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## Natural History of *Clostridioides difficile*–Related Disease Progression in the 2-Step Testing Era

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### Abstract

**Background.** The natural history of *Clostridioides difficile* progression in nucleic acid amplification test (NAAT) positive, toxin enzyme immunoassay–negative patients remains poorly described. Better understanding risk for subsequent disease may improve prevention strategies. We aimed to describe the natural history of *C. difficile* NAAT<sup>+</sup>/toxin<sup>−</sup> adults.

**Methods.** A cohort of adults (≥18 years) tested for *C. difficile* within the Duke University Health System between 15 March 2020 and 31 December 2023 were classified as NAAT<sup>−</sup>, NAAT<sup>+</sup>/toxin<sup>−</sup>, or NAAT<sup>+</sup>/toxin<sup>+</sup> and followed up to 90 days. Three time-to-event analyses were conducted. Incidence of toxin<sup>+</sup> episodes was assessed by initial test status (analysis 1). Treatment of NAAT<sup>+</sup>/toxin<sup>−</sup> adults was described using cumulative incidence curves (analysis 2). Rates of toxin<sup>+</sup> episodes and severe disease were compared between treated and untreated NAAT<sup>+</sup>/toxin<sup>−</sup> adults (analysis 3).

**Results.** The cohort included 24 474 tests and 440 toxin<sup>+</sup> episodes among 18 337 unique subjects followed for a median 71 days. NAAT<sup>+</sup>/toxin<sup>−</sup> status was associated with subsequent toxin positivity (adjusted hazard ratio [aHR], 5.06 [95% confidence interval {CI}, 3.61–7.10]), especially after antibiotic receipt (aHR, 15.71 [95% CI, 9.85–25.06]). Among 2334 NAAT<sup>+</sup>/toxin<sup>−</sup>

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**Author Contributions.** All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

episodes, 33% received presumptive treatment. Just 5% of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects progressed to toxin positivity. Presumptive treatment was associated with lower hazard of subsequent toxin positivity (aHR, 0.12 [95% CI, .05–.29]) but not fulminant disease (aHR, 1.93 [95% CI, .50–7.45]).

**Conclusions.** *Clostridioides difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status was associated with subsequent toxin positivity, especially after antibiotic receipt, though absolute risk was low overall. Further research is needed to determine whether and for whom presumptive treatment might be beneficial.

## Keywords

*Clostridioides difficile* ; multistep testing; diagnostics; natural history; toxin enzyme immunoassay

*Clostridioides difficile* infection (CDI) remains a leading cause of healthcare-associated diarrhea despite being responsible for only a minority of hospital-onset diarrhea cases overall [1, 2]. Diagnosis can be challenging. Because *C. difficile* disease is toxin-mediated, the presence of organism alone does not necessarily indicate clinical disease. Disruption of protective gut microbiota by antibiotic receipt facilitates *C. difficile* germination and toxin production, ultimately leading to diarrheal disease [3]. Nucleic acid amplification tests (NAATs), which are widely used, only detect the presence of toxigenic *C. difficile* without assessing toxin production, resulting in overdiagnosis and inappropriate treatment. Two-step testing, which combines a highly sensitive test (typically NAAT or glutamate dehydrogenase) with a more specific assay for toxin production, has proven effective at identifying subjects with true disease [4]. However, because toxin-based testing has imperfect sensitivity, clinicians may treat presumptively due to suspicion of a false-negative toxin result or due to fear of undertreating *C. difficile* disease. Thus, understanding the risk of subsequent disease among NAAT-positive (NAAT<sup>+</sup>), toxin-negative (toxin<sup>-</sup>) populations might better inform individual patients' risk/benefit assessments.

We previously found that conversion to 2-step testing was temporally associated with a decline in hospital-onset *C. difficile* rates. At least within the hospital setting, conversion to 2-step testing was associated with a parallel decline in *C. difficile*-targeted antibiotic use and no change in emergent colectomy rates [5]. While reassuring at the healthcare system level, individual patient-level outcomes remain poorly described. In this follow-up study, we sought to describe the natural history of *C. difficile*-related disease in the era of 2-step testing using individual patient-level data. Through 3 distinct time-to-event analyses, we aimed to (1) assess the risk of progression to subsequent toxin positivity among initially NAAT<sup>+</sup>/toxin<sup>-</sup> subjects, (2) describe treatment rates (presumptive or for subsequent toxin-positive [toxin<sup>+</sup>] subjects) among initially NAAT<sup>+</sup>/toxin<sup>-</sup> subjects, and (3) explore whether presumptive treatment of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects was associated with clinical outcome (eg, subsequent risk of toxin positivity or severe disease).

## METHODS

### Data Collection

We constructed a longitudinal cohort of adults (≥18 years) undergoing *C. difficile* testing within the Duke University Health System, including 3 hospitals and their affiliated

outpatient clinics, between 15 March 2020 and 31 December 2023. The study period corresponded with the onset of 2-step testing within our health system. Analysis was performed from January 2024 to May 2024. Duke University Health System's institutional review board reviewed the study design and determined it to be exempt with a waiver of consent for data collection as the study was entirely retrospective.

Antibiotic receipt was electronically queried across our entire health system, capturing inpatient administrations and outpatient prescriptions during the study period. For censoring purposes, the date of the last encounter was defined as the last in-person clinic or hospital encounter occurring within 90 days of the index *C. difficile* test. *Clostridioides difficile* status was defined by 2-step testing using NAAT (Cepheid Xpert) with reflex to toxin enzyme-linked immunosorbent assay (TechLab Quik Chek Complete) when positive. Per clinical microbiology laboratory protocols, samples were only tested if an unformed stool sample was received. NAAT<sup>+</sup>/toxin<sup>-</sup> results are accompanied by the following text: "Likely represents colonization with alternative cause of symptoms. Treatment generally not indicated unless clinical signs of severe or fulminant *C. difficile* disease. Consider Infectious Disease consult in questionable cases." Fulminant disease was defined as cases requiring rectally administered vancomycin or colectomy.

### Statistical Modeling

Three separate time-to-event analyses were conducted: (1) assessing risk of progression to toxin positivity, (2) describing treatment rates among NAAT<sup>+</sup>/toxin<sup>-</sup> individuals, and (3) exploring the association between presumptive treatment and subsequent toxin positivity and fulminant disease outcomes. In each model, follow-up time began with *C. difficile* testing and continued up to 90 days from the index test. Subjects were considered censored at either the date of their last in-person clinical encounter or the date of death if either fell within 90 days of the index test. More than 1 test-and-follow-up cycle could be included for each subject; thus, robust confidence intervals (CIs) and models appropriate for recurrent events (Prentice-Williams-Peterson gap-time and frailty models) were employed. Models were constructed using the survival and cmprsk packages in R (version 4.1.2, <https://cran.r-project.org>) [6, 7]. The proportionality of hazards was assessed for each model using a combination of graphical (log-log plots) and test-based (ZPH test with Schoenfeld residuals) methods. Variables violating the proportionality assumption were treated as stratification variables. Key potential confounders were treated as time-varying covariates, including age, chronic kidney disease, cancer, hematopoietic stem cell transplant history, 2 or more prior *C. difficile* episodes, and high-risk antibiotic receipt in the 30 days before the episode [8]. High-risk antibiotics were defined as fluoroquinolones, third- and fourth-generation cephalosporins, or clindamycin [9]. Proton pump inhibitor use was not included, partly because prior local cohorts did not find a significant association and partly because over-the-counter availability limits the utility of electronic queries for prescription data [10]. Competing risks were accounted for using the cmprsk package in analysis 2. A significance level of .05 was used for analysis 1. Analyses 2 and 3 were descriptive or exploratory, and thus *P* values were not reported.

### Analysis 1: Assessing Risk of Progression to Toxin Positivity

Data from the full cohort of tested subjects compared the risk of subsequent toxin<sup>+</sup> *C. difficile* episodes among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects relative to NAAT<sup>-</sup> and relative to recurrent disease among NAAT<sup>+</sup>/toxin<sup>+</sup> subjects. Since the assumptions inherent to various recurrent event models are not easily testable, we conducted analysis 1 in triplicate—using the Prentice-Williams-Peterson gap-time method, the frailty model method, and a traditional Cox proportional hazard model (limited to the first event only) to ensure stability. To adjust for immortality time bias (retesting is generally not permitted within 14 days of a prior NAAT<sup>+</sup>/toxin<sup>+</sup> test within our healthcare system), the follow-up period for NAAT<sup>+</sup>/toxin<sup>+</sup> subjects was adjusted to begin 14 days after the last positive test. To investigate the potential influence of informative censoring, we conducted sensitivity analyses in which censored cases were first treated as worst-case scenario (eg, progression to toxin positivity at the time of censoring) and best-case scenario (eg, remained disease free at the time of censoring). Potential interaction terms between antibiotic receipt and *C. difficile* status were explored and included when appropriate based on likelihood ratio testing.

### Analysis 2: Rates of Treatment Among NAAT<sup>+</sup>/Toxin<sup>-</sup> Subjects

To assess rates of treatment among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects—including either presumptive treatment or treatment for subsequent toxin<sup>+</sup> episode—we constructed cumulative incidence curves for the subcohort of subjects whose index *C. difficile* test was NAAT<sup>+</sup>/toxin<sup>-</sup>. Receipt of long-term *C. difficile* suppressive therapy and all-cause mortality were included as competing risks.

### Analysis 3: Association Between Presumptive Treatment of NAAT<sup>+</sup>/Toxin<sup>-</sup> Subjects and Subsequent Toxin Positivity

To explore the association between presumptive treatment of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects and later toxin positivity, we again constructed time-to-event models within the cohort of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects while excluding those placed on long-term suppression (defined as continuation of a *C. difficile*-active therapy for a duration longer than the guideline-recommended maximum of 14 days). To adjust for immortality time bias introduced by presumptive treatment, time at risk for presumptively treated subjects was set to begin at completion of the presumptive treatment course. Treatment with rectally instilled vancomycin (given in cases of severe *C. difficile* with ileus) or colectomy were selected as electronically queryable indicators of fulminant disease.

## RESULTS

The overall cohort included 24 474 tests collected from 18 337 unique subjects from March 2020 through December 2023 (Figure 1). Within the overall cohort, 20 959 tests (85.6%) were initially NAAT<sup>-</sup>, 2334 (9.5%) were initially NAAT<sup>+</sup>/toxin<sup>-</sup>, and 1181 (4.8%) were initially NAAT<sup>+</sup>/toxin<sup>+</sup>. A total of 440 subsequent toxin<sup>+</sup> *C. difficile* episodes within 90 days were captured: 195 tests (44%) among initially NAAT<sup>-</sup> subjects (90-day Kaplan-Meier event rate: 1.4% [95% CI, 1.2%–1.7%]), 83 (19%) among initially NAAT<sup>+</sup>/toxin<sup>-</sup> subjects (90-day Kaplan-Meier event rate: 5.1% [95% CI, 3.9%–6.2%]), and 162 (37%) among initially NAAT<sup>+</sup>/toxin<sup>+</sup> subjects (90-day Kaplan-Meier event rate: 19.1% [95% CI, 15.7%–

22.3%]; Supplementary Figure 1). Cohort demographics and comorbidities are summarized in Table 1. Subjects with a NAAT<sup>+</sup>/toxin<sup>+</sup> index test result were slightly older and had a slightly higher proportion of multiple prior *C. difficile* events. Otherwise, demographics and median follow-up time were similar between the overall cohort and across index test results. Healthcare contact and receipt of high-risk antibiotics were frequent overall, with 21 153 (86.4%) of subjects having been admitted in the preceding 90 days and 4175 (17.1%) receiving ≥48 hours of high-risk antibiotics in the preceding 30 days.

### Analysis 1: Assessing Risk of Progression to Toxin Positivity

Unadjusted Kaplan-Meier curves plotting *C. difficile* toxin<sup>+</sup> episode-free survival are shown in Figure 2. As there was evidence of interaction between *C. difficile* status and antibiotic receipt ( $P < .001$  by likelihood ratio test), we created a new variable representing each combination of *C. difficile* status and antibiotic receipt to allow for easier effect interpretation with proportional hazard modeling. Hazard ratios adjusted for age, select comorbidities, and prior *C. difficile* events are presented in Table 2. Relative to NAAT<sup>-</sup> subjects who did not receive antibiotics, NAAT<sup>+</sup>/toxin<sup>-</sup> status was associated with a higher risk of progression to toxin positivity (adjusted hazard ratio [aHR], 5.06 [95% CI, 3.61–7.10]). The hazard associated with NAAT<sup>+</sup>/toxin<sup>-</sup> status was numerically lower than that associated with initial NAAT<sup>+</sup>/toxin<sup>+</sup> status (aHR, 23.47 [95% CI, 17.61–31.28]). Across all statuses, high-risk antibiotic receipt increased the risk of progression to *C. difficile* toxin positivity, including a particularly high risk among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects receiving high-risk antibiotics (aHR, 15.71 [95% CI, 9.85–25.06]).

The general effects of *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status and antibiotic receipt remained stable across all 3 time-to-event models (Prentice-Williams-Peterson gap-time, frailty model, and a traditional Cox proportional hazard model limited to first events only; Supplementary Table 1). Though not the specific focus of this study, the hazard associated with initial NAAT<sup>+</sup>/toxin<sup>+</sup> status remained significant but varied in magnitude across models, likely because of smaller numbers within this subgroup. Similarly, *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status and antibiotic receipt remained significantly associated with later progression to toxin positivity across best- and worst-case censoring assumptions, though the magnitude of risk was sensitive to the assumption that all censored subjects developed disease (Supplementary Table 2).

### Analysis 2: Rates of Treatment Among NAAT<sup>+</sup>/Toxin<sup>-</sup> Subjects

Figure 3 presents a cumulative incidence plot for clinical outcomes among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects. Presumptive treatment was prescribed for 28% of episodes (95% CI, 27%–30%) within 10 days of testing, increasing slightly to 33% (95% CI, 31%–35%) by day 90 (Supplementary Table 3). A small proportion of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects received long-term suppression ( $n = 24$  [1.0%]). Progression to toxin positivity occurred in 5% (95% CI, 4%–6%) of NAAT<sup>+</sup>/toxin<sup>-</sup> episodes within 90 days of their index test.

### Analysis 3: Association Between Presumptive Treatment of NAAT<sup>+</sup>/Toxin<sup>-</sup> Subjects and Subsequent Toxin Positivity

A total of 5 toxin<sup>+</sup> episodes occurred following 690 NAAT<sup>+</sup>/toxin<sup>-</sup> events receiving presumptive treatment (90-day Kaplan-Meier event rate: 2.8% [95% CI, 0–5.8%]; Figure 4A); 83 toxin<sup>+</sup> episodes occurred following 1570 NAAT<sup>+</sup>/toxin<sup>-</sup> events not receiving presumptive treatment (90-day Kaplan-Meier event rate: 7.6% [95% CI, 5.9%–8.2%]). Using rectal vancomycin receipt or colectomy as indicators of severe *C. difficile*, just 2 colectomies occurred after presumptive treatment (90-day Kaplan-Meier event rate: 0.7% [95% CI, 0–1.7%]); 5 colectomies plus 1 instance of rectal vancomycin receipt occurred among untreated subjects (90-day Kaplan-Meier event rate: 0.6% [95% CI, 0–1.1%]; Figure 4B). One colectomy from the untreated group was for colonic necrosis occurring 6 weeks after index test and presumed to be *C. difficile* related though stool remained NAAT<sup>+</sup>/toxin<sup>-</sup> at the time of colectomy. The other 6 colectomies were all for unrelated indications (4 for diverticular disease, 1 for colon cancer, 1 for ulcerative colitis). While smaller event rates limited the ability to include all potential confounders, presumptive treatment appeared to be associated with a lower hazard of progression to toxin positivity (aHR, 0.13 [95% CI, .05–.31]) but did not appear to be associated with any reduction in risk of treatment for severe *C. difficile* disease or colectomy (aHR, 1.93 [95% CI, .50–7.45]) (Table 3). Event rates in analysis 3 were too small to adequately assess additional factors for those most likely to benefit from treatment.

## DISCUSSION

In this large-scale, single-center cohort study, *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status was associated with higher risk of progression to toxin positivity than among initially negative subjects; however, the absolute risk of progression was low (5%). The observed association between *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status and subsequent toxin positivity was consistent with prior studies [11, 12]. Antibiotic receipt was independently associated with progression to toxin positivity and also exhibited an interaction effect with *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status: receipt of antibiotics by NAAT<sup>+</sup>/toxin<sup>-</sup> subjects was associated with a particularly high risk of developing subsequent toxin positivity. The strong association between NAAT<sup>+</sup>/toxin<sup>-</sup> status and antibiotic receipt suggests this may be a particularly high-yield population for antimicrobial stewardship efforts. Prospective controlled trials are needed to prove whether stewardship interventions might prevent disease within this population.

Whether presumptive treatment of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects confers any clinical benefit remains unclear. Given that toxin testing has imperfect sensitivity for *C. difficile* disease, treatment may still be appropriate for at least some NAAT<sup>+</sup>/toxin<sup>-</sup> subjects: A recent meta-analysis found treatment may be associated with reduced overall mortality, but not recurrence risk [12, 13]. However, previous prospective studies found similar rates of diarrheal resolution among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects regardless of treatment [4]. Additionally, several large-scale studies found no difference in rates of 30-day mortality, intensive care unit admission, or colectomy upon conversion from NAAT to 2-step testing at the healthcare facility level [5, 14]. We observed a relatively low 5% (95% CI, 4%–6%) absolute rate of progression to toxin positivity overall among NAAT<sup>+</sup>/toxin<sup>-</sup> subjects. While 1 recent

prospective cohort study found a slightly higher rate of progression to toxin positivity (13%), that population was limited to recent acquisitions exclusively within hospitals or long-term care facilities [15]. Distinct study populations may explain the slight difference in observed progression rate, though our results are overall consistent in confirming that the vast majority of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects do not progress to *C. difficile* toxin positivity. Nevertheless, presumptive treatment was prescribed for nearly 30% of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects in our cohort. Though limited by smaller numbers and low event rates, presumptive treatment of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects appeared to be associated with lower risk of subsequent toxin positivity. A prior meta-analysis also found a slightly lower risk of toxin positivity when NAAT<sup>+</sup>/toxin<sup>-</sup> subjects received treatment (7.0% with treatment vs 11.6% without), though follow-up was limited to the first 60 days and the effect did not reach clinical significance [12]. Interestingly, all toxin<sup>+</sup> events in the presumptively treated group of this study occurred right at the end of the 90-day follow-up period, which raises the question of whether treatment prevents or delays disease. Vancomycin, the most commonly prescribed presumptive *C. difficile* treatment in this cohort, also damages the gut microbiota [16]. In fact, vancomycin has previously been associated with prolonged disruption of *C. difficile* colonization resistance [17]. Rates of severe disease (indicated by vancomycin enema receipt or colectomy) were very low overall and were not associated with receipt of presumptive treatment. Our results suggest that a small subgroup of NAAT<sup>+</sup>/toxin<sup>-</sup> subjects may benefit from presumptive treatment, though further prospective studies are needed to better identify this subgroup, define their optimal treatment, and ensure delay in disease is not misperceived as prevention.

Our study carries several key limitations. First, as it was based on a retrospective cohort, there is risk of residual confounding in event ascertainment and treatment decisions. Consequently, it is impossible to make any inferences about causation-only association. Second, our electronic health record queries were limited to prescriptions and laboratory testing within our health system. Any *C. difficile* episodes or antibiotic prescriptions originating outside of our healthcare system would not have been captured. Third, despite the large overall test cohort, *C. difficile* episodes remained relatively rare. As a result, some effect estimates were accompanied by wide CIs and some covariates could not be included in all models. Fourth, while we could verify antibiotic receipt for inpatient administrations, verifying adherence for outpatient antibiotic prescriptions was not possible. Fifth, generalizability may be limited as our study population exhibited a very high degree of healthcare exposure, with >85% of subjects having had a recent hospital admission.

At least within the confines of a retrospective cohort, we believe our study has multiple strengths. While prior studies assessed various aspects of the natural history of *C. difficile* NAAT<sup>+</sup>/toxin<sup>-</sup> status, we conducted 3 complementary analyses to describe a range of clinical outcomes—including risk of toxin positivity, rates of presumptive treatment, and an analysis of treatment effect on both toxin positivity and markers of severe disease. To address the potential for loss to follow-up or testing and antibiotic receipt outside our health system, we built a stringent censoring definition requiring ongoing in-person encounters during the 90-day follow-up period. By linking censoring to ongoing in-person follow-up, we aimed to increase the chances that care received during that time period would have been captured by electronic record query. Our estimated rates of CDI recurrence

among NAAT<sup>+</sup>/toxin<sup>+</sup> subjects match prospective clinical trials rather closely (19% in our study vs 15%–25% in some trials) [18]. Additionally, while time-to-event modeling carries assumptions that are difficult to assess directly, we conducted a wide range of analyses to assure robustness across an array of model types and strong censoring assumptions, with stable effect estimates for our main group of interest (NAAT<sup>+</sup>/toxin<sup>−</sup> subjects). We were careful to use models appropriate for recurrent events throughout—and always with robust CI estimates to avoid overestimating precision. Finally, testing and treatment practices may vary across clinical settings, leaving a residual source of potential confounding.

In conclusion, despite low progression rates in only 5% of those who were NAAT<sup>+</sup>/toxin<sup>−</sup>, *C. difficile* NAAT<sup>+</sup>/toxin<sup>−</sup> status was strongly associated with subsequent progression to toxin positivity, especially if accompanied by high-risk antibiotic receipt. Nearly one-third of NAAT<sup>+</sup>/toxin<sup>−</sup> subjects received presumptive *C. difficile* treatment without any apparent association with later risk of more severe toxin<sup>+</sup> disease. While the absolute risk of progression to *C. difficile* toxin positivity among NAAT<sup>+</sup>/toxin<sup>−</sup> subjects appears low overall, presumptive treatment may be associated with reduced risk of toxin positivity in a subset of initially NAAT<sup>+</sup>/toxin<sup>−</sup> subjects. Further studies are needed to better identify the NAAT<sup>+</sup>/toxin<sup>−</sup> subjects likely to benefit from presumptive treatment and define the optimal treatment.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Potential conflicts of interest.

S. C. G. reports receiving consulting fees from Gilead Sciences for serving on multiple data monitoring committees. All other authors report no potential conflicts.

All authors have 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.

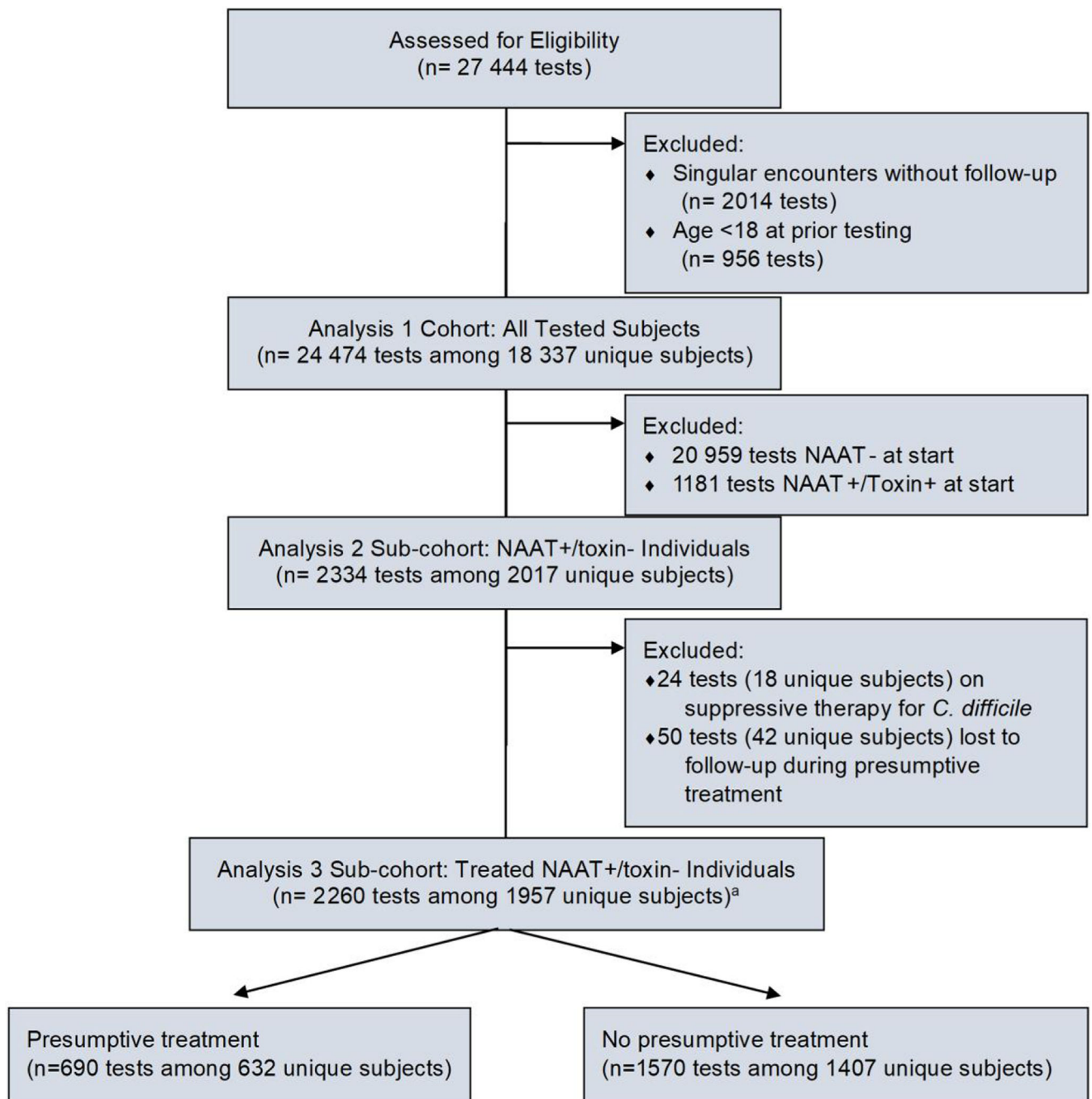
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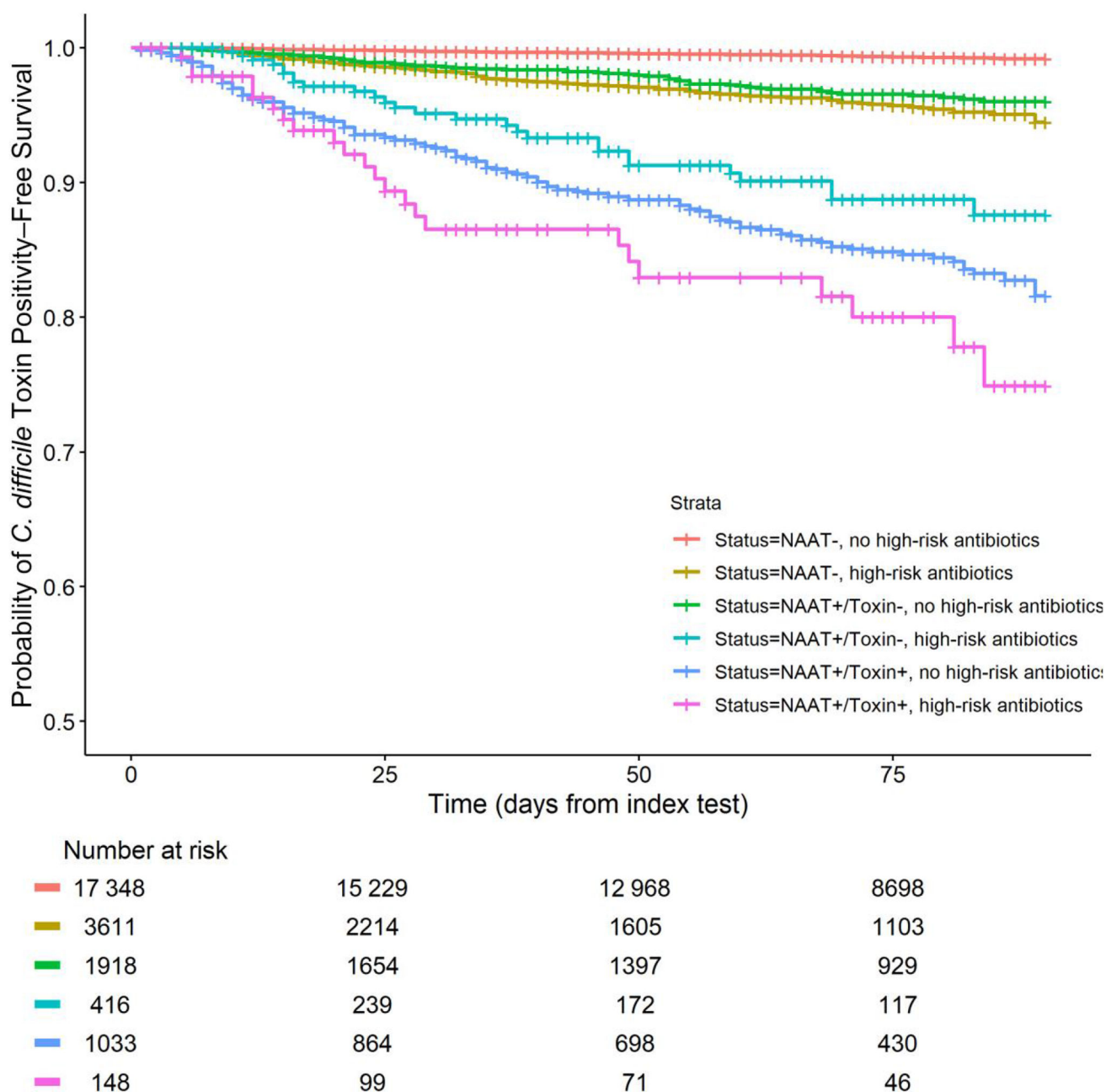
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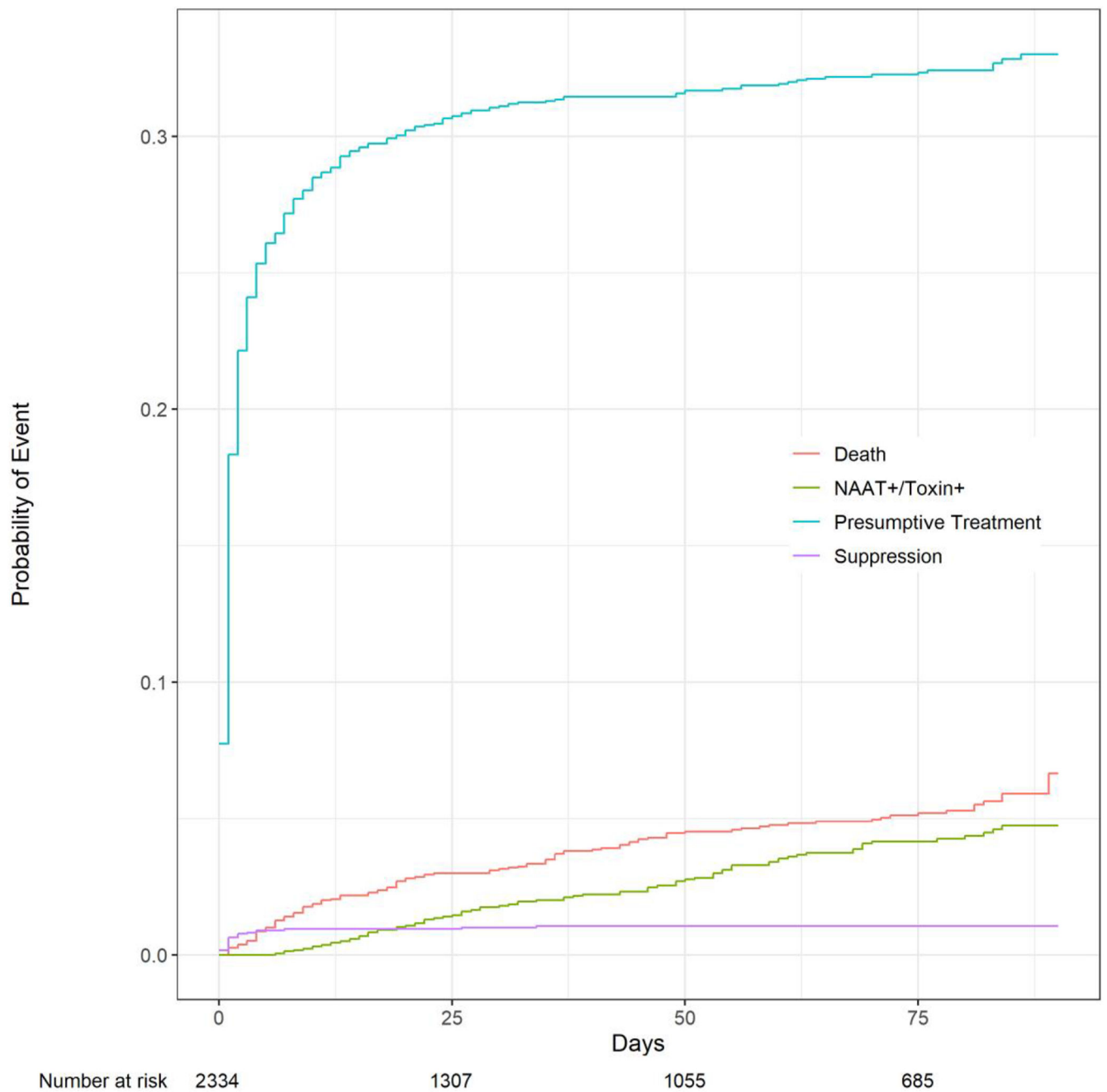


**Figure 1.**

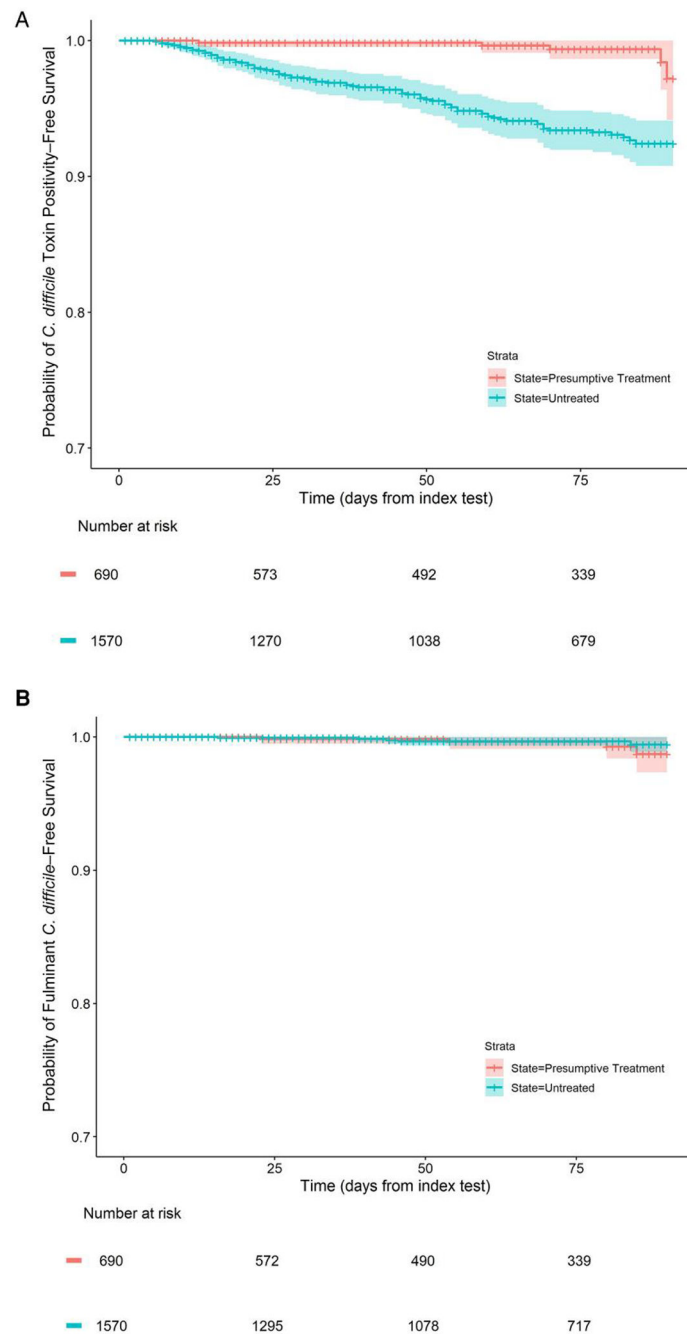
Cohort ascertainment and allocation: *Clostridioides difficile* tests conducted at Duke University Health System from 15 March 2020 to 31 December 2023. Abbreviation: NAAT, nucleic acid amplification test. <sup>a</sup>Sum of unique treated and untreated subjects may not equal sum of unique subjects in the aim 3 subcohort as 81 subjects were treated for 1 episode but not another.



**Figure 2.** Unadjusted Kaplan-Meier curve of *Clostridioides difficile* toxin positivity-free survival by initial test status and antibiotic receipt among adults tested for *C. difficile* within Duke University Health System, 15 March 2020–31 December 2023. Abbreviation: NAAT, nucleic acid amplification test.



**Figure 3.** Cumulative incidence curve—treatments received by nucleic acid amplification test (NAAT)  $^{+}/\text{toxin}^{-}$  subjects.



**Figure 4.**

Exploratory evaluation of presumptive treatment of nucleic acid amplification test (NAAT) <sup>+</sup>/toxin<sup>-</sup> patients. *A*, *Clostridioides difficile* toxin positivity-free survival among initially NAAT<sup>+</sup>/toxin<sup>-</sup> patients, by receipt of initial presumptive treatment. *B*, Fulminant *C. difficile*-free survival, by receipt of initial presumptive treatment. Fulminant *C. difficile* defined by receipt of rectal vancomycin for ileus or colectomy for toxic megacolon.

**Table 1.**  
Cohort Characteristics: *Clostridioides difficile* Tests Conducted at Duke University Health System, 15 March 2020 to 31 December 2023

| Characteristic                                             | Full Cohort (n = 24 474 Tests, 18 337 Unique Subjects) | PCR <sup>+</sup> (n = 20 959 Tests, 16 360 Unique Subjects) | PCR <sup>+</sup> /Toxin <sup>+</sup> (n = 2334 Tests, 2017 Unique Subjects) | PCR <sup>+</sup> /Toxin <sup>+</sup> (n = 1181 Tests, 992 Unique Subjects) |
|------------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Age, y, median (IQR)                                       | 62.5 (48.1–72.6)                                       | 62.2 (47.6–72.4)                                            | 63.6 (49.6–73.2)                                                            | 65.7 (53.4–74.7)                                                           |
| Sex, female                                                | 14 256 (58.2)                                          | 12 154 (58.0)                                               | 1375 (58.9)                                                                 | 727 (61.6)                                                                 |
| Race                                                       |                                                        |                                                             |                                                                             |                                                                            |
| Black or African American                                  | 6555 (26.8)                                            | 5556 (26.5)                                                 | 719 (30.8)                                                                  | 280 (23.7)                                                                 |
| White                                                      | 16 136 (65.9)                                          | 13 799 (65.8)                                               | 1497 (64.1)                                                                 | 840 (71.1)                                                                 |
| Asian                                                      | 439 (1.8)                                              | 389 (1.9)                                                   | 33 (1.4)                                                                    | 17 (1.4)                                                                   |
| Other                                                      | 805 (3.3)                                              | 724 (3.5)                                                   | 53 (2.3)                                                                    | 28 (2.4)                                                                   |
| Not reported                                               | 539 (2.2)                                              | 491 (2.3)                                                   | 32 (1.4)                                                                    | 16 (1.4)                                                                   |
| Ethnicity                                                  |                                                        |                                                             |                                                                             |                                                                            |
| Hispanic/Latino                                            | 1034 (4.2)                                             | 931 (4.4)                                                   | 62 (2.7)                                                                    | 41 (3.5)                                                                   |
| Non-Hispanic/Latino                                        | 22 589 (92.3)                                          | 19 279 (92.0)                                               | 2213 (94.8)                                                                 | 1097 (92.9)                                                                |
| Not reported                                               | 851 (3.3)                                              | 749 (3.6)                                                   | 59 (2.5)                                                                    | 43 (3.6)                                                                   |
| Comorbidities                                              |                                                        |                                                             |                                                                             |                                                                            |
| Complicated diabetes                                       | 6488 (26.5)                                            | 5432 (25.9)                                                 | 740 (31.7)                                                                  | 316 (26.8)                                                                 |
| Chronic kidney disease                                     | 9912 (40.5)                                            | 8235 (39.3)                                                 | 1137 (48.7)                                                                 | 540 (45.7)                                                                 |
| Cancer                                                     | 7905 (32.3)                                            | 6621 (31.6)                                                 | 841 (36.0)                                                                  | 443 (37.5)                                                                 |
| Liver disease                                              | 5680 (23.2)                                            | 4745 (22.6)                                                 | 657 (28.1)                                                                  | 278 (23.5)                                                                 |
| Solid organ transplant                                     | 2748 (11.2)                                            | 2322 (11.1)                                                 | 277 (11.9)                                                                  | 149 (12.6)                                                                 |
| Hematopoietic transplant                                   | 1761 (7.2)                                             | 1555 (7.4)                                                  | 126 (5.4)                                                                   | 80 (6.8)                                                                   |
| 2 or more prior <i>Clostridioides difficile</i> infections | 305 (1.2)                                              | 82 (0.4)                                                    | 30 (1.3)                                                                    | 193 (16.3)                                                                 |
| High-risk antibiotics <sup>a</sup> >48 h                   | 4175 (17.1)                                            | 3611 (17.2)                                                 | 416 (17.8)                                                                  | 148 (12.5)                                                                 |
| Admission in preceding 90 d                                | 21 153 (86.4)                                          | 18 018 (86.0)                                               | 2114 (90.6)                                                                 | 1021 (86.5)                                                                |
| Follow-up time, d, median (IQR)                            | 71 (40–84)                                             | 72 (40–85)                                                  | 70 (37–84)                                                                  | 67 (34–82)                                                                 |

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| Characteristic                     | Full Cohort (n = 24 474 Tests, 18 337 Unique Subjects) | PCR <sup>-</sup> (n = 20 959 Tests, 16 360 Unique Subjects) | PCR <sup>+</sup> /Toxin <sup>-</sup> (n = 2334 Tests, 2017 Unique Subjects) | PCR <sup>+</sup> /Toxin <sup>+</sup> (n = 1181 Tests, 992 Unique Subjects) |
|------------------------------------|--------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Subsequent toxin-positive episodes | 440 (1.8)                                              | 195 (0.9)                                                   | 83 (3.6)                                                                    | 162 (13.7)                                                                 |

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

<sup>a</sup>High-risk antibiotics defined as third- or fourth-generation cephalosporins, fluoroquinolones, or clindamycin received in the preceding 30 days.

Table 2.

Modeled Hazards of *Clostridioides difficile* Toxin Positivity Within 90 Days Among *C. difficile* Tests Conducted at Duke University Health System, 15 March 2020 to 31 December 2023

| Variable <sup>a</sup>                                  | Univariate HR (95% CI) | Multivariable HR (95% CI) | Multivariable HR With Strata (95% CI) |
|--------------------------------------------------------|------------------------|---------------------------|---------------------------------------|
| Preceding state                                        |                        |                           |                                       |
| NAAT <sup>+</sup> , no antibiotics                     | Reference              | Reference                 | Reference                             |
| NAAT <sup>+</sup> , antibiotics                        | 6.54 (4.53–9.43)       | 5.80 (4.02–8.38)          | 5.79 (4.01–8.36)                      |
| NAAT <sup>+</sup> /toxin <sup>−</sup> , no antibiotics | 5.16 (3.69–7.23)       | 5.07 (3.62–7.11)          | 5.06 (3.61–7.10)                      |
| NAAT <sup>+</sup> /toxin <sup>−</sup> , antibiotics    | 16.82 (10.61–26.67)    | 15.68 (9.83–25.02)        | 15.71 (9.85–25.06)                    |
| NAAT <sup>+</sup> /toxin <sup>+</sup> , no antibiotics | 25.34 (19.14–33.55)    | 23.46 (17.59–31.30)       | 23.47 (17.61–31.28)                   |
| NAAT <sup>+</sup> /toxin <sup>+</sup> , antibiotics    | 36.30 (22.29–59.11)    | 30.81 (18.39–51.59)       | 30.82 (18.35–51.75)                   |
| 2 or more prior CDIs                                   |                        |                           |                                       |
|                                                        | 7.00 (4.62–10.63)      | 1.24 (.82–1.86)           | 1.24 (.83–1.87)                       |
| Age                                                    |                        |                           |                                       |
|                                                        | 1.01 (1.00–1.02)       | 1.01 (1.00–1.01)          | 1.01 (1.00–1.01)                      |
| Chronic kidney disease                                 |                        |                           |                                       |
|                                                        | 1.60 (1.24–2.07)       | 1.26 (.99–1.61)           | Stratified                            |
| Cancer                                                 |                        |                           |                                       |
|                                                        | 1.60 (1.23–2.07)       | 1.06 (.82–1.38)           | 1.07 (.83–1.39)                       |
| Hematopoietic transplant                               |                        |                           |                                       |
|                                                        | 2.07 (1.39–3.09)       | 1.80 (1.22–2.68)          | Stratified                            |

Abbreviations: CDI, *Clostridioides difficile* infection; CI, confidence interval; HR, hazard ratio; NAAT, nucleic acid amplification test.

<sup>a</sup>Effect estimates obtained from Prentice-Williams-Peterson gap-time model.

**Table 3.**  
Adjusted Hazard for Outcomes Among Nucleic Acid Amplification Test–Positive, Toxin-Negative Subjects by Treatment Status

| Variable                                                                             | Univariate HR (95% CI) | Multivariable HR (95% CI) |
|--------------------------------------------------------------------------------------|------------------------|---------------------------|
| Outcome 1: Subsequent toxin positivity                                               |                        |                           |
| Presumptive treatment                                                                | 0.13 (.05–.31)         | 0.12 (.05–.29)            |
| High-risk antibiotics >48 h                                                          | 3.42 (2.10–5.56)       | 3.41 (2.08–5.57)          |
| 2 or more prior CDIs                                                                 | 1.64 (.42–6.32)        | 2.14 (.75–6.08)           |
| Age                                                                                  | 1.00 (.99–1.02)        | 1.00 (.98–1.01)           |
| Chronic kidney disease                                                               | 1.60 (1.02–2.50)       | 1.62 (.98–2.68)           |
| Cancer                                                                               | 0.97 (.62–1.52)        | 0.87 (.53–1.45)           |
| Hematopoietic transplant                                                             | 1.47 (.64–3.37)        | 1.62 (.65–4.03)           |
| Outcome 2: Need for fulminant <i>Clostridioides difficile</i> treatment or colectomy |                        |                           |
| Presumptive treatment                                                                | 1.73 (.47–6.39)        | 1.93 (.50–7.45)           |
| High-risk antibiotics >48 h                                                          | <i>a</i>               | <i>a</i>                  |
| 2 or more prior CDIs                                                                 | <i>a</i>               | <i>a</i>                  |
| Age                                                                                  | 1.04 (1.01–1.07)       | 1.05 (1.02–1.09)          |
| Chronic kidney disease                                                               | 0.30 (.06–1.44)        | 0.20 (.04–1.00)           |
| Cancer                                                                               | 2.07 (.54–7.89)        | 1.57 (.40–6.20)           |
| Hematopoietic transplant                                                             | <i>a</i>               | <i>a</i>                  |

Abbreviations: CDI, *Clostridioides difficile* infection; CI, confidence interval; HR, hazard ratio.

<sup>a</sup>Event rates within subgroup too low to permit inclusion in model.