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Effect of Nucleic Acid Amplification Testing on Population-Based Incidence Rates of *Clostridium difficile* Infection

Carolyn V. Gould,¹ Jonathan R. Edwards,¹ Jessica Cohen,^{1,2} Wendy M. Bamberg,³ Leigh Ann Clark,^{2,4} Monica M. Farley,^{4,5} Helen Johnston,³ Joelle Nadle,⁶ Lisa Winston,⁷ Dale N. Gerding,^{8,9} L. Clifford McDonald,¹ and Fernanda C. Lessa,¹ for the *Clostridium difficile* Infection Surveillance Investigators, Centers for Disease Control and Prevention

¹Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, and ²Atlanta Research and Education Foundation, Georgia; ³Colorado Department of Health, Denver; ⁴Georgia Emerging Infections Program; ⁵Division of Infectious Diseases, Emory University and Atlanta VA Medical Center, Atlanta, Georgia; ⁶California Emerging Infections Program, Oakland; ⁷Division of Infectious Diseases, University of California, San Francisco; ⁸Hines VA Hospital and ⁹Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois

Nucleic acid amplification testing (NAAT) is increasingly being adopted for diagnosis of *Clostridium difficile* infection (CDI). Data from 3 states conducting population-based CDI surveillance showed increases ranging from 43% to 67% in CDI incidence attributable to changing from toxin enzyme immunoassays to NAAT. CDI surveillance requires adjustment for testing methods.

Keywords. *Clostridium difficile*; clinical laboratory techniques; nucleic acid amplification techniques; incidence; surveillance.

Clostridium difficile infection (CDI) is an ongoing problem in healthcare, associated with high incidence, mortality, and healthcare costs [1]. The diagnosis of CDI has been problematic due to poor sensitivity of the most common testing method used by clinical laboratories, the enzyme immunoassay (EIA) for toxins A and B. As a result, many clinical laboratories have moved to more sensitive testing methods, including nucleic

acid amplification testing (NAAT) [2–5]. There are currently 5 Food and Drug Administration–approved NAAT assays targeting *C. difficile* toxin genes, utilizing either polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) [6], which appear to have similar performances [2, 5]. The higher sensitivity of these assays has led to concerns that their use will lead to higher CDI rates, particularly in the context of public reporting [7–10]. Several US states now mandate CDI reporting by healthcare facilities and publicly report their data, and as of January 2013, hospitals participating in the Centers for Medicare and Medicaid Services' Inpatient Prospective Payment System Quality Reporting Program are required to report facility-wide CDI events via the Centers for Disease Control and Prevention's National Healthcare Safety Network (NHSN) [11]. The aim of this analysis is to estimate the effect of switching from toxin EIA to NAAT on population-based incidence rates of CDI.

METHODS

The Emerging Infections Program's (EIP) CDI surveillance is a population- and laboratory-based surveillance system in selected counties in 10 US states, representing approximately 11.2 million persons. Trained surveillance epidemiologists investigate all positive *C. difficile* test reports from clinical, reference, and commercial laboratories for residents of surveillance catchment areas. A case of CDI is defined as a positive *C. difficile* toxin or molecular assay on a stool specimen from a resident ≥ 1 year of age of the surveillance catchment area without a prior positive stool in the previous 8 weeks [12].

CDI case counts and laboratory testing methods from the EIP CDI surveillance during 2009–2011 were evaluated. Only case counts were used for the analysis because we assumed that there were no changes in the populations served by the laboratories during the analysis period. Laboratories that changed their first-line testing for *C. difficile* from toxin EIA to NAAT ("switch laboratories") were compared to control laboratories in the same catchment areas that only used EIA during the evaluation period ("nonswitch laboratories"). Controls were used to adjust for any temporal changes in CDI incidence that may have occurred in each catchment area.

For switch laboratories and nonswitch laboratories in each catchment area, the medians of the ratios of CDI case counts during each consecutive month after the change to NAAT to case counts during equivalent months before the change to

Received 2 May 2013; accepted 22 July 2013; electronically published 29 July 2013.

Presented in part: IDWeek 2012, San Diego, California, 18 October 2012. Presentation 1315.

Correspondence: Carolyn V. Gould, MD, MSCR, Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion, 1600 Clifton Rd NE, MS A-31, Atlanta, GA 30333 (cgould@cdc.gov).

Clinical Infectious Diseases 2013;57(9):1304–7

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DOI: 10.1093/cid/cit492

NAAT were compared. By calculating ratios of case numbers before and after a given switch date for each laboratory, laboratories were compared to themselves, so there was no need to control for laboratory type in the analysis. To control for any potential seasonal variation in CDI incidence, equivalent months before and after switch dates were compared. Months during which laboratories changed from EIA to NAAT were not included as changes may have been implemented after the start of the month. To improve precision of the estimates, we required each group of switch laboratories and control laboratories in a given state to have at least 10 month-pairs for the catchment area to be included in the analysis. Median ratios for switch and nonswitch laboratories were compared using a one-sided nonparametric median test along with distribution-free 95% confidence intervals (CIs). The percentage of increase in CDI incidence in each catchment area attributable to a change to NAAT was calculated as the switch laboratory median ratio/nonswitch laboratory median ratio $\times 100$. The proportion of laboratory tests that were positive during the 3 months before implementation of NAAT was compared to the proportion positive during the 3 months after NAAT implementation using a Mid-P Exact test for laboratories with available information. All analyses were conducted using SAS version 9.2.

RESULTS

Eleven switch laboratories and 25 nonswitch laboratories in 3 states (California, Colorado, and Georgia) were included in the analysis. Nine laboratories switched to the Cepheid Xpert PCR assay (Cepheid, Sunnyvale, California) and 2 laboratories switched to the Meridian *illumigene C. difficile* LAMP assay (Meridian Bioscience, Cincinnati, Ohio). For each of the 3 states, the nonswitch laboratory median ratio was 1.0, indicating no apparent temporal change in CDI incidence in the catchment areas. The switch laboratory median ratios for California, Colorado, and Georgia were 1.52 (95% CI, .69–2.50), 1.43 (95% CI, 1.21–2.33), and 1.67 (95% CI, 1.50–2.06), corresponding to an attributable increase in CDI cases due to NAAT of 52%, 43%,

and 67%, respectively (Table 1). The difference between the switch laboratory median ratio and nonswitch laboratory median ratio was statistically significant at the .05 significance level for Colorado and Georgia.

Data on the percentage positive of samples tested were available from 6 of the 11 laboratories that switched from EIA to NAAT. The number of stool specimens tested for *C. difficile* decreased from 6660 during the 3 months before the switch to NAAT to 4896 during the 3 months after the switch, but the percentage positive increased from 693 of 6660 (10.4%) before the switch to 949 of 4896 (19.4%) after the switch.

DISCUSSION

This analysis of population-based surveillance data from catchment areas in 3 states demonstrated that switching from toxin EIA to NAAT for *C. difficile* diagnosis increased CDI incidence rates by 43%–67%. The absence of CDI increases in the control laboratories confirms that there was no overall temporal increase in CDI in the areas under surveillance, supporting the conclusion that the CDI increases in the switch laboratories were directly related to the introduction of NAAT methods. Other studies in individual healthcare centers have reported increases in CDI incidence rates of 57% [13] to 110% [9] after a switch from toxin EIA to PCR. Longtin et al [7] reported a 50% increase in CDI diagnoses by PCR compared to parallel testing with a 3-step algorithm (glutamate dehydrogenase [GDH] antigen + toxin EIA followed by cell culture cytotoxicity assay for GDH⁺/EIA⁻ specimens). The effects of switching to multi-step algorithms involving PCR have also been reported; Goldenberg [8] reported a 97% increase in rates after changing from toxin EIA to a 2-step algorithm (GDH antigen with PCR confirmation). Adding a reflex PCR to a 2-step algorithm of GDH antigen + toxin EIA led to a 70% increase in rates in another report [10]. We opted to examine the effect of switching one-step approaches from toxin EIA to NAAT, which enabled us to aggregate data from multiple laboratories and isolate the effect of molecular methods on *C. difficile* detection.

Table 1. Ratios of Monthly Postswitch *Clostridium difficile* Infection (CDI) Case Counts to Preswitch CDI Case Counts

State	Switch Laboratories		Nonswitch Laboratories		Median Test P Value	Attributable % Increase due to NAAT
	No. of Month-Pairs	Median Ratio (95% CI)	No. of Month-Pairs	Median Ratio (95% CI)		
California	14	1.52 (.69–2.50)	56	1.0 (1.00–1.50)	.09	52%
Colorado	24	1.43 (1.21–2.33)	161	1.0 (.85–1.06)	.0002	43%
Georgia	50	1.67 (1.50–2.06)	149	1.0 (.89–1.04)	<.0001	67%

Data are for laboratories that changed from toxin enzyme immunoassay (EIA) to nucleic acid amplification testing (switch laboratories) versus laboratories only using toxin EIA (nonswitch laboratories). Each month-pair represents 2 matching pre- and postswitch months (eg, October 2010 vs October 2009) used to calculate the postswitch to preswitch ratios. Therefore, the number of postswitch months was equal to the number of preswitch months for each state.

Abbreviations: CI, confidence interval; NAAT, nucleic acid amplification testing.

Given these reports, concerns have been raised for national and public reporting that facilities using more sensitive diagnostic methods for CDI will be penalized for having higher CDI rates [7–10]. To address this, the NHSN is collecting data from reporting facilities on testing practices used for CDI determination and will adjust for these methods in the calculation of comparative measures used for reporting [14]. In the future, as more laboratories adopt more sensitive testing methods, differences in detection will become less of an issue.

We found that the proportion of stool tests that were positive almost doubled (10.4% to 19.4%) with NAAT compared to toxin EIA. Fong et al [9] reported that laboratory positivity more than doubled after switching from toxin EIA to NAAT, whereas other studies reported more modest increases (45%–74%) [13, 15]. Notably, we found that the number of stools tested in the 3 months after implementation of NAAT was much lower than during the EIA period, likely reflecting the institution of stool rejection policies and less repeat testing with NAAT. Appropriate stool rejection policies should reduce detection of asymptomatic *C. difficile* carriage as well as costs and resources expended on duplicate testing.

Despite the increase in rates expected with molecular testing, some have suggested that rates may drop over time because of the potential benefits of NAAT for infection prevention [7, 8]. Greater sensitivity of testing could lead to more accurate diagnosis and more timely initiation of infection control measures and treatment, thereby reducing the risk of transmission. There are also potential clinical benefits of NAAT. Greater sensitivity, which obviates the need for repeat testing, may lead to decreases in length of stay, days on empiric CDI treatment and isolation, and associated costs [15–17]. One study found fewer complications among CDI cases detected by PCR but not by a 3-step nonmolecular protocol, which could reflect benefits of earlier diagnosis and treatment [7]. Alternatively, these findings could indicate increased detection of less severe CDI cases or greater detection of *C. difficile* carriers who have diarrhea from unrelated causes [7]. Further research is needed to understand whether use of NAAT for CDI diagnosis may lead to improved patient outcomes.

This analysis was limited by the number of EIP laboratories that switched from EIA to NAAT and the number of months for evaluating before and after case counts, which restricted the analysis to only 3 states. More precise estimates from a more representative sample could be generated with additional longitudinal data. We did not evaluate the effect of other testing methods (eg, 2- or 3-step testing algorithms) on rates. Finally, we used a monthly unit of analysis to generate ratios to have enough data points to obtain median values. A more aggregated (eg, bimonthly) unit of analysis would improve the stability of the estimates and could be done with ongoing surveillance.

In conclusion, based on an analysis of population-based surveillance data from 3 states, we expect that switching from toxin EIA to NAAT as a first-line testing method for CDI could increase CDI rates by as much as 67% due to greater sensitivity. Although further analysis is needed to refine these estimates, our findings can help facilities anticipate the increase in CDI incidence expected when switching to NAAT and thus help inform prevention activities. From the standpoint of public reporting, CDI surveillance requires adjustment for testing methods.

Notes

Acknowledgments. The authors acknowledge the following contributors: Zintars Beldavs, MS, Oregon Health Authority; Samir Hanna, MD, MS, Tennessee Department of Health; Gary Hollick, PhD, University of Rochester; Stacy Holzbauer, DVM, MPH, Centers for Disease Control and Prevention CEFO assigned to the Minnesota Department of Health; Carol Lyons, MPH, Connecticut Emerging Infections Program; Erin Phipps, DVM, MPH, University of New Mexico, New Mexico Emerging Infections Program; Lucy Wilson, MD, ScM, Maryland Department of Health and Mental Hygiene.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Potential conflicts of interest. D. N. G. holds patents for treatment of CDI licensed to ViroPharma; is a consultant for Merck, ViroPharma, GSK, Roche, Novartis, Optimer, Cubist, Cangene, Sanofi Pasteur, and Actelion; and holds research grants from GOJO. 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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