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Comparison of a Rapid Molecular Method, the BD GeneOhm Cdiff Assay, to the Most Frequently Used Laboratory Tests for Detection of Toxin-Producing *Clostridium difficile* in Diarrheal Feces▽

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Six hundred diarrheal stool specimens were collected from inpatients and outpatients at local university hospitals for the detection of toxigenic *Clostridium difficile* using three parallel methods, the BD GeneOhm Cdiff assay, the tissue culture cytotoxicity assay, and a commercially available enzyme-linked fluorescence immunoassay (ELFA) (Vidas *C. difficile* toxin A and B assay; bioMérieux). Toxigenic *C. difficile* culture was also performed to further clarify discordant results. During a 3-month study period, 58 (9.7%) of the 600 diarrheal samples examined were positive by the BD GeneOhm Cdiff assay, while the Vidas *C. difficile* toxin A and B assay and the cytotoxicity assay performed directly on stool samples gave 4.7% and 6.3% positivity rates, respectively. In the case of four samples, BD GeneOhm Cdiff assay results were not evaluable at first because of the presence of PCR inhibitors, but upon repeat testing from the frozen lysates, all of these samples proved to be negative. After resolution with toxigenic culture, the cytotoxicity assay proved to be positive in 55 samples (9.2%), while the ELFA was positive in 37 samples (6.2%). Results of culture and repeated cytotoxicity assays emphasized the importance of the culture method, because the use of ELFA or enzyme immunoassay without a culture method may lead to a substantial portion of toxigenic *C. difficile* strains being missed.

Toxin-producing *Clostridium difficile* strains are important pathogens among patients who are treated with antibiotics or chemotherapeutic agents not only in the hospital environment but also in the community (3, 6, 10). Since the recognition of outbreaks of *C. difficile* infection (CDI) caused by *C. difficile* PCR ribotype 027 in Canada, the United States, and several European countries, rapid and accurate diagnosis of CDI is very important to stop the spread of these strains (7, 8, 19). In addition, the increasing morbidity and mortality rates associated with CDI and the increasing number of recurrences and therapeutic failures also highlight the need for the development of a rapid and reliable detection method for toxigenic *C. difficile* in diarrheal feces (12).

Only a few laboratories routinely use the tissue culture cytotoxicity and toxin neutralization assays for the detection of toxigenic *C. difficile* in feces, because they are labor-intensive and time-consuming and standardization is very difficult. Due to their rapid turnaround time, enzyme immunoassays (EIAs) that detect toxin A and/or toxin B in stool are used in most laboratories (11, 16). To increase the sensitivity of these tests and in some instances to facilitate epidemiological investigations, culture of *C. difficile* has become essential. In spite of this, most laboratories use a single toxin detection test on feces for detection of toxigenic *C. difficile* (4). In the last 10 years, in-house PCR and real-time PCR assays have been developed to detect *C. difficile* toxin genes. These assays have shown very good sensitivity and specificity and short turnaround times (1, 17). However, widespread use of PCR methods in routine clinical microbiology is limited because these tests require special DNA extraction procedures to eliminate PCR inhibitors from fecal specimens and they cost more than do traditional testing methods.

The BD GeneOhm Cdiff assay provides a rapid method for the qualitative detection of the *C. difficile* toxin B gene (*tcdB*) in diarrheal specimens from patients suspected of having CDI. This test is based on the amplification of the *tcdB* gene and the detection of the amplified DNA using fluorogen-labeled probes. Amplification, detection, and interpretation of the results are done automatically by the SmartCycler instrument (Cepheid, Sunnyvale, CA).

Our aims were to compare the performance of the BD GeneOhm Cdiff assay to those of the tissue culture cytotoxicity assay and a commercially available enzyme-linked fluorescence immunoassay (ELFA) (Vidas *C. difficile* toxin A and B assay; bioMérieux, Marcy-l'Etoile, France), for the direct detection of toxins A and B from fecal samples.

MATERIALS AND METHODS

Stool specimens. Six hundred diarrheal stool specimens were collected from inpatients and outpatients in the local university hospital during a 3-month period. The samples were submitted by physicians to the laboratory for routine *C. difficile* testing by toxin A/B EIA, on the suspicion that these patients had CDI. On arrival at the laboratory, the stool samples were immediately tested by the ELFA (Vidas *C. difficile* toxin A and B assay; bioMérieux, Marcy-l'Etoile, France) and culture and were stored at 4°C for testing within 5 days by the BD

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TABLE 1. Comparison of the BD GeneOhm Cdiff assay to the tissue culture cytotoxicity assay for detection of toxigenic *C. difficile* from diarrheal stool after initial testing and after resolution with toxigenic culture and upon repeat PCR testing from frozen lysates

PCR result type	No. of results for cytotoxicity assay					
	Initial results			After resolution with toxigenic culture		
	Positive	Negative	Total	Positive	Negative	Total
Positive	36	22	58	53	5	58
Negative	2	536	538	2	540	542
Total	38	558	596	55	545	600

TABLE 2. Comparison of the BD GeneOhm Cdiff assay to the Vidas *C. difficile* toxin A and B assay for detection of toxigenic *C. difficile* from diarrheal stool after initial testing and after resolution with toxigenic culture and upon repeat PCR testing from frozen lysates

PCR result type	No. of results of toxin A and B assay					
	Initial results			After resolution with toxigenic culture		
	Positive	Equivocal	Negative	Positive	Negative	Total
Positive	20	9	29	29	29	58
Negative	8	30	500	8	534	542
Total				37	563	600

GeneOhm Cdiff assay and tissue culture cytotoxicity assay. The molecular method, the ELFA, the cytotoxicity assay, and culture were performed by three persons blinded to the results of other tests during this study, and a second reader also evaluated the cytotoxic activity.

***C. difficile* toxin detection using the ELFA method.** The Vidas *C. difficile* toxin A and B assay (bioMérieux, Marcy-l'Etoile, France) was used for the detection of toxins A and B directly from diarrheal stool samples and culture, and the results were available within 3 h of receipt in the laboratory.

Cytotoxicity assay on HeLa cell line. The tissue culture cytotoxicity assay was performed on all diarrheal samples using the HeLa cell line. Stool specimens were diluted in phosphate-buffered saline buffer (1:10, wt/vol) and then centrifuged at 12,000 rpm for 10 min. The supernatant was filtered through a 0.22-μm-pore-size membrane filter (Millex-GV; Millipore Co. Ltd., Billerica, MA). Twenty microliters of filtered supernatant was transferred onto the HeLa cell line grown at 37°C in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum in a 96-well microtiter plate. Prior to testing for cytotoxicity, the cell culture medium was removed and fresh medium was added without serum. The plates were incubated at 37°C for 24 h in 5% CO_2 to confirm any observed cytopathic effect; toxin neutralization with *C. difficile* goat antitoxin serum was performed (9). Broth culture of the reference strain VPI 10463 (ATCC 43255, toxinotype 0) obtained from the American Type Culture Collection (Manassas, VA) was used as a positive control in the cytotoxicity assay.

BD GeneOhm Cdiff assay. The BD GeneOhm Cdiff assay was performed according to the manufacturer's instructions. Briefly, the diarrheal stool specimen was vortexed at high speed and transferred to the sample buffer provided by the manufacturer by using a dry sterile swab. The tightly closed tube containing the broken swab was vortexed at high speed for 1 min. Forty microliters of sample buffer was transferred to the lysis tube, and 10 μl of the vortexed stool specimens was transferred to this tube. The lysis tube was vortexed at high speed for 5 min, after which it was centrifuged for about 5 s to collect the contents at the bottom of this tube. The lysis tube was incubated at 95°C for 5 min and placed immediately on ice. Two hundred twenty-five microliters of diluent was added to the Master Mix tube (provided by the manufacturer), to prepare a reaction mix sufficient for eight reactions including one negative and one positive control. The positive and negative controls were each dissolved in 225 μl sample buffer. Twenty-five microliters of Master Mix was transferred to each SmartCycler tube in a cooling block, and 3 μl of each lysed specimen and positive and negative controls was added to the labeled SmartCycler tubes. The tubes were centrifuged for 10 s and placed into an I-Core of the SmartCycler instrument.

Culture of *C. difficile*. All diarrheal samples were cultured on selective medium (*C. difficile* agar base supplemented with 7% [vol/vol] defibrinated horse blood, 250 mg/liter cycloserine, and 8 mg/liter cefoxitin) (Oxoid), and the plates were incubated at 37°C for 48 h under anaerobic conditions (85% N_2, 10% CO_2, 5% H_2; Bactron; Sheldon Manufacturing Inc.). The culture isolates were identified on the basis of characteristic *C. difficile* colony morphology, odor, fluorescence, and Gram stain; in the case of any discrepancy, the API 20A (bioMérieux, Marcy-l'Etoile, France) identification system was used. Pure isolated *C. difficile* colonies were subcultured in chopped meat broth and incubated for 48 h under anaerobic conditions. The supernatant was tested using the tissue culture cytotoxicity assay on the HeLa cell line; this was considered a toxigenic culture.

RESULTS

A total of 600 diarrheal feces samples collected from inpatients and outpatients were examined for the presence of toxigenic *C. difficile*. Initially, four samples were inhibitory to PCR, but upon repeat testing of samples from frozen lysates, all of these samples proved to be negative. During the study period, 9.7% of the specimens were positive by the BD GeneOhm Cdiff assay. The tissue culture cytotoxicity assay and the Vidas *C. difficile* toxin A and B assay had positivity rates of 6.3% and 4.7%, respectively, from direct testing of stool samples. On initial testing, 36 samples (6%) were positive and 536 (90%) were negative by the BD GeneOhm Cdiff assay and the tissue culture cytotoxicity assay (Table 1). Samples with results discordant between the two methods were resolved by toxigenic culture. Seventeen samples which were positive by the BD GeneOhm Cdiff assay but negative by the cytotoxicity assay were confirmed as truly positive by toxigenic culture (Table 1). In five cases, the BD GeneOhm Cdiff assay gave positive results while the cytotoxicity assay and toxigenic culture were negative. Two samples were positive by the cytotoxicity assay but negative by the BD GeneOhm Cdiff assay; these were confirmed as true positives by toxigenic culture. With the use of the tissue culture cytotoxicity assay and resolution of discordant specimens with toxigenic culture, the sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) of the BD GeneOhm Cdiff assay after discordant result analysis were 96.4%, 99.1%, 92%, and 100%, respectively (Table 1).

Of the 600 samples, 39 gave equivocal results by the Vidas *C. difficile* toxin A and B assay; in these cases cytotoxicity assay and culture were used to confirm these results (Table 2). Of 39 diarrheal samples, 30 proved to be negative by the BD GeneOhm Cdiff assay, cytotoxicity test, and culture, while nine specimens gave positive results by real-time PCR and toxigenic culture (Table 2). Concordant results were observed for 20 positive and 500 negative samples (Table 2). Six samples were positive by only the Vidas *C. difficile* toxin A and B assay.

Nontoxigenic *C. difficile* strains were isolated from 19 diarrheal samples; these samples were negative by the BD GeneOhm Cdiff assay and Vidas *C. difficile* toxin A and B assay. The majority of toxin-positive samples were collected from patients in the intensive care unit (34.6%). Two out of eight samples from the dermatology unit and one out of four samples from the surgery department proved to be positive for

toxigenic *C. difficile*; these represented units in which physicians usually request insufficiently low numbers of *C. difficile* tests as part of the routine enteric laboratory diagnostic testing.

DISCUSSION

On the basis of our results and those of Stamper et al., the BD GeneOhm Cdiff assay provides better sensitivity and specificity for the detection of *C. difficile* than do toxin A/B enzyme-linked immunosorbent assays directly from diarrheal stool specimens (15). In addition, the prevalence of positive samples directly from stool by real-time PCR was higher (9.7%) than in the case of the cytotoxicity assay (6.3%), which is considered the "gold standard" for the detection of toxigenic *C. difficile*. The overall prevalence of toxin-producing *C. difficile* strains determined by toxigenic culture (i.e., culture plus cytotoxicity assay) was 9.2%. These results emphasized the importance of culture method, because 2.9% of toxigenic *C. difficile* strains were detected from broth culture, and the cytotoxicity assay gave false-negative results directly from stool; thus, these direct-cytotoxin-negative but cytotoxigenic-culture-positive samples represent true CDI cases. In addition, the cytotoxicity test for the detection of toxigenic *C. difficile* is available only once or twice per week, and in these cases, pure broth culture of the isolated strain required setup of the test, which meant an additional delay in the diagnosis of CDI of at least 4 days. The five samples that were positive only by the BD GeneOhm Cdiff assay and from which *C. difficile* could not be isolated could be considered false positive by PCR. However, the presence of toxigenic *C. difficile* in these samples cannot be excluded completely, because the stringent requirements for anaerobic culture and the alcohol shock treatment during sample processing may have reduced the number of viable bacteria and spores and led to culture failure; in these cases, further confirmation would be necessary to determine whether these samples are false or true positives. The six samples that were positive only by the Vidas *C. difficile* toxin A and B assay may be due to the cross-reactivity with *Clostridium sordellii* toxin; however, there is no evidence for this, or perhaps this result simply represents poor specificity of the EIA. A relatively high number of diarrheal samples (5%) showed equivocal results by the Vidas assay; in these cases, the cytotoxicity assay and culture or new sample collection may provide more reliable results.

The superior performance of the BD GeneOhm Cdiff assay was shown in two cases in which the PCR assay gave positive results earlier than did the Vidas *C. difficile* toxin A and B assay. Case 1 was a 39-year-old male patient who had antibiotic-associated diarrhea after an appendectomy. His first diarrheal sample was submitted for laboratory testing on 3 November 2008, at which time the BD GeneOhm Cdiff assay, the cytotoxicity assay, and culture results for toxigenic *C. difficile* were all positive; but the Vidas *C. difficile* toxin A and B assay was negative. Because of persistent diarrhea, detection of toxins A and B was performed 10 days later, after the patient had been discharged from the hospital, by the Vidas *C. difficile* toxin A and B assay, which gave a positive result. In case 2, a 75-year-old patient was treated in the infectious disease unit for severe diarrhea. Four samples were sent to our laboratory (on 5, 14, and 27 October 2008 and 13 November 2008) for detection of toxigenic *C. difficile* because of persistent diarrhea despite the adequate antibiotic therapy. Three samples were negative, and one sample gave an equivocal result with the Vidas toxin A/B assay. The cytotoxicity assay result from direct stool testing was positive on 5 and 27 October. In contrast, all four specimens were positive by the BD GeneOhm Cdiff assay. *C. difficile* was grown from all samples, and toxin production was confirmed by the cytotoxicity assay.

Several studies have demonstrated the performance of PCR or real-time PCR assays for the detection of *C. difficile* genes from diarrheal stool samples (2, 5, 13, 14, 17, 18). In 2002, Guilbault et al. used PCR for the detection of the nonrepeating region of the *tcdB* gene from 59 stool specimens (5). Compared to the reference cytotoxicity assay, this method demonstrated 100% specificity and 91.5% sensitivity, results were available within 6 h, and none of the samples tested were inhibited (5). Bélanger et al. later developed a real-time PCR assay targeting the major toxin genes (*tcdA* and *tcdB*) (2). The turnaround time including sample preparation was about 1.5 h, and the assay was about 100-fold more sensitive than the PCR method described by Guilbault et al. (2, 5). van den Berg et al. published two studies using real-time PCR assays for the detection of the *tcdB* gene (17, 18). However, compared with the cytotoxicity assay, the sensitivity and the specificity of this test (87.1% and 96.5%, respectively) were lower than the observations by Guilbault et al. and showed the highest concordance with toxigenic culture (5, 17, 18). These studies showed that a remarkable number of samples were positive by immunoassay but were negative by tissue culture cytotoxicity, toxigenic culture, and real-time PCR. In our study, six samples (1%) were false positive by the Vidas *C. difficile* toxin A and B assay, and the number of equivocal results was quite high. This was of concern because not only can this delay accurate diagnosis of *C. difficile*-associated disease but also it is associated with a higher cost due to the repeated testing or a new sample collection. Similar sensitivity and specificity (86% and 97%, respectively) were found by Sloan et al. in a study where real-time PCR was applied for the detection of the *tcdC* gene and compared to toxigenic culture (14).

Stamper et al. examined 404 stool samples using the BD GeneOhm Cdiff assay, the cell culture cytotoxicity assay, and toxigenic culture, and the prevalence of positive specimens was 10% by both PCR and cytotoxin production (15). Compared with the cytotoxicity assay as the reference method, the sensitivity, specificity, PPV, and NPV of the BD GeneOhm Cdiff assay were 90.9%, 95.2%, 70.2% and 98.8%, respectively. In our study, the following results were obtained: the sensitivity, specificity, PPV, and NPV were 96.4%, 99.01%, 92%, and 100%, respectively. The PCR inhibition rate in our study was 0.6% (4/600); similarly, Stamper et al. detected inhibitors in three samples. Two samples in our study were negative by only real-time PCR, while cytotoxicity and Vidas assays and toxigenic culture were positive; similar findings were reported by Stamper et al., who found four samples which proved to be negative by real-time PCR. A possible explanation of this is that some strains may carry an aberrant *tcdB* gene, and this may result in the failure of primer annealing, or the copies of the target in the stool sample were below the detection limit of the PCR assay.

Although the cost of the BD GeneOhm Cdiff assay is higher than that of immunoassays, the use of a more sensitive test can

provide more reliable results for detection of toxigenic *C. difficile*. With prompt and accurate diagnosis of CDI, the total cost of the hospital care and the duration of patient hospitalization may be reduced. In addition, unnecessary antibiotic therapy may be avoided and the number of further possible nosocomial infections may also decrease. Using the PCR assay, it is also feasible to avoid false-negative, direct EIA results originating from the possible degradation of *C. difficile* toxins in fecal specimens. Specimens can be kept at room temperature up to 48 h before testing; thus, temperature does not influence the test result within this period of time, which is very important mainly if transport at 4°C is not available, e.g., in the case of outpatients. The BD GeneOhm Cdiff assay contains an internal control; thus, the presence of PCR-inhibitory substances can be detected too. The assay is cost-effective if at least eight reactions including one positive and one negative control are run, because reconstituted reagents remain stable at 2 to 8°C for 3 h.

In conclusion, the BD GeneOhm Cdiff assay is a rapid and reliable tool for detection of toxigenic *C. difficile* from diarrheal specimens and is superior to all known traditional methods such as the cytotoxicity assay, various immunoassays, and in-house PCRs.

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No conflict of interest exists.

REFERENCES

1. **Alonso, R., C. Munoz, S. Gros, D. Garcia de Viedma, T. Pelaez, and E. Bouza.** 1999. Rapid detection of toxigenic *Clostridium difficile* from stool samples by a nested PCR of toxin B gene. J. Hosp. Infect. **41:**145–149.
2. **Bélanger, S. D., M. Boissinot, N. Clairoux, F. J. Picard, and M. G. Bergeron.** 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. J. Clin. Microbiol. **41:**730–734.
3. **Delaney, A. C., S. Dial, A. Barkun, and S. Suissa.** 2007. Antimicrobial drugs and community-acquired *Clostridium difficile*-associated disease (CDAD), UK. Emerg. Infect. Dis. **13:**761–763.
4. **Delmée, M., J. Van Broeck, A. Simon, M. Janssens, and V. Avesani.** 2005. Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea: a plea for culture. J. Med. Microbiol. **54:**187–191.
5. **Guilbault, C., A. C. Labbé, L. Poirier, L. Busque, C. Béliveau, and M. Laverdiére.** 2002. Development and evaluation of a PCR method for detection of the *Clostridium difficile* toxin B gene in stool specimens. J. Clin. Microbiol. **40:**2288–2290.
6. **Hirschhorn, L., Y. Trnka, A. Onderdonk, M. Lee, and R. Platt.** 1994. Epidemiology of community-acquired *Clostridium difficile*-associated diarrhea. J. Infect. Dis. **169:**127–133.
7. **Loo, V. G., L. Poirier, M. A. Miller, M. Oughton, M. D. Libman, S. Michaud, A. M. Bourgault, T. Nguyen, C. Frenette, M. Kelly, A. Vibien, P. Brassard, S. Fenn, K. Dewar, T. J. Hudson, R. Horn, P. Rene, Y. Monczak, and A. Dascal.** 2005. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N. Engl. J. Med. **353:**2442–2449.
8. **McDonald, L. C., G. E. Killgore, A. Thompson, R. C. Owens, S. V. Kazakova, S. P. Sambol, S. Johnson, and D. N. Gerding.** 2005. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N. Engl. J. Med. **353:**2433–2441.
9. **Nagy, E., and J. Földes.** 1991. Electron microscopic investigation of lysogeny of *Clostridium difficile* strains isolated from diarrhea cases and from healthy carriers. APMIS **99:**321–326.
10. **Norén, T., T. Åkerlund, E. Bäck, L. Sjöberg, I. Persson, I. Alriksson, and L. G. Burman.** 2004. Molecular epidemiology of hospital-associated and community-acquired *Clostridium difficile* infection in a Swedish county. J. Clin. Microbiol. **42:**3635–3643.
11. **O'Connor, D., P. Hynes, M. Cormican, E. Collins, G. Corbett-Feeney, and M. M. Cassidy.** 2001. Evaluation of methods for detection of toxins in specimens of feces submitted for diagnosis of *Clostridium difficile*-associated diarrhea. J. Clin. Microbiol. **39:**2846–2849.
12. **Pépin, J., M. E. Alary, L. Valiquette, E. Raiche, J. Ruel, K. Fulop, D. Godin, and C. Bourassa.** 2005. Increasing risk of relapse after treatment of *Clostridium difficile* colitis in Quebec, Canada. Clin. Infect. Dis. **40:**1591–1597.
13. **Peterson, L. R., R. U. Manson, S. M. Paule, D. M. Hacek, A. Robicsek, R. B. Thomson, and K. L. Kaul.** 2007. Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of *C. difficile*-associated diarrhea. Clin. Infect. Dis. **45:**1152–1160.
14. **Sloan, L. M., B. J. Duresko, D. R. Gustafson, and J. E. Rosenblatt.** 2008. Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. J. Clin. Microbiol. **46:**1996–2001.
15. **Stamper, P. D., R. Alcabasa, D. Aird, W. Babiker, J. Wehrlin, I. Ikpeama, and K. C. Carroll.** 2009. Comparison of a commercial real-time PCR assay for *tcdB* detection to a cell culture cytotoxicity assay and toxigenic culture for direct detection of toxin-producing *Clostridium difficile* in clinical samples. J. Clin. Microbiol. **47:**373–378.
16. **Turgeon, D. K., T. J. Novicki, J. Quick, L. Carlson, P. Miller, B. Ulness, A. Cent, R. Ashley, A. Larson, M. Coyle, A. P. Limaye, B. T. Cookson, and T. R. Fritsche.** 2003. Six rapid tests for direct detection of *Clostridium difficile* and its toxins in fecal samples compared with the fibroblast cytotoxicity assay. J. Clin. Microbiol. **41:**667–670.
17. **van den Berg, R. J., L. S. B. van Coppenraet, H. J. Gerritsen, H. P. Endtz, E. R. van der Vorm, and E. J. Kuijper.** 2005. Prospective multicenter evaluation of a new immunoassay and real-time PCR for rapid diagnosis of *Clostridium difficile*-associated diarrhea in hospitalized patients. J. Clin. Microbiol. **43:**5338–5340.
18. **van den Berg, R. J., N. Vaessen, H. P. Endtz, T. Schülin, E. R. van den Vorm, and E. J. Kuijper.** 2007. Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study. J. Med. Microbiol. **56:**36–42.
19. **Warny, M., J. Pepin, A. Fang, G. Killgore, A. Thompson, J. Brazier, E. Frost, and L. C. McDonald.** 2005. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet **366:**1079–1084.