ORIGINAL ARTICLE

Prevalence of Clostridium difficile toxin in diarhoeal stool samples of patients from a tertiary hospital in North Eastern Penisular Malaysia

Siti Asma' Hassan, MBBS, MMed, Norlela Othman, MPath, Fauziah Mohd Idris, MPath, Zaidah Abdul Rahman, MPath, Nurahan Maning, MPath, Rosliza Abdul Rahman, Dip MLT, Chan Guan Tiong, Dip MLT

Clinical Microbiologist, Hospital Universiti Sains Malaysia, Kampus Kesihatan, Department of Medical Microbiology and Parasitology, Kubang Kerian, Kota Bharu, Kelantan 16150, Malaysia

SUMMARY

This study describes the prevalence of *Clostridium difficile* toxin (CDT) in loose stool samples from inpatients aged more than two years of a tertiary hospital. A total of 175 samples that had been examined were from stool samples that were sent to the Medical Microbiology & Parasitology Laboratory for various clinical indications. The toxin was detected by a commercial immunochromatograhic test, and the patients' demography, clinical features, treatment and outcomes were analyzed from their medical records.

***Clostridium difficile* toxin was positive in 24 (13.7%) of the stool samples. Male and female were 11 (45.8 %) and 13 (54.2 %) respectively, with the majority of them aged more than 50 years. Most were from medical wards (n=21, 87.5%), with the rest from surgical wards (n=2, 8.3%) and intensive care units (n=1, 3.4%). All the CDT positive patients had history of prior antibiotic usage within 6 weeks before the detection of the toxin. The mean duration of antibiotics usage was 17.75 (±13.75) days, while the mean duration of diarrhea was 5.21((± 5.85) days. Eighteen patients had underlying medical illnesses that were diabetes mellitus, chronic renal disease, hypertension, ischaemic heart disease, cerebrovascular disease and malignancy; with seven of them being CDT positive while on chemotherapy. Stool occult blood test was positive in 15 patients whereas presence of pus cells in the CD positive stool samples were detected in 21 patients. The duration of hospitalization among the patients was 27.96 (± 23.22) days.**

KEY WORDS:
Clostridium difficile toxin, Loose stool, Risk factors

INTRODUCTION

Clostridium difficile was first described in 1935 as part of the intestinal microflora in neonates but was not identified as a causative agent of human disease until 1978[1]. The toxin-mediated *C. difficile* infection (CDI) is the main cause of infectious diarrhoea that develops following hospitalization and antibiotic treatment. *C. difficile* can only colonize the gut if the normal intestinal microbiota is disturbed; which in most cases are due to antibiotic exposure. Prior antibiotic therapy is a major risk factor for CDI as it confers selection pressure on *C. difficile* strains and/or induces toxin production. However, in some cases, antibiotic exposure appears not to be necessary for the development of CDI[2].

The clinical outcomes range from asymptomatic colonization to mild diarrhoea and to more severe disease syndromes that include pseudomembranous colitis. Symptoms of CDI may start on the first day of antibiotic therapy and up to 8 weeks after termination of therapy[3]. Complications of the infection include toxic megacolon, bowel perforation, sepsis, shock and death.

Based on annual data from the state of Ohio in 2006 (Ohio Department of Health), United States (US) hospitals and long-term care facilities had about 500,000 CDI cases per year, with an estimated 15,000 to 20,000 death. Worldwide, CDI cases are also increasing[4,5]. Prevalence of CDI in Taiwan was estimated to be around 12.4%[6]. Unfortunately, our local data regarding CDI prevalence is not yet available. In 2005, a *C. difficile* strain BI/NAP1/027 was identified to be responsible for a large number of infections in North America and Canada[7,8]. The emergence and spread of *C. difficile* BI/NAP1/027 correlates with acquired resistance to the fluoroquinolone antibiotics i.e gatifloxacin and moxifloxacin, thus making treatment and management of CDI more challenging. The use of cephalosporins, to which virtually all *C. difficile* strains are resistant, has been implicated as a CDI risk factor. Other risk factors include exposure to stomach acid-reducing agents, such as H2 blockers and proton pump inhibitors8.

This study investigated the prevalence of *C. difficile* toxins (CDT) in loose stool samples. The demographic and clinical parameters of the patients were also analyzed.

MATERIALS AND METHODS

Loose stool samples from inpatients of Hospital Universiti Sains Malaysia (HUSM) that were sent to Medical Microbiology and Parasitology Laboratory from June to October 2008 were analyzed for the presence of *C. difficile* toxin A & toxin A/B. Stool samples that were sent for typhoid clearance investigation and those from patients below the age of 2 years old were excluded from the study. The samples were processed within 24 hours of receipt using a commercial immunochromatographic technique (ICT) kit (Remel Xpect®;

This article was accepted: 8 May 2012
Corresponding Author: Siti Asma' Hassan, Clinical Microbiologist, Hospital Universiti Sains Malaysia, Kampus Kesihatan, Department of Medical Microbiology and Parasitology, Kubang Kerian, Kota Bharu, Kelantan 16150, Malaysia Email: sitiasma@kb.usm.my

Oxoid, UK). Previous studies from the United Kingdom showed the median sensitivities and specificities of Remel Xpect® was 0.82 (0.75 – 0.89) and 0.96 (0.95 – 0.98) respectively which was comparable to other commercially available kits for reliable detection of CDT[10,11]. Cell cytotoxity assay were also performed. The demographic and clinical history of the patients were obtained from medical records and analyzed based on known risk factors for CDI.

Definitions: Loose stool sample was defined as stool sample which follows the shape of the collecting container. Diarrhoea was defined as more than 3 loose stools passed within 1 day[6]. Antibiotic associated diarrhoea (AAD) was considered in patients with history of taking antibiotic within 6 weeks prior to the onset of diarrhea. CDI was defined as a patient with diarrhea and CDT detected in the stool samples either by ICT or cell cytotoxicity assay[11]. The duration of antibiotic was defined from the first day of antibiotic receipt to the last day of antibiotic exposure, regardless of how many types of antibiotic administered. Enigmatic AAD was considered in patient who have antibiotic associated diarrhoea but negative for CDT.

Statistical analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for window (version 12.0, SPSS, Inc, USA) to determine the frequency of distributions and chi-squared values. P-values of < 0.05 were considered significant. Ethical approval had been obtained for this study.

RESULTS

All together, 175 stool samples were analyzed during the 5-month period. 105 out of 175 patients met the criteria for AAD. Twenty-four of the AAD patients had CDT in their stool samples (Figure 1). None of the patients who had non-antibiotic associated diarrhea (70/175) were positive for CDT. The remaining number of AAD patients (n=81) were negative for CDT and categorized as enigmatic AAD. Using a study power and precision of 80% and 0.05 respectively, the prevalence of CDT in the studied population was found to be 13.7% (95% CI 8.6 – 18.8). The prevalence of possible toxin A-B+ as detected by the ICT kit was 2.9% (95 % CI 0.4 – 5.0). The patients' demography data is as shown in Table I. Both male and female were almost equal in distribution. Malay predominates as they are the main population in the studied community. Majority of CDT positive patients were more than 50 years old and from the medical ward.

Table I: Characteristics of patients with positive Clostridium difficile toxin

Characteristic	No.	%
Sex		
Male	11	45.8
Female	13	54.2
Race		
Malay	19	79.2
Chinese	4	16.7
Indian	1	4.1
Age group (years)		
3–12	5	20.8
13–49	9	37.5
> 50	10	41.7
Wards		
Medical	21	87.5
Surgical	2	8.3
ICU	1	4.2
Clinical parameter		
Previous history of taking antibiotic		
Yes	24	100
Mean duration of hospitalization	27.96 (± 23.22)	
Mean duration of antibiotic (current admission)	17.75 (±13.75)	
Mean duration of diarrhoea	5.21 (±5.85)	
Received chemotherapy	7	29.2
Do not received chemotherapy	17	70.8
On nasogastric tubing	3	12.5
Not on nasogastric tubing	21	87.5
Underlying medical illness	18	75.0
No underlying med. illness	6	25.0

Table II: Clinical parameters of patients with CDT

Clinical parameter	AAD with CDT (N=24) Mean (SD)	Enigmatic AAD (N=81) Mean (SD)	Mean Diff (95% CI)	t-stats (df)	P Value
Duration of hospitalization in days	27.96 (23.22)	21.48 (20.84)	6.48 (-3.38, 16.34)	1.303 (103)	0.196
Duration of antibiotic treatment in days	17.75 (±13.75)	15.15 (14.37)	2.60 (-3.96, 9.16)	0.786 (103)	0.433
Duration of diarrhoea in days	5.21 (5.85)	4.28 (4.46)	0.92 (-1.29, 3.14)	0.828 (103)	0.410
Clinical parameters	Possible CDI N (%)	Enigmatic AAD N (%)	Total	RR(96% CI)	P Value
Underlying Medical illness					
With	18 (17.1)	47 (44.8)	65 (61.9)	1.85 (0.800,	0.133
Without	6 (5.7)	34 (32.4)	40 (38.1)	4.258)	
Total	24 (22.9)	81 (77.1)	105 (100)		
Previous antibiotic treatment (within 6 weeks)					
Yes	9 (8.6)	14 (13.3)	23 (21.9)	2.14 (1.078,	0.035[b]
No	15 (14.3)	67 (63.8)	82 (78.1)	4.243)	
Total	24 (22.9)	81 (77.1)	105 (100)		
NG tubing					
With	3 (2.9)	17 (16.2)	20 (19.0)	0.607 (0.201,	0.554[a]
Without	21 (20.0)	64 (61.0)	85 (81.0)	1.837)	
Total	24 (22.9)	81 (77.1)	105 (100)		
Chemotherapy					
Received	7 (6.7)	23 (21.9)	30 (28.6)	1.029 (0.476,	0.941
Not received	17 (16.2)	58 (55.2)	75 (71.4)	2.227)	
Total	24 (22.9)	81 (77.1)	105 (100)		

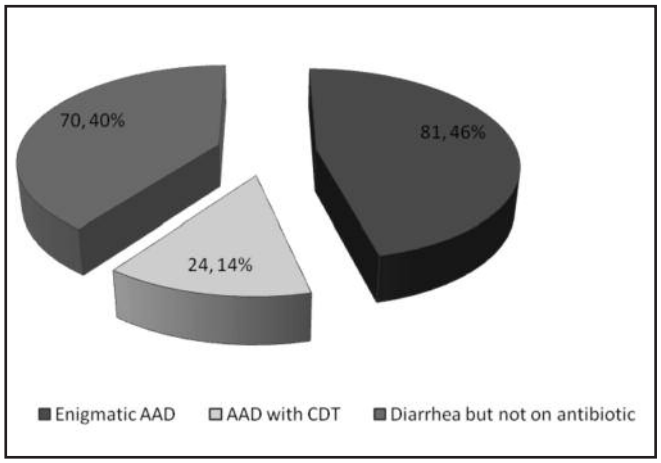

Fig. 1: Distribution of patients with diarrhea in relation to prior antibiotic treatment.

Among the 24 patients with positive CDT, majority (n=18, 87.5 %) had underlying medical illness which were diabetes mellitus, chronic renal failure, hypertension, ischaemic heart disease, cerebrovascular disease and malignancy. Seven of the patients were on chemotherapy and three had history of nasogastric intubation. The mean duration of hospitalization in patient whose antibiotic treatment was started during admission was 27.96 (± 23.22) days, while the mean duration of antibiotic treatment was 17.75 (±13.75) days. The mean duration of diarrhea in these patients were 5.21 (±5.85) days.

There was a significant association between history of previous antibiotics treatment and positive detection of CDT (P<0.035). The risk for acquisition of CDT was found to double in patients with prior antibiotics treatment compared to those without the treatment, and 1.85 times more in a patient with concurrent medical illness. However, histories of underlying medical illness or receiving chemotherapy or presence of nasogastric tubing were found not to have any significant association with presence of CDT in the stool samples (Table II). Only 6 (25 %) had received chemotherapy and 3 (12.5 %) had had nasogastric intubation.

DISCUSSION

Hospitalization with discharged diagnosis of CDI had significantly increased in many parts of the world [13,14]. For instance in Asia, the incidence of CDI in Singapore rose sharply from 1.49 cases per 10,000 patient-days to 6.64 cases per 10,000 patients-days over a period of 6 years from 2001-2006[15]. The percentage of CDT positives samples were also noted to increased from 7% to 11% during the same study period. Another study done in Taiwan reported the prevalence of CDT among 48 patients with antibiotic associated diarrhea at the National Taiwan University Hospital as 12.5 %6 .Korea and China showed prevalence of 10.8% and 9.5% respectively16.In developing countries like India, the positivity for CDT was 30% patients in the antibiotic receiving group compared to only 7% in those not receiving the antibiotics[12]. The presence of CDT among patients with diarrhea in our study in Malaysia was found to be 13.7% (95% CI 8.6 – 18.8), which is comparable to the prevalence of CDT estimated in other Asian country.

The majority of our CDT positive patients were older than 50 years old; only five (20.8 %) AAD patients were in the paediatric age group of 3-12 years old. *C. difficile* was found to be an important pathogen responsible for AAD in children of 5-12 year age group in a study in India[13]. Even though the organism can be part of the intestinal normal flora of the younger age group, it is more often than not being associated with diarhoea in antibiotic receiving children. Most of our patients had underlying medical illness and had been administered multiple drugs including broad spectrum antibiotics such as aminoglycosides, carbapenem, third and fourth generation cephalosporin, tazobactam-piperacillin and fluoroquinolones group. Older age group and severe underlying medical illness are both known risk factors for the acquisition of *C. difficile*, and possibly CDI[17, 18, 19].

Other risk factors reviewed in our study were history of previous antibiotic treatment (that is, at least in the 6 weeks from date of hospitalization), nasogastric intubation, history of chemotherapy, prolonged hospitalization and extended antibiotic treatment. The mean duration of hospitalization and antibiotic treatment were 27.96 (± 23.22) days and 17.75 (±13.75) days respectively, which showed that patients with CDT positive in our local setting had prolonged hospitalization and longer duration of antibiotic treatment. These findings were consistent with findings from other hospitals, that is a CDT-positive result was more likely to occur in those with prolonged hospital admissions (>14 days) than in those who had shorter hospital stays (<7 days)[12]. Our study further supports the significance of broad spectrum antibiotics as one of the risk factors for CDAD. However, chemotherapy and nasogastric intubation were not significant risk factors.

The current study also compared the clinical characteristics between diarrhea patients with positive CDT and those with negative CDT; the latter being classified as enigmatic AAD patients. Even though only 37.5 % of our CDT patients had previous history of antibiotic treatment, we found that they were more likely to acquire CDT when compared to those without antibiotic treatment. More than 60 % of patients with CDT were positive for stool occult blood and pus cell tests, making these two laboratory parameters an important feature in the diagnosis of CDI.

Laboratory test requested by clinicians for the detection of CDT were received for only 11 (45.8%) of the CDT positive patients. More than half of the loose stool samples received was not for CDT detection but for other microbiological testing. Thus, we would recommend that clinicians have a high index of suspicion for CDI whenever inpatients presented with diarrhea, so that stool samples are collected for laboratory detection of CDT.

CONCLUSION

The detection of CDT in the diagnosis of CDI requires vigilance by both clinicians and microbiologists in looking out for possible infected patients. Even though the prevalence of CDT among our diarrhea patient is lower than those estimated by US CDC, our study's result could probably be an underestimation. Antibiotic usage is a known risk factor for CDI; thus wisely used antibiotics may results in lowered number of CDI cases with subsequent less hospital cost associated with hospital stays and required treatment.

LIMITATION

This study did not detail the analysis of each antibiotic in relation to positive CDT the patients were exposed to, nor antifungal exposure; the latter being part of empiric treatment in controlling infection or in cases where fungal infection has presumptively sets in. We also did not differentiate between colonization and infection by C difficile, as CDT screening was done on all the loose stool samples regardless of clinical diagnosis. Confirmation of possible toxin B+ cannot be done because of contamination in the stored culture.

ACKNOWLEDGMENT

USM short term grant (304/PPSP/6131520), Medical Microbiology and Parasitology Laboratory in the Department of Medical Microbiology and Parasitology USM, Hospital Universiti Sains Malaysia.

REFERENCES

1. Bartlett JG. Clostridium difficile: history of its role as an enteric pathogen and the current state of knowledge about the organism. Clin Infect Dis1994; 18(Suppl.4), S265-S272.
2. Kuijper EJ, and Dissel JT. Spectrum of Clostridium difficile infections outside health care facilities CMAJ 2008;7; 179(8): 747-8.
3. Mogg GA, Keighley MR, Burdon DW et al. Antibiotic-associated colitis - a review of 66 cases. Br J Surg 1979; 66: 738-42.
4. Burckhardt F, Friedrich A, Beier D,Eckmanns T. Clostridium difficile surveillance trends, Saxony, Germany. Emerg Infect Dis 2008; 14: 691–692.
5. Soler P, Nogareda F,Cano R. Rates of Clostridium difficile infection in patients discharged from Spanish hospitals, 1997–2005. Infect Control Hosp Epidemiol 2008; 29: 887-9.
6. Hsu MS, Wang JT, Huang WK, *et al.* Prevalence and clinical features of Clostridium difficile associated diarrhoea in tertiary hospital in Northern Taiwan. Journal of Microbiol Immuno Infect 2005; 32: 242-8.
7. McDonald LC, Killgure GE, Thumpson A et al. An epidemic, toxin gene-variant strain of Clostridium difficile. N. Engl. J. Med 2005; 353: 2433-41.
8. Labbe AC, Poirier L, Maccannell D, *et al.* Clostridium difficile infections in a Canadian tertiary care hospital before and during a regional epidemic associated with the BI/NAP1/027 strain. Antimicrob. Agents Chemother 2008; 52: 3180-7.
9. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of Clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case–control studies. CMAJ 2004; 171.
10. Tim Planche, Adamma Aghaisu, Richard Holliman, *et al.* Diagnosis of Clostridium difficile infection by toxin detection kits: a systematic review. The lancet.com/infection Vol 8 December 2008.
11. Summers A, Snow K, Dunham L, Craft D. Comparative evaluation of six commercially available assays for the detection of Clostridium difficile toxins in fecal specimens. Proceedings of the American Society of Microbiology. 106th General Meeting, Orlando, FL, USA; May 21-25. Abstract 107876.
12. Vaishnavi C, Kochhar R, Bhasin DK, Thapa BR, Singh K. Detection of Clostridium difficile toxin by an indigenously developed latex agglutination assay. Trop Gastroenterol 1999: 20: 33-5.
13. Barbut F, Mastrantonio P, Delmée M et al. Prospective study of Clostridium difficile-associated disease in Europe with phenotypic and genotypic characterization of the isolates. Clin Microbiol Infect 2007; 13: 1048-57.
14. McDonald LC, George E. Killgore, Angela Thompson at al. An Epidemic, Toxin Gene–Variant Strain of Clostridium difficile. N Engl J Med 2005; 353: 2433-2441December 8, 2005.
15. Lim PL, Timothy MS, Ling LM, *et al.* Increasing incidence of Clostridium difficile associated disease, Singapore. Emerging Inf Diseases 2008; 14: 9.
16. 11th Western Pacific Congress on Chemotherapy and Infectious Diseases.Taiwan 2008.
17. Sunenshine RH, McDonald LC. Clostridium difficile–associated disease: new challenges from an established pathogen. Cleveland Clin J Med 2006; 73: 187-97.
18. Pepin J, Saheb N, Coulombe MA, *et al.* Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile–associated diarrhea: a cohort study during an epidemic in Quebec. Clin Infect Dis 2005; 41: 1254-60.
19. Loo VG, Poirier L, Miller MA, *et al.* A predominantly clonal multiinstitutional outbreak of Clostridium difficile–associated diarrhea with high morbidity and mortality. N Engl J Med 2005; 353: 2442-9.