

# NAP1 Strain Type Predicts Outcomes From *Clostridium difficile* Infection

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(See the Editorial Commentary by Aronoff on pages 1401–3.)

**Background.** Studies are conflicting regarding the importance of the fluoroquinolone-resistant North American pulsed-field gel electrophoresis type 1 (NAP1) strain in *Clostridium difficile* infection (CDI) outcome. We describe strain types causing CDI and evaluate their association with patient outcomes.

**Methods.** CDI cases were identified from population-based surveillance. Multivariate regression models were used to evaluate the associations of strain type with severe disease (ileus, toxic megacolon, or pseudomembranous colitis within 5 days; or white blood cell count  $\geq 15\,000$  cells/ $\mu\text{L}$  within 1 day of positive test), severe outcome (intensive care unit admission after positive test, colectomy for *C. difficile* infection, or death within 30 days of positive test), and death within 14 days of positive test.

**Results.** Strain typing results were available for 2057 cases. Severe disease occurred in 363 (17.7%) cases, severe outcome in 100 (4.9%), and death within 14 days in 56 (2.7%). The most common strain types were NAP1 (28.4%), NAP4 (10.2%), and NAP11 (9.1%). In unadjusted analysis, NAP1 was associated with greater odds of severe disease than other strains. After controlling for patient risk factors, healthcare exposure, and antibiotic use, NAP1 was associated with severe disease (adjusted odds ratio [AOR], 1.74; 95% confidence interval [CI], 1.36–2.22), severe outcome (AOR, 1.66; 95% CI, 1.09–2.54), and death within 14 days (AOR, 2.12; 95% CI, 1.22–3.68).

**Conclusions.** NAP1 was the most prevalent strain and a predictor of severe disease, severe outcome, and death. Strategies to reduce NAP1 prevalence, such as antibiotic stewardship to reduce fluoroquinolone use, might reduce CDI morbidity.

**Keywords.** *Clostridium difficile*; clinical outcomes; strain typing; epidemiology.

Increases in incidence and severity of *Clostridium difficile* infection (CDI) have been reported in the past decade and were initially attributed to the emergence of a

“hypervirulent” strain, the North American pulsed-field gel electrophoresis type 1 (NAP1) strain, also described as polymerase chain reaction (PCR) ribotype 027 and restriction endonuclease analysis (REA) group BI [1–3]. This strain demonstrates increased toxin production in vitro and increased fluoroquinolone resistance compared with previously described strains.

Subsequent reports of the relationship between the NAP1 strain and patient outcomes have been in conflict. Although some studies suggest that infection with the NAP1 strain is associated with more severe disease [4–6], others reported no association [7–11]. These studies used different outcome measures (clinical

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severity [10] as defined by clinical practice guidelines [12]; a composite of intensive care unit [ICU] admission, colectomy, and death [5, 7, 9]; and 14- or 30-day mortality [4, 6, 8, 11]), involved small sample sizes, or have focused on cases of infection from a single institution or community.

We sought to clarify the role of *C. difficile* strain type by determining the relationship between strain type and disease outcomes using a geographically diverse dataset from the United States. We used outcome measures similar to those in other studies to facilitate comparisons with prior reports.

## METHODS

### CDI Surveillance and Study Population

Data were obtained from the Centers for Disease Control and Prevention (CDC) Emerging Infections Program (EIP) *C. difficile* surveillance, which has been described elsewhere [13, 14]. The EIP CDI surveillance system is an active population-based and laboratory-based surveillance system that began in 2009 in selected counties of 6 US states (California, Colorado, Connecticut, Georgia, Minnesota, New York), expanded to 2 additional US states in 2010 (Tennessee and Oregon), and in 2011 expanded to an additional 2 states (Maryland and New Mexico). At each EIP site, trained surveillance officers investigate all positive *C. difficile* toxin assay or molecular assay reports from clinical, reference, and commercial laboratories for residents of surveillance catchment areas. A CDI case is defined as a positive *C. difficile* stool specimen in a surveillance area resident aged 1 year or older who did not have a positive test in the previous 8 weeks.

Cases are classified as community-associated if a positive specimen was collected as an outpatient or within 3 days of an acute care admission, without documentation of an overnight stay in a healthcare facility during the 12 weeks prior to stool collection; otherwise, cases are classified as healthcare-associated. Healthcare-associated cases are further classified as healthcare facility-onset if they occurred during a long-term-care facility/nursing home stay or >3 calendar days after hospital admission; otherwise they are classified as community-onset healthcare facility-associated [15]. All CDI cases classified as either community-associated or community-onset healthcare facility-associated underwent a full medical record review to collect information on symptoms, coinfections, clinical comorbidities (Charlson index) [16], and outcomes, and a 10% sample of the healthcare facility-onset cases was fully reviewed.

A convenience sample of clinical laboratories in each catchment area ( $n = 37$  laboratories) submitted all stool specimens from CDI patients with full medical record review to 3 reference laboratories (Edwards Hines Jr Veterans Affairs, New York State Department of Health, and Minnesota Department of Health Public Health Laboratory) for culture of *C. difficile* [17]. Recovered isolates were sent to the CDC for molecular

typing by pulsed-field gel electrophoresis (PFGE). PFGE patterns were analyzed using BioNumerics version 5.10 (Applied Maths, Austin, Texas) and grouped into pulsed-field types using Dice/unweighted pair group method with arithmetic mean clustering. An 80% similarity threshold was used to assign North American PFGE (NAP) types [18]. Isolates also underwent PCR to detect the presence of *tcdA*, *tcdB*, and binary toxin (*cdtA* and *cdtB*) genes [19].

For this analysis, we limited the data to the CDI cases with stool specimens collected between 1 January 2009–31 December 2011. Only cases with full medical record review and strain typing results available were included. Cases whose isolates were negative for both *tcdA* and *tcdB* ( $n = 89$ ) were excluded. During 2009–2011, only 8 EIP sites collected stool specimens (California, Colorado, Connecticut, Georgia, Minnesota, New York, Oregon, and Tennessee). These 8 EIP sites represented a surveillance catchment area of 9 667 103 persons in 2011.

### Outcomes of Interest

Three separate outcome measures were evaluated: severe CDI disease, severe CDI outcome, and death within 14 days of infection. The definition of severe disease, adapted from current clinical practice guidelines [12], was development of ileus, toxic megacolon, or pseudomembranous colitis within 5 days of the positive *C. difficile* stool specimen or serum white blood count  $\geq 15\ 000$  cells/ $\mu\text{L}$  within 1 calendar day of collection of the stool specimen. Severe outcome was defined as ICU admission within 7 days after stool collection, colectomy for CDI, or death within 30 days of stool collection, in accordance with a recent study from Walk et al [7]. Death within 14 days was also evaluated based on the recent study from Walker et al [5].

### Statistical Analysis

Multiple imputation was used to impute missing race (12.6% of cases) based on the distribution of known race by age, sex, and surveillance site. Analysis of imputed datasets was performed using PROC MIANALYZE (SAS Institute, Cary, North Carolina) to account for the uncertainty associated with imputation. Baseline differences between groups were evaluated using  $\chi^2$  or Fisher exact tests for categorical variables, as appropriate, and Wilcoxon rank-sum tests for continuous variables. Because a linear relationship between increasing Charlson index and outcome variables was seen (up to a Charlson comorbidity index of 3), the Charlson index was treated as an ordinal variable with levels 0, 1, 2, and  $\geq 3$ .

For the outcomes studied, initial analyses of the association between individual variables and the outcome of interest were first performed with a univariate logistic regression model; a separate multivariate logistic regression model was then constructed for each of 3 outcome measures of interest using stepwise backward selection. Variables with  $P \leq .25$  in univariate

analysis were eligible for inclusion in the corresponding multivariate model. Possible confounding variables (ie, change of  $\geq 10\%$  to the estimated odds ratio for NAP1 strain) were added to respective multivariate models regardless of  $P$  values. Charlson index was also included in all models regardless of  $P$  value. To confirm the results found in multivariate models, analyses stratifying the data by age (patients  $\leq 50$  vs  $>50$  years of age) and by epidemiologic classification (community-associated vs healthcare-associated) were performed. A sensitivity analysis was also performed excluding the EIP site contributing the largest number of NAP1 cases from models. A 2-tailed  $P$  value  $< .05$  was considered statistically significant. All analyses were performed with SAS software, version 9.3 (SAS Institute).

### Human Subjects Review

The CDC and local institutional review boards approved the study. A waiver of informed consent was granted because the study posed no greater than minimal risk to participants.

## RESULTS

### Description of *Clostridium difficile* Infection Cases

During 2009–2011, strain typing results were available for 2057 of the 14 091 total CDI cases identified. Two EIP sites (New York and Minnesota) contributed  $>50\%$  of cases with strain typing results (Table 1). Compared with CDI cases without strain typing results but with clinical data available ( $n = 5324$ ), those with strain typing results were similar with respect to age ( $P = .41$ ) and the 3 clinical outcomes of interest: severe disease ( $P = .05$ ), severe outcome ( $P = .90$ ), and death within 14 days ( $P = .18$ ). Although differences in sex ( $P = .04$ ) and race ( $P < .0001$ ) between CDI cases with and those without strain typing results reached statistical significance, the relative differences were  $<6\%$  between groups.

**Table 1. *Clostridium difficile* Infection Cases With Strain Typing Results by Emerging Infections Program Site, 2009–2011**

EIP Site	CDI Cases, All Strains ( $n = 2057$ )		NAP1 Strain ( $n = 585$ )	
	No.	% of Total	No.	% of Total
California	114	5.5	29	5.0
Colorado	346	16.8	103	17.6
Connecticut	184	9.0	82	14.0
Georgia	105	5.1	32	5.5
Minnesota	469	22.8	56	9.6
New York	689	33.5	242	41.4
Oregon	39	1.9	4	0.7
Tennessee	111	5.4	37	6.3

Abbreviations: CDI, *Clostridium difficile* infection; EIP, Emerging Infections Program.

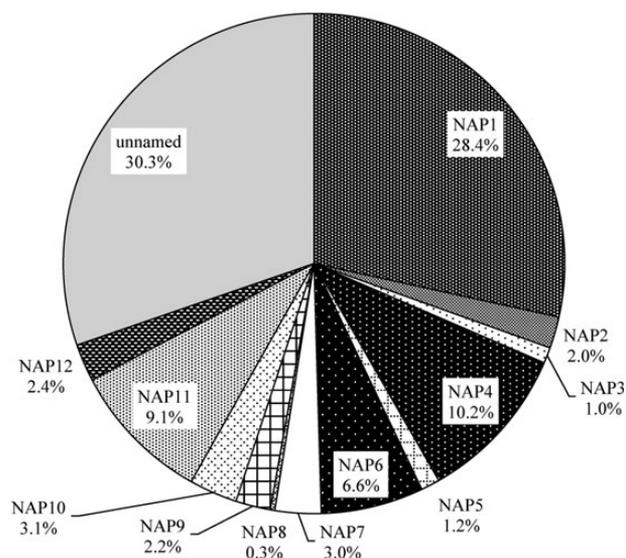
Among the 2057 CDI cases included in the analysis, the majority (52.8%) were community-associated by design. Overall, the most common strain types were NAP1 (28.4% of cases), NAP4 (10.2%), NAP11 (9.1%), and NAP6 (6.6%) (Figure 1). Of the 585 NAP1 cases, 17 (2.9%) were negative for binary toxin. Compared with these 3 most common NAP types, and compared with all others, the NAP1 type was associated with a greater odds of severe disease in unadjusted analysis (Figure 2). Therefore, remaining analyses compare the NAP1 strain to all other strain types (ie, non-NAP1 strains).

NAP1 strain was associated with older age ( $P < .0001$ ), healthcare-associated epidemiologic classification ( $P < .0001$ ), emergency department visits ( $P = .003$ ) in the prior 12 weeks, Charlson index ( $P < .0001$ ), and prior receipt of antibiotics ( $P < .0001$ ) in univariate analysis (Table 2). Inflammatory bowel disease ( $P = .0003$ ) and prior immunosuppressive treatment ( $P = .04$ ) were associated with non-NAP1 strains.

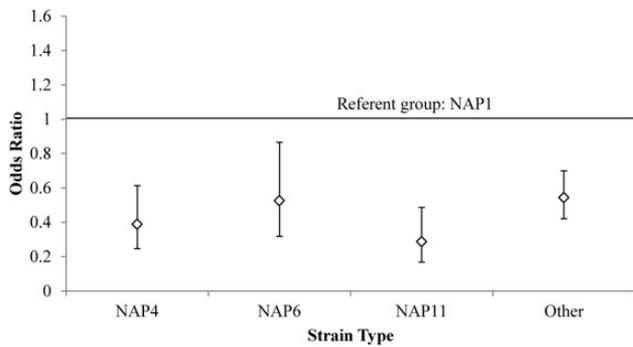
### Outcomes

#### Severe Disease

Criteria for severe disease were met for 363 cases (17.7%) with strain typing results. The majority of these cases (86.0%) met criteria for severe disease because of elevated white blood cell count alone. Infection with the NAP1 strain was significantly associated with severe disease in multivariate analysis (adjusted odds ratio [AOR], 1.74; 95% confidence interval [CI], 1.36–2.22) after controlling for age, epidemiologic classification, prior emergency department visits and hospitalizations,



**Figure 1.** Distribution of North American pulsed-field gel electrophoresis (PFGE) types among *Clostridium difficile* cases with strain typing results ( $n = 2057$ ). The “unnamed” strain type consists of many ( $>200$ ) unrelated PFGE patterns.



**Figure 2.** Univariate odds ratios for severe disease by North American pulsed-field gel electrophoresis strain type. NAP1 is the referent group, as indicated by the horizontal line at the odds ratio of 1.0. *P* values for all odds ratios depicted are  $<.05$ .

Charlson index, prior immunosuppressive treatment, and prior antibiotic use (Table 3).

### Severe Outcome

Severe outcomes occurred for 100 cases (4.9%). Of these cases, 41 were admitted to an ICU within 7 days after infection, 6 underwent colectomy, and 70 died within 30 days of infection. Cases developing severe outcomes were more likely to be infected with a NAP1 strain compared with those who did not develop severe outcomes (46.0% vs 27.5%,  $P < .0001$ ). NAP1 strain remained a predictor of severe outcomes in multivariate analysis (AOR, 1.66; 95% CI, 1.09–2.54) after controlling for older age, white race, healthcare-associated epidemiologic classification, Charlson index, and prior antibiotic use (Table 4).

### Death Within 14 Days

Fifty-six deaths occurred within 14 days of stool collection. The NAP1 strain was more common among those who died within 14 days than among those who survived (51.8% vs 27.8%,  $P < .0001$ ). The 14-day mortality of NAP1 cases was 5.0% and for non-NAP1 cases was 1.8%. In particular, in cases with NAP7/8, which corresponds to ribotype 078, the 14-day mortality was 1.5% (95% CI, .04%–8.0%; 1 of 66). In univariate analyses, older age (odds ratio [OR], 5.6; 95% CI, 2.87–10.86), healthcare-associated epidemiologic classification (OR, 5.41; 95% CI, 2.43–12.07 for community-onset healthcare facility-associated vs community-associated; OR, 10.38; 95% CI, 4.59–23.43 for healthcare facility-onset vs community-associated), Charlson index (OR 2.68; 95% CI, 1.94–3.70), prior proton pump inhibitor use (OR, 2.18; 95% CI, 1.28–3.71), and prior antibiotic use (OR, 2.31; 95% CI, 1.30–4.11) were also associated with death within 14 days. In multivariate analysis, age, race, epidemiologic classification, and Charlson index were retained in the final model. After controlling for these risk factors, NAP1

**Table 2. Univariate Comparison of Baseline Characteristics of *Clostridium difficile* Infection Due to the NAP1 Strain Compared to Non-NAP1 Strains**

Characteristic	NAP1 Strain, No. (%) (n = 585)	Non-NAP1 Strain, No. (%) (n = 1472)	<i>P</i> Value
<b>Demographic variable</b>			
Age >65 y	305 (52.1)	586 (39.8)	$<.0001$
Female sex	346 (59.2)	909 (61.8)	.27
White race	505 (86.4)	1262 (85.8)	.74
<b>Epidemiologic classification</b>			
Healthcare facility-onset	111 (19.0)	211 (14.3)	$<.0001$
Community-onset, healthcare facility-associated	232 (39.7)	416 (28.3)	
Community-associated	242 (41.4)	845 (57.4)	
<b>Healthcare exposures</b>			
Chronic hemodialysis	39 (6.7)	68 (4.6)	.06
Surgery during 12 wk prior to infection	130 (22.2)	285 (19.4)	.15
ED visits during 12 wk prior to infection	224 (38.3)	462 (31.4)	.003
<b>Charlson comorbidity index</b>			
0	206 (35.2)	689 (46.8)	$<.0001$
1	81 (13.9)	169 (11.5)	
2	85 (14.5)	161 (10.9)	
$\geq 3$	213 (36.4)	453 (30.8)	
<b>Other underlying conditions</b>			
Diverticular disease	51 (8.7)	121 (8.2)	.71
Inflammatory bowel disease	11 (1.9)	90 (6.1)	.0002
<b>Medications during 14 d prior to infection</b>			
Proton pump inhibitor	192 (32.8)	466 (31.7)	.61
H2 blocker	52 (8.9)	146 (9.9)	.48
Any immunosuppressive treatment	82 (14.0)	261 (17.7)	.04
Any antibiotic	345 (59.0)	691 (46.9)	$<.0001$

Abbreviation: ED, emergency department.

strain remained a significant predictor of 14-day mortality (AOR, 2.12; 95% CI, 1.22–3.68; Table 5).

### Sensitivity Analyses

In analyses stratified by age group, NAP1 infection remained a predictor of poor outcomes in both younger ( $\leq 50$  years) and older patients ( $>50$  years) (data not shown). When stratifying by epidemiologic class (healthcare- vs community-associated), NAP1 remained significantly associated with the 3 outcomes of interest for healthcare-associated cases, whereas for community-associated cases, all NAP1 ORs were  $>1.0$  but *P* values were  $>.05$ . Finally, the associations between the NAP1 strain and severe disease, severe outcome, and 14-day mortality remained significant after excluding cases from the EIP site (New York) that contributed the largest number of NAP1 cases.

**Table 3. Multivariate Analysis for Severe *Clostridium difficile* Disease Among Cases With Strain Typing Results, 2009–2011**

Risk Factors <sup>a</sup>	AOR (95% CI)	P Value
Cases with strain typing results (n = 2057)		
Age >65 y	1.69 (1.31–2.18)	<.0001
Healthcare-associated epidemiologic classification <sup>b</sup>	1.75 (1.32–2.34)	.0001
Emergency department visit during 12 wk prior to infection	1.31 (1.01–1.69)	.04
Charlson index	1.08 (.98–1.20)	.14
Medications during 14 d prior to infection		
Immunosuppressive treatment	1.42 (1.05–1.92)	.02
Any antibiotic	1.38 (1.08–1.76)	.01
NAP1 strain	1.74 (1.36–2.22)	<.0001

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.

<sup>a</sup> Candidate variables included in the severe disease model: age; epidemiologic classification; surgery or emergency department visit during 12 weeks prior to infection; diverticular disease; Charlson index; proton pump inhibitor use, immunosuppressive treatment, or antibiotic use during 14 days prior to infection; strain type.

<sup>b</sup> Healthcare facility-onset and community-onset healthcare facility-associated epidemiologic classes did not have significantly different odds of severe disease and were collapsed into a single “healthcare-associated” category.

## DISCUSSION

We found NAP1 to be the most common strain, accounting for more than one-quarter of cases in our dataset. NAP1 was associated with greater odds of severe disease than other NAP types in unadjusted analysis and was also associated with older age

**Table 4. Multivariate Analysis for Severe Outcome of *Clostridium difficile* Infection Among Cases With Strain Typing Results, 2009–2011**

Risk Factors <sup>a</sup>	AOR (95% CI)	P Value
Cases with strain typing results (n = 2057)		
Age >65 y	1.71 (1.06–2.76)	.03
White race	0.49 (.29–.85)	.01
Healthcare-associated epidemiologic classification <sup>b</sup>	2.90 (1.63–5.19)	.0003
Charlson index	1.71 (1.38–2.13)	<.0001
Any antibiotic during 14 d prior to infection	1.63 (1.04–2.56)	.03
NAP1 strain	1.66 (1.09–2.54)	.02

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.

<sup>a</sup> Candidate variables included in the severe outcome model: age; race; epidemiologic classification; chronic hemodialysis; emergency department visit during 12 weeks prior to infection; Charlson index; proton pump inhibitor use, H2 blocker use, or antibiotic use during 14 d prior to infection; strain type.

<sup>b</sup> Healthcare facility-onset and community-onset healthcare facility-associated epidemiologic classes did not have significantly different odds of severe outcome and were collapsed into a single “healthcare-associated” category.

**Table 5. Multivariate Analysis for 14-Day Mortality After *Clostridium difficile* Infection Among Cases With Strain Typing Results, 2009–2011**

Risk Factors <sup>a</sup>	AOR (95% CI)	P Value
Cases with strain typing results (n = 2057)		
Age >65 y	2.98 (1.45–6.11)	.003
White race	0.46 (.23–.95)	.04
Epidemiologic classification		
Healthcare facility-onset	3.80 (1.62–8.94)	.002
Community-onset healthcare facility-associated	2.33 (1.01–5.36)	.05
Community-associated (reference)		
Charlson score	2.03 (1.44–2.86)	<.0001
NAP1 strain	2.12 (1.22–3.68)	.008

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.

<sup>a</sup> Candidate variables included in the model: age; race; epidemiologic classification; chronic hemodialysis; emergency department visit during 12 weeks prior to infection; Charlson index; proton pump inhibitor use, H2 blocker use, or antibiotic use during 14 d prior to infection; strain type.

and a variety of healthcare exposures. After controlling for potential confounders, the NAP1 strain remained a significant predictor of severe disease, severe outcome, and 14-day mortality. Our results represent the largest study to date to examine the association between strain type and disease outcomes. Furthermore, inclusion of cases from a wide geographic spread reduces bias due to regional variation in *C. difficile* strain type or patient characteristics.

We chose the outcome measures of severe outcome and 14-day mortality to facilitate comparisons with 2 recent studies on CDI outcome related to the NAP1 strain. Our findings agree with those in a recent report from the United Kingdom showing that the NAP1/027 strain is associated with increased 14-day mortality [5]. However, our results differ from a recent study from the United States by Walk et al that found no association between the NAP1/027 strain and severe outcome [7]. In the US study, the number of patients with NAP1 was small (approximately 40) and <50 patients met the outcome measure. Although the US study reported a lack of association, the odds for severe outcome were increased among NAP1/027 cases, albeit not achieving statistical significance. Thus, as suggested by others [20, 21], lack of association between the NAP1/027 strain and severe outcome reported in the US study might be largely related to differences in sample size.

The proportion of patients who died in our study (3.8%) is lower than that reported from other studies of CDI outcomes [5, 22]. This discrepancy is likely a result of a larger proportion of community-associated CDI cases in our study, which are associated with better outcomes than healthcare-associated cases [14]. This is unlikely to bias our study toward detection of

association between strain type and outcomes, given that we adjusted for epidemiologic classification as a confounder in our analyses.

We found baseline differences between the patient populations infected by *C. difficile* NAP1 vs non-NAP1 strains. Although we adjusted for patient comorbidities, unmeasured patient-level confounders might still account for some of the relationship we found between the NAP1 strain and patient outcomes. However, our findings persisted in analyses stratified by age, supporting that *C. difficile* strain is an important predictor of patient outcome independent of patient age. Associations between NAP1 strain and patient outcomes did not remain significant for community-associated cases when stratifying the data by epidemiologic classification. This lack of significance is likely related to low statistical power, as outcomes of interest were uncommon among community-associated cases (10.8% for severe disease, 1.5% for severe outcomes, and 0.7% for death within 14 days), and the NAP1 strain was less prevalent in the community.

Our analysis therefore provides additional support for the conclusion that infection by the NAP1 strain adversely affects patient outcomes. The practical implications of this finding remain to be determined. Host-related factors play an important role in the development of CDI, and other studies have suggested that clinical scores or biomarkers based on the immune response of the host (eg, albumin, serum white blood count, C-reactive protein), rather than strain type, should be the basis for decisions about severity of disease for treatment [12, 23, 24].

Nevertheless, strategies that account for strain-specific factors might complement treatment strategies based on host response and further reduce morbidity due to CDI. The specific virulence factors possessed by the NAP1/027 strain that lead to worsened outcomes remain to be more clearly elucidated. As such research progresses, vaccines being developed for *C. difficile* might target such factors.

In addition, antimicrobial stewardship might further aid in preventing infections from the NAP1 strain. Although more judicious antimicrobial use would likely reduce *C. difficile* infections in general, including those caused by NAP1, stewardship efforts might also be leveraged to have a greater impact on NAP1 prevalence. For example, given that the NAP1/027 strain is more resistant than other strains to the fluoroquinolones [1, 3, 5, 25, 26], antimicrobial stewardship aimed at reducing the overall use of fluoroquinolones might also reduce the prevalence of infections from NAP1 and decrease patient morbidity from CDI. Indeed, fluoroquinolone use has been found to be a risk factor for infection by the NAP1/027 strain [27–29], and the development of fluoroquinolone resistance by the NAP1/027 strain has been suggested to be the primary genetic factor facilitating its spread [29]. Fluoroquinolone restriction has also been reported to be an important component of efforts to control

outbreaks of *C. difficile* from the NAP1/027 strain [30, 31], but further research is needed to determine the utility of the application of this strategy to nonoutbreak settings.

We should note the following limitations of our study. First, all clinical data collected were obtained by retrospective review of medical charts, potentially leading to underestimation of mortality rates if patients died soon after discharge. Second, we were not able to control for differences in treatment, which might affect patient outcomes. However, as noted earlier, because our data encompass a diverse geographic area, it is unlikely that our findings are driven by individual institutional treatment practices. Third, we could not fully evaluate the potential role of the NAP7/8/ribotype 078 strain, which has also been reported to have increased virulence [5, 32], on outcomes due to limited numbers of these cases in our dataset. Fourth, our analysis might not be representative of all *C. difficile* infections. For example, only a sample of healthcare facility-onset CDI cases are fully reviewed. However, even though patients with strain typing results represent a convenience sample of the total, comparison to patients without strain typing results suggests that our sample is representative of those cases with full medical record review. Fifth, we do not have data on all cases about the type of diagnostic test that was used to identify each CDI case (eg, toxin assay vs nucleic acid amplification test [NAAT]). As CDI cases detected by toxin assays have been reported to have higher mortality than cases detected solely by NAAT [33], we could not account for this potential effect modifier of CDI outcomes.

In conclusion, analysis of a large, geographically diverse set of CDI cases from the United States corroborates that the *C. difficile* NAP1 strain type is an important determinant of patient outcomes. Disease from *C. difficile* results from a complex interplay between host-related factors and pathogen-specific factors. Efforts to reduce the burden of CDI likely will need to consider both.

## Notes

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## References

1. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med* **2005**; 353:2442–9.
2. Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* **2005**; 366:1079–84.
3. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med* **2005**; 353:2433–41.
4. Labbé AC, Poirier L, MacCannell D, et al. *Clostridium difficile* infections in a Canadian tertiary care hospital before and during a regional epidemic associated with the BI/NAP1/027 strain. *Antimicrob Agents Chemother* **2008**; 59:3180–7.
5. Walker AS, Eyre DW, Wyllie DH, et al. Relationship between bacterial strain type, host biomarkers and mortality in *Clostridium difficile* infection. *Clin Infect Dis* **2013**; 56:1589–600.
6. Miller M, Gravel D, Mulvey M, et al. Health care-associated *Clostridium difficile* infection in Canada: patient age and infecting strain type are highly predictive of severe outcome and mortality. *Clin Infect Dis* **2010**; 50:194–201.
7. Walk ST, Micic D, Jain R, et al. *Clostridium difficile* ribotype does not predict severe infection. *Clin Infect Dis* **2012**; 55:1661–8.
8. Goldenberg SD, French GL. Lack of association of *tcdC* type and binary toxin status with disease severity and outcome in toxigenic *Clostridium difficile*. *J Infect* **2011**; 62:355–62.
9. Cloud J, Noddin L, Pressman A, Hu M, Kelly C. *Clostridium difficile* strain NAP-1 is not associated with severe disease in a nonepidemic setting. *Clin Gastroenterol Hepatol* **2009**; 7:868–73.
10. Sirard S, Valiquette L, Fortier L. Lack of association between clinical outcome of *Clostridium difficile* infections, strain type, and virulence-associated phenotypes. *J Clin Microbiol* **2011**; 49:4040–6.
11. Wilson V, Cheek L, Satta G, et al. Predictors of death after *Clostridium difficile* infection: a report on 128 strain-typed cases from a teaching hospital in the United Kingdom. *Clin Infect Dis* **2010**; 50:e77–81.
12. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect Control Hosp Epidemiol* **2010**; 31:431–55.
13. Centers for Disease Control and Prevention. Measuring the scope of *Clostridium difficile* infection in the United States. Available at: <http://www.cdc.gov/hai/eip/clostridium-difficile.html>. Accessed 2 July 2013.
14. Lessa FC. Community-associated *Clostridium difficile* infection: how real is it? [epub ahead of print]. *Anaerobe* **2013**; 24:121–3.
15. McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK. Recommendations for surveillance of *Clostridium difficile*-associated disease. *Infect Control Hosp Epidemiol* **2007**; 28:140–5.
16. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chron Dis* **1987**; 40:373–83.
17. Lowy I, Molrin DC, Leav BA, et al. Treatment with monoclonal antibodies to *Clostridium difficile* toxins A and B prevent recurrent infection. *New Engl J Med* **2010**; 362:1–9.
18. Killgore G, Thompson A, Johnson S, et al. 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* **2008**; 46:431–7.
19. Persson S, Torpdahl M, Olsen KE. New multiple PCR method for the detection of *Clostridium difficile* toxin A (*tcdA*) and toxin B (*tcdB*) and the binary toxin (*cdtA/cdtB*) genes applied to a Danish strain collection. *Clin Microbiol Infect* **2008**; 11:1057–64. Erratum: *Clin Microbiol Infect* **2009**; 15:296.
20. Gerding DN, Johnson S. Does infection with specific *Clostridium difficile* strains or clades influence clinical outcome? *Clin Infect Dis* **2013**; 56:1601–3.
21. Walker AS, Eyre DW, Crook DW, Wilcox MH, Peto TE. Regarding “*Clostridium difficile* ribotype does not predict severe infection.” *Clin Infect Dis* **2013**; 56:1845–6.
22. Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper E. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study. *Clin Infect Dis* **2013**; 56:1108–16.
23. Fujitani S, George WL, Murthy AR. Comparison of clinical severity score indices for *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* **2011**; 32:220–8.
24. Bauer MP, Hensgens MP, Miller MA, et al. Renal failure and leukocytosis are predictors of a complicated course of *Clostridium difficile* infection if measured on day of diagnosis. *Clin Infect Dis* **2012**; 55(suppl 2): S149–53.
25. Walkty A, Boyd DA, Gravel D, et al. Molecular characterization of moxifloxacin resistance from Canadian *Clostridium difficile* clinical isolates. *Diagn Microbiol Infect Dis* **2010**; 66:419–24.
26. Vardakas KZ, Konstantelias AA, Loizidis G, Rafailidis PI, Falagas ME. Risk factors for development of *Clostridium difficile* infection due to BI/NAP1/027 strain: a meta-analysis. *Int J Infect Dis* **2012**; 16:e768–73.
27. Pépin J, Saheb N, Coulombe M-A, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. *Clin Infect Dis* **2005**; 49:1254–60.
28. Kazakova SV, Ware K, Baughman B, et al. A hospital outbreak of diarrhea due to an emerging epidemic strain of *Clostridium difficile*. *Arch Int Med* **2006**; 166:2518–24.
29. He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nat Genet* **2013**; 45:109–13.
30. Kallen AJ, Thompson A, Ristaino P, et al. Complete restriction of fluoroquinolone use to control and outbreak of *Clostridium difficile* infection at a community hospital. *Infect Control Hosp Epidemiol* **2009**; 30:264–72.
31. Aldeyab MA, Devine MJ, Flanagan P, et al. Multihospital outbreak of *Clostridium difficile* ribotype 027 infection: epidemiology and analysis of control measures. *Infect Control Hosp Epidemiol* **2011**; 32:210–9.
32. Goorhuis A, Bakker D, Corver J, et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin Infect Dis* **2008**; 47:1162–70.
33. Planche TD, Davies KA, Coen PG, et al. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicenter diagnostic validation study of *C. difficile* infection. *Lancet Infect Dis* **2013**; 13:936–45.