Characteristics and Demographic Distributions of Toxigenic *Clostridium difficile* Strains in Rajavithi Hospital, 2009-2015

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Background: *In recent years, toxigenic Clostridium difficile infection (CDI) has been the cause of C. difficile-associated diseases (CDAD), resulting in increasing hospitalization, community infection outbreak and mortality all over the world.*
Objective: *To investigate the prevalence of toxigenic C. difficile strains in Rajavithi Hospital.*
Material and Method: *This was a retrospective study of data from C. difficile toxin tests at Rajavithi Hospital's microbiology laboratory from January 1, 2009 to June 30, 2015.*
Results: *Of the 5,821 stool specimens tested for C. difficile toxin, 561 (9.6%) were positive for toxigenic C. difficile strains (10.2%, 7.6%, 5.6%, 9.6%, 9.7%, 15.4% and 12.0% respectively from year 2009 to 2015). The samples of all 453 unrepeated patients with this toxigenic C. difficile strain were analyzed. Two hundred and fifty-six (56.5%) of the patients were female, and the highest prevalence (58.3%) of toxigenic C. difficile strains was found in those aged over 65 years old. Overall, 7.7% of toxigenic C. difficile strains were isolated from OPD while the other 92.3% were from IPD. The majority of these strains (73.0%) were isolated in patients from the internal medicine wards, followed by surgery wards, intensive care units (ICUs), gynecology wards, radiation oncology wards and orthopedic wards at 8.9%, 6.2%, 2.4%, 2.2% and 1.1%, respectively.*
Conclusion: *The prevalence of toxigenic C. difficile strains at Rajavithi Hospital was not high. The occurrence of these strains, recorded for each month of the study period, showed an irregular trend, but by far the highest frequency was found in older age groups. Rapid and accurate detection is necessary to enable immediate treatment and to stop the spread of infection. In future, these strains will be studied at a molecular level and antimicrobial susceptibility testing will be performed and the results entered in a database of toxigenic C. difficile strains in Thailand.*

Keywords: *Clostridium difficile, prevalence, toxin, Thailand*

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Clostridium difficile is an anaerobic, gram-positive, spore-forming bacillus that is frequently found in the intestinal flora of babies (up to 50%) but rarely in adults (less than 3%)[1]. Pathogenic strains of *C. difficile* produce two different toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin), which are the main virulence factors of these organisms[1]. Toxigenic *C. difficile* strains, Toxin A and B (A+B+)[1], Toxin B only (A-B+)[2] or binary toxin[3], are the major causes of *C. difficile*-associated diseases (CDAD)[4] of which the most common is antimicrobial-associated diarrhea (AAD)[5]. In addition to diarrhea symptoms, this disease can lead to pseudomembrane colitis (PMC), toxic megacolon and sepsis[1,4]. The clinical manifestations of *C. difficile* infection (CDI) range from asymptomatic or mild diarrhea to severe colitis and death[1], especially from binary toxins, ribotype 027[3] and variant strains, and ribotype 017[2], which are hypervilurent *C. difficile* strains and cause severe illness resulting in increased mortality and infection outbreak.

The risk factors of acquiring CDAD are undergoing prolonged antibiotic therapy, both inside and outside hospitals, being elderly, or being immunocompromised[6].

In recent years, the incidence of both hospital-acquired CDI[1,3] and community-acquired CDI has increased markedly[7,8].

In Thailand, there have still been few reports about the incidence of CDAD[9-11]. The present study investigated the prevalence of toxigenic *C. difficile*

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strains isolated at Rajavithi Hospital, Bangkok, Thailand from 2009 to 2015, as well as patients' characteristics and sources of infection.

Material and Method

The protocol of this research was reviewed and approved by the ethics committee of Rajavithi Hospital (No. 009/2558).

Collection of data

From January 1, 2009 to June 30, 2015, a study was performed at Rajavithi Hospital, a 1,200-bed tertiary care centre in Bangkok, of hospitalized patients who were over 15 years old. All patients' data, including their stool samples, which were sent to Rajavithi Hospital's Microbiology section for detection of *C. difficile* toxin, were collected, and all positive results were studied. Incidence of the same pathogen in the same patient was defined as an isolate. Demographic data including sex, age and hospital units were analyzed. In classification of hospital units, outpatient departments (OPD) included emergency rooms and observation rooms, while inpatient departments (IPD) were hospitalization wards.

Methods of detecting C. difficile toxin

Before March 1, 2014, Rajavithi Hospital's Microbiology section used the immunochromatographic technique (Enzyme immunoassay (EIA)) in the form of The Xpect *Clostridium difficile* toxin A/B test kit (Remel, Lenexa, USA) and the RIDAQUICK *Clostridium difficile* Toxin A/B (R-Biopharm AG, Germany). The sensitivity and specificity of the Xpect test were 86.3% and 96.2%, and those of the RIDAQUICK test were 100% and 91.0%, respectively. After March 1, 2014, Rajavithi's Microbiology section started to use real-time polymerase chain reaction (PCR) for detection of the *C. difficile* toxin B gene (*tcdB*) using BD MAX™ CDIFF assay (Becton Dickinson, Franklin Lakes, NJ). The sensitivity and specificity of this test were 96.3% and 92.4%, respectively.

Stool collection and storage

A liquid or soft stool specimen was collected in a clean container and sent to Rajavithi Hospital's Microbiology laboratory. If the stool sample could not be tested immediately, it was stored at 4°C for no more than 24 hours.

Statistic analysis

Data were collected using the MLAB program and analyzed using percentages.

Results

From January 1, 2009 to June 30, 2015 (6.5 years), 5,821 stool specimens were tested for *C. difficile* toxin using the immunochromatography assay, in the form of the Xpect or RIDAQUICK test, or the real-time PCR assay, the BD-Max *C. difficile* test.

A total of 561 (9.6%) specimens were positive for *C. difficile* toxin (toxigenic *C. difficile* strains). Of these, 10.2% (52/508) were found in 2009, 7.6% (53/700) in 2010, 5.6% (61/1,086) in 2011, 9.6% (109/1,141) in 2012, 9.7% (118/1,213) in 2013, 15.4% (124/805) in 2014 and 12.0% (44/368) in 2015 (from January 1 to June 30) (Table 1). The distribution pattern of toxigenic *C. difficile* strains in each year was irregular (Fig. 1). All 453 positive cases of toxigenic *C. difficile* strain were examined. Of the 561 positive specimens, 256 (56.5%) were from females and 197 (43.5%) were from males; 36.7% were from patients aged more than 75 years (22.1% female and 14.6% of male); 21.6% were

Table 1. Percentage of toxigenic *Clostridium difficile* strains isolated at Rajavithi Hospital, 2009-2015

Year	No. of total tested	No. positive of *C. difficile* toxin, n (%)
2009	508	52 (10.2)
2010	700	53 (7.6)
2011	1,086	61 (5.6)
2012	1,141	109 (9.6)
2013	1,213	118 (9.7)
2014	805	124 (15.4)
2015	368	44 (12.0)
Total	5,821	561 (9.6)

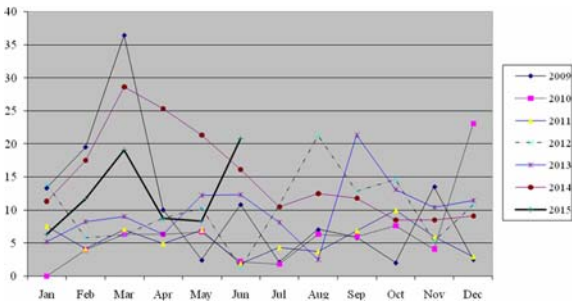

Fig. 1 Percentage of toxigenic strains of *Clostridium difficile* isolated at Rajavithi Hospital, 2009-2015, each month.

from people the age group 65-74 years (11.9% female and 9.7% male); 27.8% were from 45-64 year-olds (14.1% female and 13.7%); and 13.9% were from patients aged 15-44 years old (8.4% females and 5.5% males) (data not shown) (Fig. 2).

Of the 453 toxigenic *C. difficile* strains, 35 (7.7%) were isolated in the outpatient department (OPD), and the other 418 (92.3%) were isolated in the inpatient department (IPD). The ratio of OPD to IPD was 0.08 (35/418).

Of the 35 OPD cases, 37.1% were patients over 75 years old, 14.3% were in the age group 65-74 years, 28.6% were patients aged 45-64 years old and 20.0% were in the age range 15-44 years old (data not shown). Of the 418 IPD cases, the highest number of toxigenic *C. difficile* strains was found in stool samples of patients hospitalized in the medical wards (73.0%) followed by surgical (8.9%), intensive care units (ICUs) (6.2%), gynecological (2.4%), radiation oncological (2.2%) and orthopedic wards (1.1%) (Table 2).

Discussion

The prevalence of toxigenic *C. difficile* strains at Rajavithi Hospital was 9.6%, which was higher than that found in North Indian strains (7.1%)[12], but lower than found in the study by Wongwanich S (18.6%)[9]. However, a trend towards increasing prevalence of toxigenic *C. difficile* at Rajavithi Hospital was observed, with an increase from 8.3% in May 2015 to 20.8% in June 2015.

As in other studies[13], one of the risk factors for CDI was being over 65 years old, and in our study, this was the prominent (58.3%) age range.

The ratio of OPD to IPD cases at Rajavithi was 0.08, almost as low as that found in another study performed in a Korean tertiary hospital (0.06)[14]. In Canada, the US and Europe, the ratio of community-

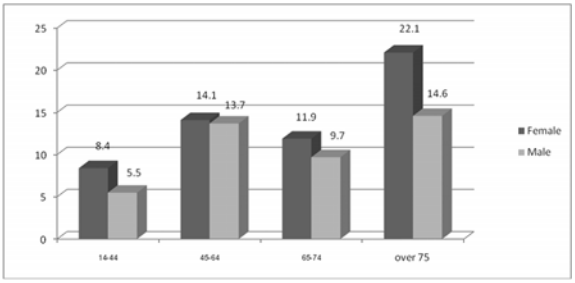

Fig. 2 Percentage of toxigenic strains of *Clostridium difficile* isolated at Rajavithi Hospital, 2009-2015, by Sex and Age.

Table 2. Percentage of toxigenic strains of *Clostridium difficile* isolated at Rajavithi Hospital, 2009-2015, by Wards

Year	No. positive of *C. difficile* Toxin	OPD (%)	IPD (%)							
			Total	Med	Sur	ICU	Gyn	Rad	Ort	Other
2009	51	2	49	32	7	1	1	-	-	8
2010	43	2	41	29	1	1	-	3	1	6
2011	46	2	44	31	3	4	1	-	-	5
2012	76	0	76	57	8	3	2	2	1	3
2013	95	19	76	57	5	6	4	1	1	2
2014	101	8	93	71	7	9	2	3	1	-
2015	41	2	28	28	6	2	-	-	1	2
Total	453	35 (7.7)	418 (92.3)	305 (73)	37 (8.9)	26 (6.2)	10 (2.4)	9 (2.2)	5 (1.1)	26 (6.2)

onset CDI (CO-CDI) to healthcare-associated CDI (HA-CDI) ranged from 0.23 to 5.9[15]; however, the authors have found little CDI in the community in Thailand. As in the study by Khanna S et al[16], there was a higher proportion of younger (15-44 years) patients in OPD compared to IPD, at 20% and 13.9% respectively; however, those aged over 75 years made up the largest proportion (37.1%) of patients in OPD.

In the research by Chaudhry R et al[12], most toxigenic *C. difficile* was found in the medical wards (72%) primarily hematology/oncology wards (67.5%), followed by gastrointestinal surgery, neurology and nephrology and other medical wards. This was comparable with the findings of our study, which identified most of this infection in the medical wards (73%).

In Thailand, most laboratories use EIA tests for toxigenic *C. difficile* detection, and these often give false negative results due to their low sensitivity; hence, this infection can be misdiagnosed, leading to improper treatment. The use of molecular tests yields higher sensitivity[17]. The limitations of this study included absence of information about antibiotic treatment, clinical data of patients, results of treatment and unclassified toxin profiles and ribotypes.

Conclusion

The prevalence of toxigenic *C. difficile* strains at Rajavithi Hospital between 2009 and 2015 was not high, averaging approximately 10%. The majority of these strains were found in the medical wards in each year of the study period, and their incidence displayed an irregular pattern. Unfortunately, in the last two months of this study, May and June 2015, their prevalence increased dramatically from 8.3% to 20.8%. Owing to this increasing incidence of CDI in both hospitals and the community, further studies need to be performed to characterize these toxigenic *C. difficile* strains by toxin profiles: A+B+, or A-B+, or binary toxin, ribotypes 017 or 027; to identify cultures using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF); to compare the specificity of peaks of proteins with ribotypes for surveillance of CDI; and to test antimicrobial susceptibility. Importantly, we need databases at Rajavithi Hospital to enable the Infectious Control Committee (ICC) to provide accurate treatment for this disease and to carry out surveillance of the organisms in order to limit infection.

What is already known on this topic ?

The incidence of *Clostridium difficile*-associated disease (CDAD) is increasing worldwide and has different geographic distributions.

CDAD causes major nosocomial diarrhea infection because of imbalance of bacteria in the intestines due to prolonged use of antibiotics.

In recent years, there have been many reports of CDAD causing community diarrhea infection and related with environment.

Risk factors of CDAD are advanced age (over 65 years), prolonged use of antibiotics, and prolonged hospital stay.

Toxins of CDAD include three types; A+B+, A-B+, and hypervirulent/binary toxins which are 16-25 times more virulent and cause mortality.

Hypervirulent ribotype 027 strains are mostly found in America and Europe.

Variant hypervirulent ribotype 017, A-B+, are found mostly found in Asia.

There have been many reports of antimicrobial susceptibility testing of CDAD, and the treatment drugs of choice are vancomycin and metronidazole.

A few reports found that *Clostridium difficile* was resistant to metronidazole.

What this study adds ?

There are few reports of CDAD in Thailand. The present study showed the prevalence of toxigenic *Clostridium difficile* in a large tertiary 1,200-bed adult centre, Rajavithi Hospital, isolated between 2009 and 2015 (6.5 years).

The present study showed the distribution of toxigenic *Clostridium difficile* including sex, age group, and source of infection.

The prevalence of toxigenic *Clostridium difficile* at Rajavithi Hospital, Bangkok, Thailand, was approximately 10%, showing an irregular pattern, and at present, it has not reached outbreak levels.

Acknowledgement

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Potential conflict of interest

None.

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คุณลักษณะและแผนภาพการกระจายของเชื้อ Clostridium difficile สายพันธุ์ที่สร้างสารพิษในโรงพยาบาลราชวิถี ระหว่างปี พ.ศ. 2552-2558

ประกายทิพย์ ทองคุ้ม, สุวัฒนา กาญจนหฤทัย, สุริวรรณ จันทรคุปตังกูร, สมศักดิ์ ราหุล

ภูมิหลัง: *ในปัจจุบันพบการติดเชื้อ Clostridium difficile สายพันธุ์ที่สร้างสารพิษ เป็นสาเหตุของเกิดโรคติดเชื้อ C. difficile ทั้งการติดเชื้อภายในโรงพยาบาลและชุมชน การระบาดและเป็นสาเหตุของการเสียชีวิตเพิ่มขึ้นทั่วโลก*

วัตถุประสงค์: *เพื่อทำการประเมินสถานการณ์ความชุกของเชื้อ C. difficile สายพันธุ์ที่สร้างสารพิษในโรงพยาบาลราชวิถี*

วัสดุและวิธีการ: *การศึกษาข้อมูลย้อนหลังของการตรวจหาสารพิษของเชื้อ C. difficile ของงานจุลชีววิทยา โรงพยาบาลราชวิถี ระหว่างวันที่ 1 มกราคม พ.ศ. 2552 ถึง 30 มิถุนายน พ.ศ. 2558*

ผลการศึกษา: *สิ่งส่งตรวจประเภทอุจจาระของผู้ป่วยที่ส่งตรวจหาสารพิษของเชื้อ C. difficile จำนวน 5,821 ตัวอย่าง พบให้ผลบวกสำหรับสารพิษของเชื้อ C. difficile เป็นการพบเชื้อ C. difficile สายพันธุ์ที่สร้างสารพิษจำนวน 561 สายพันธุ์ คิดเป็นร้อยละ 9.6 โดยในปี พ.ศ. 2552 พบร้อยละ 10.2 พ.ศ. 2553 พบร้อยละ 7.6 พ.ศ. 2554 พบร้อยละ 5.6 พ.ศ. 2555 พบร้อยละ 9.6 พ.ศ. 2556 พบร้อยละ 9.7 พ.ศ. 2557 พบร้อยละ 15.4 และ พ.ศ. 2558 พบร้อยละ 12.0 จากการวิเคราะห์ข้อมูลของผู้ป่วยที่พบเชื้อ C. difficile สร้างสารพิษแบบไม่ซ้ำคนจำนวน 453 คน พบ 256 คน คิดเป็นร้อยละ 56.4 เป็นเพศหญิง พบเชื้อ C. difficile สายพันธุ์สร้างสารพิษในกลุ่มอายุมากกว่า 65 ปีมากที่สุดคิดเป็นร้อยละ 58.3 แยกเชื้อสายพันธุ์นี้ได้จากผู้ป่วยนอกร้อยละ 7.7 จากหอผู้ป่วยในร้อยละ 92.3 โดยพบเชื้อสายพันธุ์นี้มากที่สุดในหอผู้ป่วยอายุรกรรมร้อยละ 73.0 หอผู้ป่วยศัลยกรรม หอผู้ป่วยหนัก หอผู้ป่วยนรีเวช ห้องผู้ป่วยรังสีรักษา และหอผู้ป่วยออร์โธปิดิกส์ร้อยละ 8.9, 6.2, 2.4, 2.2 และ 1.1 ตามลำดับ*

สรุป: *ความชุกของเชื้อ C. difficile สายพันธุ์สร้างสารพิษที่โรงพยาบาลราชวิถียังไม่สูงมาก แนวโน้มของอุบัติการณ์การพบเชื้อสายพันธุ์เหล่านี้ ในแต่ละเดือนระหว่างปี พ.ศ. 2552-2558 ไม่แน่นอนอย่างไรก็ตามเชื้อสายพันธุ์เหล่านี้พบมากในผู้สูงอายุ ดังนั้นการทดสอบที่ไว และถูกต้องจึงมีความจำเป็นอย่างยิ่งต่อการรักษาที่ถูกต้องและการป้องกันการแพร่กระจายของเชื้อ การศึกษาในอนาคตทำการวิเคราะห์แยกเชื้อ C. difficile สายพันธุ์สร้างสารพิษที่โรงพยาบาลราชวิถีในระดับโมเลกุลและทำการทดสอบความไวของเชื้อสายพันธุ์นี้ต่อยาต้านจุลชีพ ทั้งนี้เพื่อเป็น ฐานข้อมูลของการติดเชื้อนี้ในประเทศไทยต่อไป*