The value of repeat *Clostridium difficile* toxin testing during and after an outbreak of *C difficile*-associated diarrhea

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BACKGROUND: The recent increase in *Clostridium difficile*-associated diarrhea (CDAD) has led to questions about the reproducibility and sensitivity of *C difficile* toxin testing (CDTT). While there have been recommendations to repeat CDTT following a negative result, previous studies have failed to show a benefit. However, no studies were performed during an outbreak of CDAD. The value of repeat CDTT after an initial negative result in patients tested during and after an outbreak of CDAD is reported in the present study, as well as the reproducibility of CDTT when multiple samples are received and tested on the same day.

METHODS: The results of CDTT, performed using a cell cytotoxicity assay between April 1, 2001, and March 31, 2008, were retrieved and searched for patients who had repeat samples tested after an initial negative result. The result and the number of days after a negative result were determined using the date of the most recent negative test. The cumulative positivity rate was calculated by adding all of the repeat positive test results for the days in question and dividing by the total number of tests performed during that time.

RESULTS: A total of 8661 patients submitted 14,991 stool specimens for CDTT during the study period. There were 3095 samples that tested positive (20.6%) for the toxin. The results were divided into two time periods to reflect the CDAD outbreak, which began in April 2002: period 1 (outbreak) was from April 1, 2002, to March 31, 2006, and period 2 was from April 1, 2006, to March 31, 2008. The rate of positivity was 24.2% during period 1, and 11.6% during period 2 (P<0.001). Repeat CDTT was performed 619 times on samples received on the same day as the initial specimen, and only three (0.5%) were discordant. A total of 1630 samples were retested within one to seven days of a negative result, and 103 (6.3%) tested positive (7.8% period 1 and 2.9% period 2; P=0.002). The likelihood of a positive result on repeat testing in the first three days after a negative result was low (0.9%, 7% and 4%, respectively). The cumulative positivity for repeat testing performed in the first three days was 0.9%, 3.3% and 3.5%, respectively, and did not differ significantly at day 3 during the period of high CDTT positivity (P=0.110).

CONCLUSIONS: The value of repeat CDTT, performed using a cell cytotoxicity assay, was low in the first three days after an initial negative result and was unchanged during a CDAD outbreak.

Key Words: Clostridium difficile; *Toxin testing*

Utilité de la répétition des tests de dépistage des toxines de *Clostridium difficile* durant et après une éclosion de diarrhée associée à cette bactérie

HISTORIQUE : La récente augmentation des cas de diarrhée associée à *C. difficile* (DACD) a soulevé des questions à propos de la reproductibilité et de la sensibilité des tests de dépistage des toxines de *C. difficile* (TDTCD). Il a été recommandé de répéter les TDTCD après un résultat négatif, mais des études antérieures ne sont pas arrivées à confirmer l'avantage de cette mesure. Or, aucune n'a été réalisée durant une éclosion de DACD. La présente étude évalue l'utilité des TDTCD répétés après un résultat initial négatif chez des patients durant et après une éclosion de DACD, de même que leur reproductibilité lorsque plusieurs échantillons sont reçus et testés le même jour.

MÉTHODE : Les résultats des TDTCD effectués par analyse de l'activité cytotoxique entre le 1er avril 2001 et le 31 mars 2008 ont été rétrouvés et recensés pour les patients chez qui les tests avaient été répétés après un résultat initial négatif. Le résultat et le nombre de jours suivant un test négatif ont été déterminés à partir de la date du plus récent test négatif. Le taux cumulatif de positivité a été calculé par la somme de tous les résultats de tests répétés positifs pour les jours en question, divisée par le nombre total de tests effectués pendant cette période.

RÉSULTATS : En tout, 8 661 patients ont soumis 14 991 spécimens de selles pour TDTCD durant la période de l'étude. Trois mille quatre-vingt-quinze échantillons ont produit des résultats positifs (20,6 %) à l'égard des toxines. Les résultats ont été divisés entre deux périodes pour refléter l'éclosion de DACD débutée en avril 2002 : Période 1 (éclosion), du 1er avril 2002 au 31 mars 2006, et Période 2, du 1er avril 2006 au 31 mars 2008. Le taux de positivité a été de 24,2 % durant la Période 1 et de 11,6 % durant la Période 2 (p < 0,001). Le TDTCD a été répété 619 fois sur les échantillons reçus le même jour que le spécimen initial et seulement trois résultats (0,5 %) étaient discordants. En tout, 1 630 échantillons ont été retestés dans les sept jours suivant un résultat négatif et 103 (6,3 %) se sont révélés positifs (7,8 % à la Période 1 et 2,9 % à la Période 2, p = 0,002). La probabilité d'un résultat positif lors de la reprise du test dans les trois premiers jours suivant un résultat négatif a été faible (0,9 %, 7 % et 4 %, respectivement). La positivité cumulative des tests répétés au cours des trois premiers jours a été de 0,9 %, 3,3 % et 3,5 %, respectivement, et n'a pas été significativement différente au Jour 3, durant la période de forte positivité des TDTCD (p = 0,110).

CONCLUSIONS : L'utilité des TDTCD répétés par analyse de l'activité cytotoxique a été faible au cours des trois premiers jours suivant un résultat initial négatif et est demeuré inchangé durant l'éclosion de DACD.

There has been a worldwide increase in the incidence of *Clostridium difficile*-associated diarrhea (CDAD) (1). The toxins produced by *C difficile* can be detected using various methods including enzyme immunoassay (EIA), cell cytotoxicity assay (CTA) or polymerase chain reaction. There is some controversy regarding the sensitivity and specificity of the various toxin assays, which has led to the recommendation for repeat toxin testing at least once after a negative result (2) or use of a two-step method of detection, incorporating bacterial

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antigen or culture (3,4). Because the positivity rate for toxin detection is approximately 10% in nonoutbreak periods, this strategy could result in excessive and expensive repeat testing. Previous studies (5-12) investigating the value of repeat *C difficile* toxin testing (CDTT) have shown that between 0% and 11.2% of specimens that originally tested negative would test positive if repeated within five to 10 days of the first sample. The range in values may be attributed to the CDTT methodology used.

Beginning in 2002, the province of Quebec experienced a CDAD outbreak, with CDTT positivity rates approaching 30% (13). During this period, many microbiology laboratories received repeat specimens based on the clinical impression that the initial results were not accurate. There were anecdotal reports of specimens from patients with severe CDAD having initially negative CDTT results that were positive on repeat testing. This led physicians to request multiple stool samples that were sent on the same day or within a few days of the first sample. In one recent study (14), 57% of patients had more than one sample sent within a 24 h period. To examine the utility of repeat CDTT, the present study was designed to review all results over a continuous six-year period. Additionally, a comparison of repeat CDTT during and after a CDAD outbreak was performed.

METHODS

A Monarch data retrieval program was used to retrospectively search the laboratory results for all patients who had samples sent for CDTT between April 1, 2002, and March 31, 2008. During this period of time, all CDTT was performed solely by CTA using a standardized protocol. St Mary's Hospital Center is a 316-bed McGill University-affiliated teaching hospital in Montreal, Quebec, that provides College of American Pathologists-accredited laboratory services for a predominately adult population, with external clients that include several rehabilitation hospitals, a large mental health institution, more than 30 medical clinics and more than 300 community physicians. The vast majority of samples received for CDTT originate from this hospital and the institutions that it services. The date of sample testing was determined by the laboratory accession number, and this date was also used as the date of sample acquisition. The results were divided into two periods to reflect the CDAD outbreak that began at St Mary's Hospital Center during the 2002/2003 fiscal year (Figure 1). Period 1 (outbreak) encompassed April 1, 2002, to March 31, 2006 (48 months) and period 2 from April 1, 2006, to March 31, 2008 (24 months). Patients who had an initial negative CDTT and underwent repeat testing anytime during the two study periods were examined. All results of patients with multiple initial samples tested on the same day were analyzed. Equivocal and weakly positive CDTT results were classified as positive results. Patients who had an initial negative CDTT result and had subsequent samples sent were studied. The number of days from the most recent negative result was determined. Once a positive result was obtained, no further samples were included in the analysis, with the exception of patients sending multiple samples on the same day.

Cumulative positivity was determined by adding the number of repeat positive test results by the total number of repeat tests performed over the indicated period. For example, the total number of repeat positive tests for days 1 and 2 was divided by the total number of repeat tests performed on days 1 and 2 to determine the cumulative repeat positivity result. Clinical information about the patients who were tested was not available. Two-tailed χ^2 analysis and Fisher's exact test were used to compare the different time periods.

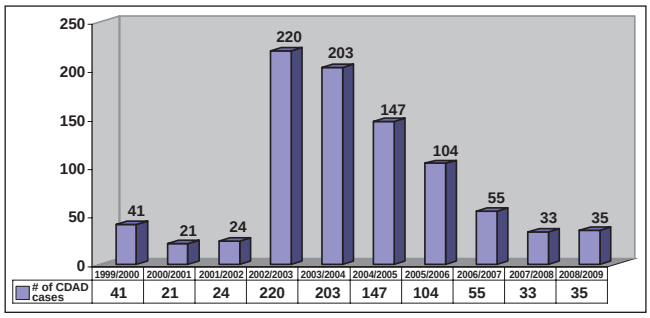

Figure 1) *Nosocomial cases of* Clostridium difficile*-associated diarrhea (CDAD) at St Mary's Hospital Center (Montreal, Quebec) between 1999 and 2009*

RESULTS

Between April 1, 2002, and March 31, 2008, a total of 14,991 stool samples from 8661 patients were tested for *C difficile* toxin using a CTA. The testing results are summarized in Table 1. The overall positivity rate for the entire period was 20.6%, with a markedly higher rate during period 1 (outbreak) compared with period 2 (24.2% versus 11.6%, respectively; P<0.001). There were 619 samples received on the same day as the initial specimen. Repeat testing (Table 2) showed a discordance of only 0.5%. A total of 1630 samples were retested within one to seven days of a negative result (Figure 2). Of these, 103 (6.3%) tested positive (7.8% period 1 and 2.9% period 2; P=0.002). The likelihood of a positive result on repeat testing in the first three days after a negative result was low (0.9%, 7% and 4%, respectively). The cumulative positivity for repeat testing performed in the first three days was 0.9%, 3.3% and 3.5%, respectively (Figure 3). As expected, the percentage of positive repeat tests was higher during the outbreak period from days 4 to 7. There were 12.5% new positive results on day 7 (16.5% period 1 versus 4.7% period 2; P=0.056). The cumulative positivity rate for the seven days was 6.3% (8.5% period 1 versus 2.9% period 2; P<0.001).

DISCUSSION

The increased incidence of CDAD has been noted worldwide (1). During 2003, the province of Quebec was particularly affected, with a reported estimated quadrupling of cases from one region (13) – St Mary's Hospital Center experienced a 10-fold increase in the number of nosocomial cases of CDAD (Figure 1). Establishing a diagnosis of CDAD usually requires the demonstration of toxin production in stool obtained from patients with diarrhea. Various methods have been used to perform CDTT, with each having some limitation. EIA testing lacks sensitivity when compared with CTAs, but has more rapid turnaround times (8). The current gold standard for CDTT is cultivation of stool for *C difficile*, followed by efforts to detect toxin production from the isolates (3).

This is labour intensive and time consuming. As a result, most clinical laboratories use EIA with or without initial screening for the

TABLE 1
Results of *Clostridium difficile* toxin testing

	Patients, n	Specimens, n	Negative, n	Positive, n	Weak positive, n	Equivocal, n	% Positive
Period 1	5922	10,759	8153	2524	60	22	24.2*
Period 2	2739	4232	3743	466	18	5	11.6
Total	8661	14,991	11,896	2990	78	27	20.6

**P<0.001 compared with period 2*

TABLE 2
Repeat *Clostridium difficile* toxin testing on the same day

Initially negative	Negative on repeat	Positive on repeat
Period 1	321	2
Period 2	98	0
Initially positive	**Positive on repeat**	**Negative on repeat**
Period 1	173	1
Period 2	24	0

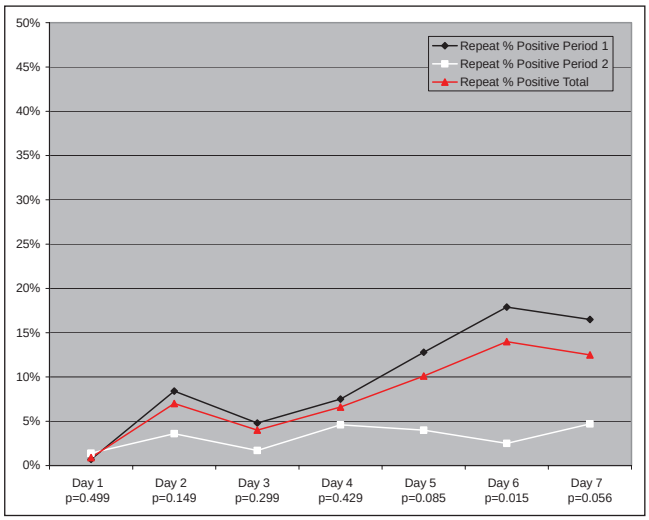

Figure 2) *Percentage of positive* Clostridium difficile *toxin tests on repeat testing*

presence of *C difficile* antigen. The knowledge that current CDTT may not detect all cases of CDAD has resulted in the recommendation that a repeat sample should be sent if the initial result is negative (2). Clinicians have also been uneasy relying on a single negative CDTT result for patients at risk.

This has led to numerous additional samples sent to the laboratory after an initial negative result (14). Other studies (4-6) using different methods have shown that there is little value in repeating CDTT within seven days of an initial result. In a smaller study (4) using CTA, the overall positivity rate was only 7.4%, and repeat testing of previously negative patients in the first seven days was positive in only two patients. A larger study (12) using EIA had a high initial positivity rate of 16.6%. A single repeat test was positive in 4.9% of samples, while a third test was positive in another 3%. These patients were studied for only five days per episode. Additional studies showed that repeat testing in the first three days after a negative result produced 1.8%, 3.8% and 2.6% new positives (8), while in another study (10), one in 78 (0.8%) initially negative repeat tests became positive. Although all these studies consistently show the minimal benefit of performing repeat testing after a negative result, none of the studies were performed during a CDAD outbreak. The present study compared CDTT during and after a major outbreak of CDAD at St Mary's Hospital Center. There was negligible benefit in repeating CDTT on specimens received on the same day. Only three of 619 (0.5%) samples repeated on the same day gave discordant results, which is a positive reflection on the reliability of the manner of testing. In addition, the laboratory has been accredited by the College of American Pathologists since 1995, and there have not been any discordant results while performing yearly proficiency testing for *C difficile* toxin detection.

There was a slight increase in the number of new positive results during the first three days of retesting, but the totals did not differ significantly. The greatest increase occurred during the second day and

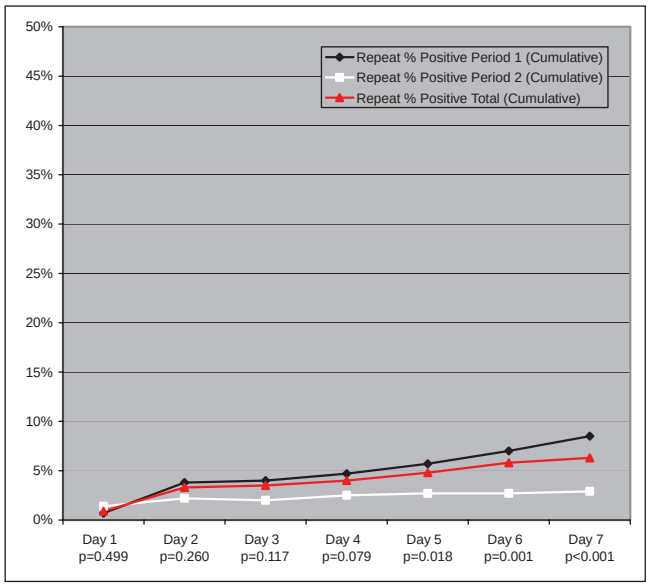

Figure 3) *Percentage of positive* Clostridium difficile *toxin tests on repeat testing (cumulative)*

is difficult to explain. Only four of these repeat tests performed on the second day yielded weak positive or equivocal results; thus, the increase cannot be explained by including borderline results. Another less likely explanation comes from a recent study (15) using EIA, which suggests that some of the positive repeat tests may reflect false test results. Some of the patients in that study were asymptomatic at the time of repeat testing. False-positive results have been reported for EIA, but are rare with CTA (16).

In real time, using CTA, it often takes two days to finalize a result and, thus, many of the repeat specimens were ordered before the initial test result was known. At least 421 initially negative samples were repeated before the final result was made available. The laboratory has a policy of rejecting repeat tests following a positive result for a period of six weeks. This policy also states that only a single sample per patient per day will be processed. During this study period, many samples were rejected because of these restrictions. Unfortunately, at the bench level, samples continued to be accepted that should have been rejected. Based on the present and earlier studies, it would seem reasonable to restrict repeat CDTT for at least three days after the initial negative test result. For patients whose clinical condition is unstable, empirical treatment for CDAD may be warranted, particularly for those evaluated using an EIA and who are at high risk for disease.

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REFERENCES

1. Kelly CP, LaMont JT. *Clostridium difficile* – more difficult than ever. N Engl J Med 2008;359:1932-40.
2. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 2002;346:334-9.
3. Reller ME, Lema CA, Perl TM, et al. Yield of stool culture with isolate toxin testing versus a two-step algorithm including stool toxin testing for detection of toxigenic *Clostridium difficile*. J Clin Microbiol 2007;415:3601-5.

4. Ticehurst JR, Aird DZ, Dam LM, et al. Effective detection of toxigenic *Clostridium difficile* by a two-step algorithm including tests for antigen and cytotoxin. J Clin Microbiol 2006;44:1145-9.
5. Borek AP, Aird DZ, Carroll KC. Frequency of sample submission for optimal utilization of the cell culture cytotoxicity assay for the detection of *Clostridium difficile* toxin. J Clin Microbiol 2008;43:2994-5.
6. Renshaw AA, Stelling JM, Doolittle MH. The lack of value of repeated *Clostridium difficile* cytotoxicity assays. Arch Pathol Lab Med 1996;120:49-52.
7. Aichinger E, Schleck CD, Harmsen WS, et al. Nonutility of repeat laboratory testing for detection of *Clostridium difficile* by use of PCR or enzyme immunoassay. J Clin Microbiol 2008;46:3795-7.
8. Cardona DM, Rand KH. Evaluation of repeat *Clostridium difficile* enzyme immunoassay testing. J Clin Microbiol 2008;46:3686-9.
9. Manabe YC, Vinetz JM, Moore RD, et al. *Clostridium difficile* colitis. An efficient clinical approach to diagnosis. Ann Intern Med 1995;123:835-40.
10. Mohan SS, McDermott BP, Parchuri S, Cunha BA. Lack of value of repeat stool testing for *Clostridium difficile* toxin. Am J Med 2006;119:356-8.
11. Drees M, Snydman DR, O'Sullivan CE. Repeated enzyme immunoassays have limited utility in diagnosing *Clostridium difficile*. Eur J Clin Microbiol Infect Dis 2008;27:397-9.
12. Nemat H, Khan R, Ashraf MS, et al. Diagnostic value of repeated enzyme immunoassays in *Clostridium difficile* infection. Am J Gastroenterol 2009;104:2035-41.
13. Pépin J, Valiquette L, Alery ME, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: A changing pattern of severity. N Engl J Med 2004;171:466-72.
14. Morelli MS, Rouster SD, Giannella RA, Sherman KE. Clinical application of polymerase chain reaction to diagnose *Clostridium difficile* in hospitalized patients with diarrhea. Clin Gastroenterol Hepatol 2004;43:2994-5.
15. Hacek DM, Robicsek A,Thomson RB, Peterson LR. A myth of *Clostridium difficile* infection diagnosis: The illusion of repeat EIA testing. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, September 12 to 15, 2009.
16. Planche T, Aghaizu A, Holliman R, et al. Diagnosis of *Clostridium difficile* infection by toxin detection kits: A systematic review. Lancet Infect Dis 2008;8:777-84.