recent estimates that hospital-onset CDI contributes annually to more than 6000 deaths, 300 000 excess hospital-days, and no fewer than $850 million in added hospital costs [2]. Data from 2008 revealed nearly $4.8 billion in added expenses associated with CDI in US acute-care facilities [3]. This epidemic has exacted a disproportionate toll on older adults. Approximately 92% of CDI--related deaths occur in patients aged 65 or older, making CDI the 18th leading cause of death in this population [4]. In addition, in the United States, the annual rate of CDI-related hospitalizations per 100 000 people in patients ≥85 years of age was noted to exceed that of all other age groups combined [5]. The tremendous growth of CDI as a problem both within hospitals and community settings has motivated significant interest in identifying risk factors, particularly modifiable ones, which are important drivers of transmission, disease severity, and outcome.

In the wake of the dramatic rise in the incidence and severity of CDI, investigators soon identified a novel strain of *C. difficile* responsible for the new epidemic [6, 7]. The strain would become known by the methods used to type it: restriction endonuclease analysis (REA) type BI, North American Pulsed-field Gel Electrophoresis (NAP) type 1, or polymerase chain reaction (PCR) ribotype 027, hereafter referred to as the NAP1 strain. More recent studies have refined our understanding of the outbreak, revealing 2 distinct epidemic lineages of NAP1 strain, not one as previously thought, which emerged in North America and spread globally [8]. Studies to characterize this emergent pathogen identified features absent from usual, endemic (historical) *C. difficile* isolates: most notably fluoroquinolone resistance and features suggesting unique virulence characteristics [6, 9].

Initial studies of the epidemic *C. difficile* NAP1 isolates revealed that these strains expressed a binary toxin, not present in all disease-causing strains, and harbored genetic mutations in the *tcdC* gene, encoding a putative negative regulator of expression of the 2 major cytotoxins, tcdA and TcdB [7, 9]. What is more, in vitro studies documented greater expression of these cytotoxins by the NAP1 strains compared to historical isolates [9]. Subsequent investigations have attempted to further characterize unique virulence characteristics of the NAP1, both genotypically and phenotypically, expanding the concept that epidemic NAP1 strains are hypervirulent. Compared with historic, nonepidemic strains, the characteristics of hypervirulent strains include increased transmissibility [10], increased toxin production [9], enhanced sporulation [11], greater tcdB potency [12], expression of binary toxin (thought to assist in colonization) [13], and increased resistance to antimicrobials [6] and disinfectants [14]. The evidence in support of these unique virulence properties largely stems from in vitro and animal studies and is not without some contradictory results [15–17]. Data also suggest that not all contemporary NAP1 isolates are alike in their virulence potential, implying that genetic signatures other than the common typing methods might better identify hypervirulence [17, 18].

The *C. difficile* epidemic witnessed the emergence of NAP1 strains in an older and sicker patient population [4]. This simultaneous shift, in both pathogen and host, has made it challenging to attribute the rise in CDI severity solely to the pathogen itself (ie, adopting the

Received 21 February 2014; accepted 25 February 2014; electronically published 5 March 2014.

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Clinical Infectious Diseases 2014;58(10):1401–3

DOI: 10.1093/cid/ciu141

hypervirulence concept). Advanced age, frailty, and comorbidity are important risk factors for poor outcomes due to CDI [19], and this is the population most afflicted by the *C. difficile* NAP1 strains [20]. The tight epidemiological association between NAP1 *C. difficile* strains and geriatric populations has not been completely explained. It can be speculated that antibiotic use drove the emergence of the NAP1 strains [8] at a time when both the age and medical complexity of hospitalized patients has been increasing [21].

The extent to which enhanced virulence features of the NAP1 strains contribute to the increased severity and poor outcomes of CDI in elderly populations has been difficult to untangle, given the "hypersusceptibility" of older, institutionalized adults. Relatively large studies linking NAP1 strains to adverse disease outcome have not adjusted comprehensively for host factors known to influence disease severity, such as age, comorbidity, co-administered medications, and functional status [20, 22], resulting in ongoing debate regarding the importance of hypervirulence features to disease severity in older adults [23].

The new study by See and colleagues in this issue of *Clinical Infectious Diseases* improves our understanding of the potential contribution of strain type on disease severity in CDI and is a welcomed and important contribution [24]. This study was a large, retrospective review of cases collected through surveillance by the Centers for Disease Control and Prevention (CDC)'s Emerging Infections Program. In total, 2057 CDI cases were included, from which strains were available for typing by pulsed-field gel electrophoresis, with NAP1 being the most prevalent. There was a nearly even mix of healthcare- and community-associated cases. The authors applied univariate and multivariate logistic regression analyses to investigate the association between strain type and 3 separate outcome measures: severe CDI disease (defined by the development of ileus, toxic megacolon, or pseudomembranous colitis within 5 days of diagnosis, or serum white blood count $\geq$15 000 cells/mm^3 within 1 day of collection of the stool specimen), severe outcome (intensive care unit admission within 7 days after stool collection, colectomy for CDI, or death within 30 days of stool collection), and death within 14 days of infection [24].

The major finding of See and collaborators was that infection caused by NAP1 strains was associated with a significant increased risk for all 3 major endpoints: severe disease, severe outcome, and death [24]. These associations held even when adjusted for several important covariates, an important point, because there was a significant imbalance in covariates between NAP1-infected patients and all other cases [24]. Clearly, patients infected with NAP1 strains significantly differed in many regards from patients infected with other strains. They were older, more often suffered from healthcare-associated CDI, experienced a greater number of emergency room visits in the 12 weeks before diagnosis, had higher degrees of comorbidity, and were more likely to have received antibiotics prior to diagnosis. These important differences likely reflect the fact that NAP1 strains circulate in nosocomial settings. Regardless, the association of NAP1 infection with disease severity and outcome held after the authors adjusted for these potential confounders, providing more evidence that unique host and microbial features contribute to the myriad of outcomes in healthcare-associated CDI.

In addition to adjusting for individual covariates, See et al stratified their analysis by age group, finding that NAP1 infection remained a predictor of poor outcomes in both younger ($\leq$50 years) and older patients (>50 years) [24]. Interestingly, however, when the investigators stratified by epidemiologic class (healthcare- vs community-associated), NAP1 remained significantly associated with the 3 outcomes of interest for healthcare-associated cases only, but not for community-associated episodes. This was despite a relatively large number of NAP1 cases in community-associated CDI (n = 242 patients), suggesting that NAP1 strains induce adverse disease outcomes only in sicker (more vulnerable) patients with healthcare-associated CDI. However, the authors rightly point out that the smaller number of deaths and severe infections observed in community-associated infections limited the power to detect a true association between strain type and severity or outcome.

In addition to accounting for potentially important confounders, this investigation by See and colleagues had other strengths, most notably the large number of cases and broad geographic and epidemiological diversity. Of course, the study design was limited by its retrospective nature. The great imbalance in several covariates between NAP1-infected and non-NAP1-infected patients (above) begs the question of whether additional, unaccounted for (but clinically important) covariates might have been imbalanced between these patient populations (a point duly noted by the authors). It is hard to imagine what such covariates might be, but even relatively weak confounders can become important if they are significantly imbalanced between groups and when the population size is large. For example, if NAP1-infected patients were less able to perform routine activities of daily living (ie, were functionally impaired), this might have contributed to their worsened disease severity and outcome, as shown recently in a separate study [19].

On balance, this work by See and colleagues is important and informative. It is one of the stronger and more compelling arguments that strain type is an independent contributor to disease severity and outcome in healthcare-associated CDI. What remains unclear is how this information should be translated into clinical care. Management decisions based on host factors (age, healthcare-association,

comorbidities, concomitant medications, etc) are likely to remain the most important drivers of care in the near future. Prospective studies will need to be conducted to determine the extent to which tailoring therapy to certain *C. difficile* strains improves this approach. It is difficult to know from existing data whether it makes economic sense to determine strain type for routine patient care (outside of purposes for infection control and epidemiological studies).

In the future, whole genome sequencing of clinical *C. difficile* strains may reveal specific microbial determinants of disease severity that can be targeted for therapy or prevention, moving the field well past simple typing strategies. Vaccine approaches may also benefit from greater understanding of how genetic variability among strains influences virulence [12]. Finally, there are other aspects of disease pathogenesis that deserve attention as we search for modifiable influences on disease outcome. For example, it is unclear how information regarding patients' gastrointestinal microbiome or metabolome can be used to shape treatment and prevention decisions.

In summary, *C. difficile* remains an important cause of colitis, particularly in older adults. Although NAP1 strains are clearly associated with increased disease severity and outcomes, the extent to which this reflects the hypervirulence of the bacteria or the hypersusceptibility of infected patients remains an open question, with evidence implicating both host and microbe in this dangerous dance.

Notes

Financial support. D. M. A. received research support from the National Institutes of Health, grant 5U19AI090871.

Potential conflicts of interest. Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed

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