

## A Nosocomial Outbreak of Norovirus Infection Masquerading as *Clostridium difficile* Infection

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**Noroviruses (NoVs) are increasingly being recognized as important enteric pathogens. At a university-based hospital, we investigated a nosocomial outbreak of NoV infection that was originally attributed to *Clostridium difficile*. We describe here the unique challenges of the identification of NoVs as the true etiologic pathogen in an outbreak occurring in a health care setting, where *C. difficile* infection is endemic, as well as the important lessons learned.**

In the United States, noroviruses (NoVs) are the most common cause of foodborne diseases [1, 2] and are the etiologic agents of ~23 million cases of gastroenteritis each year [3]. Because of their low infectious dose, environmental stability, and resistance to most cleaning agents, NoVs are frequently associated with epidemic outbreaks [4, 5]. Although a self-limited illness, NoV gastroenteritis results in a tremendous public health burden, leading to temporary closures of affected institutions, the overwhelming of capabilities to manage infected persons, and substantial financial loss [6–8]. We describe here a challenging outbreak that was originally attributed to *Clostridium difficile* and the important lessons learned from this outbreak.

**Methods.** The outbreak occurred in the Michael E. DeBakey Veterans Affairs Medical Center, a 357-bed university-

affiliated teaching hospital in Houston, Texas. Psychiatry unit 1 has 22 patient beds, and the adjacent psychiatry unit 2 has 28 beds.

We were initially asked to investigate an outbreak of *C. difficile* infection that occurred from 8 March through 24 March 2008. The initial outbreak stool samples collected tested positive for *C. difficile* toxin by ELISA (Premier *C. Difficile* Toxin A+B; Meridian Bioscience). However, because of the rapid dissemination of disease, a failure to identify *C. difficile* infection in subsequent cases, a lack of response to conventional therapy for *C. difficile* infection, and a history of NoV outbreak in psychiatry unit 1 (in 2004), an investigation of NoV infection was also performed. Case subjects were defined as admitted patients or hospital health care workers (HCWs) who (1) had contact with the psychiatry or adjacent units or with existing case subjects and (2) had acute gastroenteritis manifesting as vomiting or diarrhea through 31 March 2008. Diarrhea was defined as  $\geq 2$  loose stools within a 24-h period. A standardized questionnaire was used to collect information from case subjects, including demographic information, symptom characteristics, and epidemiological risk factors.

Stool samples were tested for *C. difficile* toxins A and B by ELISA (Premier *C. Difficile* Toxin A+B). All available stool samples were also cultured on cycloserine-cefoxitin-fructose agar (CCFA) for *C. difficile* after alcohol shock treatment with 100% ethanol, to kill vegetative cells. *C. difficile* isolates were further characterized by testing for 16S rRNA, *tcdA* (toxin A), *tcdB* (toxin B), *tcdC* (putative negative regulator of toxin A and B production), and *cdtA* and *cdtB* (binary toxin) genes, as described elsewhere [9]. Evaluation of the presence of *tcdC* deletions associated with the epidemic BI/NAP1/027 strains was performed using microsatellite DNA technology [10, 11].

Stool samples from prospective case subjects were sent to the Center for Infectious Diseases laboratory at the University of Texas–Houston School of Public Health and to the Houston Department of Health and Human Services for evaluation for NoVs. The Center for Infectious Diseases laboratory performed conventional RT-PCR for NoV detection, as described elsewhere [12], and the Houston Department of Health and Human Services laboratory performed real-time RT-PCR. Sequence analysis for NoV-positive specimens was performed using methods that have been described elsewhere [13], with genotypes determined by comparing nucleotide sequences with those of prototype strains.

To evaluate for *C. difficile* contamination, environmental sampling of the 2 psychiatry units was performed using sterile

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cotton swabs moistened in Luria-Bertani broth. Swabs of the surfaces of toilets and other items commonly contaminated with *C. difficile*—such as sinks, bathroom railings, bed railings, door knobs, windowsills, floors, and chairs in case patients' rooms and common living areas—were collected [14–16]. The swab samples were cultured on CCFA for *C. difficile* after alcohol shock treatment.

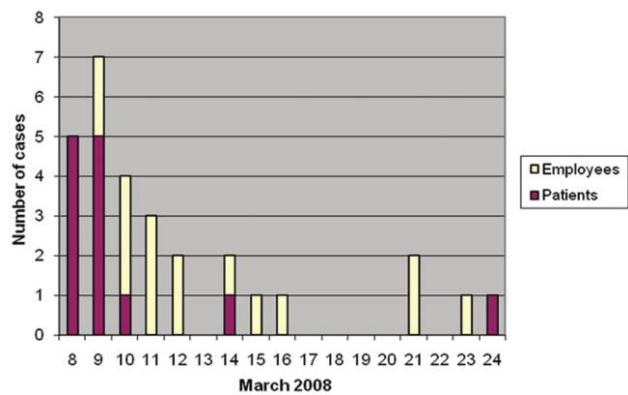
**Results.** From 8 March through 24 March 2008, a total of 13 admitted patients and 16 HCWs met the case definition (figure 1). There was initial confusion concerning the etiologic agent of the outbreak, because 5 stool samples from HCWs tested positive for *C. difficile* toxin. However, the 12 subsequent stool samples tested revealed no evidence of *C. difficile* infection. Stool samples from the remaining 12 case subjects were unavailable for testing. At least 1 case subject with presumed *C. difficile* infection was given metronidazole without clinical response, as nausea, vomiting, and diarrhea persisted.

Three of the 5 *C. difficile*-positive stool samples were eventually shown to contain NoV RNA. Five of 5 tested stool samples from admitted patients and 7 of 12 tested stool samples from HCWs revealed the presence of NoVs. Results from the Houston Department of Health and Human Services and the Center for Infectious Diseases laboratory were completely consistent. Genotype was determined for 4 of the 12 NoV strains detected in this outbreak. The 4 NoV strains had capsid nucleotide sequences 100% identical to the GII.4 NoV Hu/GII-4/C5-159/South Korea strain (GenBank accession number EU003965).

Secondary transmission among the admitted patients was believed to be facilitated by close quarters, shared bathrooms and living areas, and encouragement to actively socialize with other patients. All ill HCWs either worked on the affected wards or interacted with case patients. Once the outbreak in psychiatry unit 1 was recognized, new admissions were halted, but patients in this unit were allowed to have access to the adjacent psychiatry unit 2 for group therapy, where potentially 4 HCWs became infected.

Interestingly, *C. difficile* was isolated on CCFA under anaerobic conditions from 1 *C. difficile* toxin-positive stool sample from a HCW and from 2 *C. difficile* toxin-negative stool samples from admitted patients. The *C. difficile* 16S rRNA gene was detected in all 3 isolates. The presence of the *tcdA*, *tcdB*, and *tcdC* genes were demonstrated in only 1 *C. difficile* isolate from an admitted patient, but the stool sample tested negative for toxin A and B production by ELISA. The hypervirulent *C. difficile* strain was not detected (there was no evidence of the *tcdC* deletion or of binary toxin production). Both admitted patients with *C. difficile* detection by culture were infected with NoVs.

Environmental sampling for contamination of the psychiatric units with *C. difficile* spores yielded no *C. difficile* isolates. It



**Figure 1.** Epidemic curve of the outbreak of norovirus infection.

should be noted, however, that cleaning and disinfection measures had been initiated before samples could be obtained from these areas.

Rapid implementation of infection-control measures by the hospital infection-control team limited the extent of this outbreak and led to its early termination. These interventions included closure of the psychiatry units to new admissions, instruction of ill HCWs to not report to work until at least 72 h after the resolution of symptoms, and careful surveillance for new exposures and cases. Disinfection of patients' rooms involved initial cleaning with an all-purpose cleaner (35% 2-butoxyethanol and 5%–20% nonionic surfactant), followed by the application of a bleach-impregnated disposable cloth to environmental surfaces 3 times daily. Strict hand-hygiene practice (with soap and water) was reinforced and monitored by the infection-control staff.

**Discussion.** During outbreaks of acute gastroenteritis in health care facilities, where patients are at risk for *C. difficile* infection, detection of *C. difficile* can create confusion regarding the causative agent. Increased collection and testing of stool samples for *C. difficile*, in addition to false-positive results associated with *C. difficile* assays, may be reflected by increased rates of *C. difficile* detection [17–19]. In the outbreak of NoV infection analyzed here, testing individuals who lacked traditional risk factors for *C. difficile* infection (such as HCWs) likely led to *C. difficile* false-positive results. However, there was no evidence of increased *C. difficile* testing during this outbreak compared with that during the previous and subsequent months. Asymptomatic *C. difficile* colonization also contributed to the confusion during this outbreak in a hospital setting, where *C. difficile* infection is endemic. Interactions between distinct enteric pathogens such as *C. difficile* and NoVs have not been studied, and it is unknown whether *C. difficile* infection may somehow augment the pathogenesis of NoV infections or vice versa. Distinguishing the true enteric pathogen responsible for an outbreak has important therapeutic implications,

such as the use of supportive treatment for NoV infections and antibiotics for *C. difficile* infections.

We believe that coincidental *C. difficile* colonization and false-positive results were encountered in this NoV outbreak because of fecal sampling to detect potential cases and a heightened awareness of diarrheal cases. Five of 7 case subjects for whom *C. difficile* was detected by either ELISA or stool culture were shown to be infected with NoVs, and at least 1 of these case subjects was given appropriate antimicrobial therapy for *C. difficile* infection but failed to improve clinically.

Two clusters of cases are apparent from the epidemic curve, with the second cluster occurring 5 days after the 25th case. Although the incubation period for NoV infection is typically 24–48 h, NoV persistence in fecal shedding [20] and on environmental surfaces [21] may have led to the second group of cases. Prolonged NoV fecal shedding of up to several weeks after infection has been well described [20]. Infected HCWs were allowed to return to work 72 h after the resolution of symptoms, in accordance with Centers for Disease Control and Prevention recommendations [22]. These recommendations regarding the resumption of duties by HCWs recovering from acute NoV gastroenteritis may need to be reevaluated.

We expect that, as recognition of NoVs as a cause of acute gastroenteritis increases with improvements in and greater accessibility to molecular diagnostic testing, the importance of this enteric pathogen will continue to become more apparent. A better understanding of the significance of the detection of multiple potential pathogens (such as NoVs and *C. difficile*) during a single outbreak is critical to improving implementation of the optimal therapeutic and preventive strategies for patients.

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## References

1. Lynch M, Painter J, Woodruff R, et al. Surveillance for foodborne-disease outbreaks—United States, 1998–2002. *MMWR Surveill Summ* **2006**; *55*:1–42.
2. Bresee JS, Widdowson M-A, Monroe SS, Glass RI. Foodborne viral gastroenteritis: challenges and opportunities. *Clin Infect Dis* **2002**; *35*: 748–53.
3. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis* **1999**; *5*:841–2.
4. Le Guyader FS, Atmar RL. Binding and inactivation of viruses on and in food, with a focus on the role of the matrix. In: Koopmans M, Bosch A, Cliver D, eds. *Food-borne viruses: a multidisciplinary review of progress and challenges in food-borne viruses research*. Washington, DC: American Society for Microbiology Press (in press).
5. National Institute of Allergy and Infectious Diseases Biodefense Research. NIAID category A, B, and C priority pathogens. Available at: <http://www3.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/research/CatA.htm>. Accessed 10 January 2008.
6. Johnston CP, Qiu H, Ticehurst JR, et al. Outbreak management and implications of a nosocomial norovirus outbreak. *Clin Infect Dis* **2007**; *45*:534–40.
7. White C. Scale of norovirus outbreak may have been exaggerated. *BMJ* **2008**; *336*:66.
8. Lopman BA, Brown DW, Koopmans M. Human caliciviruses in Europe. *J Clin Virol* **2002**; *24*:137–60.
9. Borriello SP, Honour P. Simplified procedure for the routine isolation of *Clostridium difficile* from feces. *J Clin Pathol* **1981**; *34*:1124–7.
10. Kassai-Jäger E, Ortutay C, Tóth G, Vellai T, Gáspári Z. Distribution and evolution of short tandem repeats in closely related bacterial genomes. *Gene* **2008**; *410*:18–25.
11. Guo DC, Milewicz DM. Methodology for using a universal primer to label amplified DNA segments for molecular analysis. *Biotechnol Lett* **2003**; *25*:2079–83.
12. Anderson AD, Garrett VD, Sobel J, et al. Multistate outbreak of Norwalk-like virus gastroenteritis associated with a common caterer. *Am J Epidemiol* **2001**; *154*:1013–9.
13. Green KEA. Caliciviridae: the noroviruses. In: Knipe DEA, ed. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; **2007**: 949–79.
14. Verity P, Wilcox MH, Fawley W, Parnell P. Prospective evaluation of environmental contamination by *Clostridium difficile* in isolation side rooms. *J Hosp Infect* **2001**; *49*:204–9.
15. Wilcox MH, Fawley WN, Wigglesworth N, Parnell P, Verity P, Freeman J. Comparison of the effect of detergent versus hypochlorite cleaning on environmental contamination and incidence of *Clostridium difficile* infection. *J Hosp Infect* **2003**; *54*:109–14.
16. Dubberke ER, Reske KA, Noble-Wang J, et al. Prevalence of *Clostridium difficile* environmental contamination and strain variability in multiple health care facilities. *Am J Infect Control* **2007**; *35*:315–8.
17. Martin AJ, Collins CJ, Ruddy R, Drudy D, Hannan MM, Kyne L. Simultaneous control of norovirus and *Clostridium difficile* outbreaks due to enhanced infection prevention and control measures. *J Hosp Infect* **2008**; *68*:180–1.
18. Bignardi GE, Staples K, Majmudar N. A case of norovirus and *Clostridium difficile* infection: casual or causal relationship? *J Hosp Infect* **2007**; *67*:198–200.
19. Barrett SP, Holmes AH, Newsholme WA, Richards M. Increased detection of *Clostridium difficile* during a norovirus outbreak. *J Hosp Infect* **2007**; *66*:394–5.
20. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. *Emerg Infect Dis* **2008**; *14*:1553–7.
21. D'Souza DH, Sair A, Williams K, et al. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int J Food Microbiol* **2006**; *108*:84–91.
22. LeBaron CW, Furutan NP, Lew JF, et al. Viral agents of gastroenteritis: public health importance and outbreak management. *MMWR Recomm Rep* **1990**; *39*:1–24.