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ORIGINAL ARTICLE

Prevalence and molecular epidemiology of *Clostridium difficile* infection in Indonesia

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Abstract

Clostridium difficile has not been studied in detail in Asia, particularly Southeast Asia. We thus performed a prevalence study across four hospitals in Central Java province, Indonesia. Stool samples were collected from patients with diarrhoea and tested by enzyme immunoassay for glutamate dehydrogenase (GDH) and toxin A/B (C DIFF QUIK CHEK COMPLETE, TechLab). Specimens were cultured and molecular typing was performed. In total, 340 samples were tested, of which 70 (20.6%) were GDH positive, with toxin detected in 19 (5.6%). Toxigenic *C. difficile* was isolated from 37 specimens (10.9%), while a further 36 (10.6%) nontoxigenic isolates were identified. The most common strain was ribotype 017 (24.3% of 74 isolates), followed by nontoxigenic types QX 224 (9.5%), and QX 238 and QX 108 (both 8.1%). The high prevalence of *C. difficile* highlights a need for ongoing surveillance of *C. difficile* infection in Indonesia.

Keywords: *Clostridium difficile*, epidemiology, Indonesia, molecular epidemiology, prevalence
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Introduction

Clostridium difficile causes hospital- and community-acquired infections ranging in severity from self-limiting diarrhoea to life-threatening toxic megacolon and pseudomembranous colitis. The main risk factors for *C. difficile* infection (CDI) are recent antimicrobial exposure, hospitalization, residence in a healthcare facility and advanced age [1].

The symptoms of CDI are mediated by toxins A (enterotoxin) and B (cytotoxin), encoded by the genes *tcdA* and *tcdB* respectively [2]. A third binary toxin, CDT, is encoded by the genes *cdtA* and *cdtB,* which are found less frequently than *tcdA* and *tcdB* [2]. Toxigenic strains cause disease, always carry *tcdB* and generally *tcdA* and less frequently produce binary toxin; however, asymptomatic colonization with toxigenic strains is also possible. Nontoxigenic strains do not carry *tcdA* and *tcdB;* nor do they cause disease. Therefore, detection of *C. difficile* alone is insufficient to diagnose CDI because toxin A and/or B must be detected in stool and diarrhoea must be present [3]. PCR detection of *tcdB* can identify toxigenic *C. difficile* but will not distinguish colonization from infection.

Recent major outbreaks of CDI in North America and Europe were attributed to a binary toxin-positive ($A^+B^+CDT^+$) strain of *C. difficile* (ribotype (RT) 027) [4]. Outbreaks caused by this strain have highlighted the need for worldwide surveillance of CDI and causative strains. The relatively few reports about *C. difficile* from Asia show a predominance of a toxin A–negative toxin B–positive (A^-B^+) strain, RT 017, while CDT^+ strains are rare [5]. However, the limited investigations of CDI in Asia, particularly Southeast Asia [5], may have given an incomplete picture. Other diarrhoea-causing pathogens are more commonly tested for as a result of poor awareness of

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TABLE 1. Results of EIA and culture analysis in Indonesian inpatients

EIA result	Culture	Toxin profile	Site, *n* (%)				
			1 (n = 148)	2 (n = 98)	3 (n = 49)	4 (n = 45)	Total (n = 340)
$GDH^+/Toxin^+$	Positive	$A^+B^+CDT^-$	1 (0.7)	0	2 (4.1)	1 (2.2)	4 (1.2)
		$A^-B^+CDT^-$	12 (8.1)	1 (1.0)	0	0	13 (3.8)
		$A^-B^-CDT^-$	4 (2.7)[a]	1 (1.0)[a]	0	0	5 (1.5)[a]
	Negative		1 (0.7)[a]	1 (1.0)	0	0	2 (0.6)[a]
$GDH^+/Toxin^-$	Positive	$A^+B^+CDT^-$	6 (4.1)	0	5 (10.2)	1 (2.2)	12 (3.5)[b]
		$A^-B^+CDT^-$	6 (4.1)	2 (2.0)	0	1 (2.2)	9 (2.6)
		$A^-B^-CDT^-$	10 (6.8)[c]	9 (9.2)	9 (18.4)	3 (6.7)	31 (9.1)[c]
	Negative		1 (0.7)	1 (1.0)	0	0	2 (0.6)
Negative			112 (75.7)	84 (85.7)	35 (71.4)	39 (86.7)	271 (79.5)

EIA, enzyme immunoassay; GDH, glutamate dehydrogenase.
[a]Five specimens contained one toxigenic and one nontoxigenic strain.
[b]One specimen contained two distinct toxigenic strains.
[c]Two specimens contained one toxigenic and one nontoxigenic strain.

CDI among Asian physicians [6]. In addition, limited resources mean diagnostic tests for CDI are often lacking or inadequate. For example, in a study from the Philippines, patients with CDI were incorrectly diagnosed with amoebiasis [7]. In addition, some studies of CDI performed in the early 2000s used enzyme immunoassays (EIAs) for toxin A to detect *C. difficile,* which likely resulted in underdiagnosis of CDI, given the high proportion of RT 017 strains in Asia [5].

There are very few reports on CDI in Indonesia. A prevalence of 1.3% by toxin A EIA was reported in community and hospital clinics in Jakarta in 1999 [8], and eight Indonesian strains of *C. difficile* isolated from healthy individuals, six of which were A^-B^+, were described in 1993 [9]. Reports of inappropriate antibiotic prescribing [10] and free access to antibiotics without prescriptions suggest that CDI may be common in Indonesia. Therefore, we aimed to investigate the prevalence and molecular epidemiology of CDI in hospital inpatients in Indonesia.

Methods

This prospective study was conducted from July 2014 to February 2015 in four hospitals in Central Java province, as follows: site 1, Semarang, 1070 beds; site 2, Jepara, 420 beds; site 3, Purwokerto, 730 beds; and site 4, Semarang, 240 beds. Diarrhoeal (loose or watery) stool specimens were tested at all sites by EIA for glutamate dehydrogenase (GDH) and toxin A/B using C DIFF QUIK CHEK COMPLETE (TechLab, USA). GDH-positive specimens were sent to Western Australia on transport swabs in Cary-Blair medium (Medical Wire & Equipment Co. Ltd., UK) for culture and molecular analysis. Culture, PCR ribotyping and toxin gene detection of *C. difficile* were performed as previously described [11].

Results

In total, 340 samples were tested, of which 19 (5.6%) were GDH positive/toxin positive. A further 51 (15.0%) were GDH positive/toxin negative (Table 1). *C. difficile* was not recovered by culture from four specimens, two of which were toxin positive. Eight specimens yielded two unique isolates, resulting in isolation of 74 unique *C. difficile* strains. A *tcdB*-positive *C. difficile* strain was isolated from 20 toxin-negative specimens. Overall, 38 unique strains were *tcdB* positive, with two isolated from the same specimen, giving a prevalence of toxigenic *C. difficile* of 37 (10.9%) of 340. Six other specimens yielded one toxigenic and one nontoxigenic strain each. The overall prevalence of nontoxigenic *C. difficile* was 36 (10.6%) of 340. The most common toxigenic strain was RT 017 (A^-B^+, *n* = 18, 24.3% of isolates), then QX 134 (A^-B^+, *n* = 3, 4.1%), RT 053 and QX 215 (both A^+B^+, *n* = 3, 4.1%). Nontoxigenic types QX 224 (*n* = 7, 9.5%), QX 238 and QX 108 (*n* = 6, both 8.1%) were also common (Table 2). No CDT^+ strains were identified.

Discussion

The prevalence of *C. difficile* in Indonesia was relatively high compared to neighbouring countries. While Australia (7.2% toxigenic *C. difficile*) [12] has a prevalence comparable to many developed countries, Singapore (7–11% toxin positive) [13] and Malaysia (13.7%) [14] had higher prevalence by toxin EIA, a relatively insensitive test. A recent study in Thailand identified similar high proportions of toxigenic (9.2%) and nontoxigenic *C. difficile* (15.6%) among 422 patients with diarrhoea [11].

The high prevalence of *C. difficile* in Indonesia, a country with widespread inappropriate antibiotic usage, is concerning. Until

TABLE 2. Molecular types of Indonesian *Clostridium difficile* isolates, collected in Central Java province, July 2014 to February 2015

Isolate	Ribotype	*n* (%)
$A^-B^+CDT^-$	017	18 (24.3)
	QX 134	3 (4.1)
$A^+B^+CDT^-$	053	3 (4.1)
	QX 215	3 (4.1)
	014/020	2 (2.7)
	043	2 (2.7)
	103	2 (2.7)
	QX 076	2 (2.7)
	002	1 (1.4)
	QX 024	1 (1.4)
	QX 593	1 (1.4)
$A^-B^-CDT^-$	QX 224	7 (9.5)
	QX 238	6 (8.1)
	QX 108	6 (8.1)
	QX 083	2 (2.7)
	QX 561	2 (2.7)
	QX 011	1 (1.4)
	QX 012	1 (1.4)
	QX 053	1 (1.4)
	QX 104	1 (1.4)
	QX 107	1 (1.4)
	QX 153	1 (1.4)
	QX 206	1 (1.4)
	QX 510	1 (1.4)
	QX 571	1 (1.4)
	QX 594	1 (1.4)
	Other	2 (2.7)
	Total	74 (100.0)

recently, limited resources made CDI diagnostics inadequate, and anaerobic culture facilities facilitating molecular analysis were lacking, making surveillance difficult; however, culture facilities and molecular typing were recently established at site 1 (Dr Kariadi Hospital). It would be beneficial to carry out surveillance to monitor infection rates and movement of strains, and expand on the findings of this study.

One limitation of our study was that the prevalence may have been underestimated because GDH-negative samples, some of which may have been falsely negative, were not cultured. Furthermore, given ongoing debates about diagnosis of CDI [3], it is difficult to determine how many *tcdB*-positive patients were colonized and how many had a true infection.

The predominant molecular type in our study, RT 017 (Table 2), is commonly found throughout Asia, including China [5], and particularly in neighbouring Thailand [11] and Singapore [15]. The low prevalence of CDT^+ isolates is in line with previous Asian studies [5]. The high prevalence of nontoxigenic strains of *C. difficile* was interesting, and further studies should explore the role of such strains in Southeast Asia. Colonization with a nontoxigenic strain of *C. difficile* protects against colonization with other potentially virulent strains of *C. difficile* [16], and this may be occurring in Asia leading to lower rates of disease. The identification of molecular types which are frequently found in neighbouring countries supports the need for surveillance of international spread of *C. difficile* strains.

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Conflict of interest

None declared.

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