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Review Article

Epidemiology of *Clostridium* (*Clostridioides*) *difficile* Infection in Southeast Asia

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Abstract. This review describes the current understanding of *Clostridium* (*Clostridioides*) *difficile* infection (CDI) in southeast Asia regarding the prevalence of CDI, *C. difficile* detection methods, antimicrobial susceptibility profiles, and the potential significance of a One Health approach to prevention and control. Our initial focus had been the Indochina region, however, due to limited studies/surveillance of CDI in Indochina, other studies in southeast Asian countries and neighboring Chinese provinces are presented here for comparison. *Clostridium* (*Clostridioides*) *difficile* infection is one of the most common causes of hospital-acquired gastroenteritis worldwide. Since its discovery as a cause of pseudomembranous colitis in 1978, *C. difficile*-related disease has been more prevalent in high-income rather than low-income countries. This may be because of a lack of knowledge and awareness about the significance of *C. difficile* and CDI, resulting in underreporting of true rates. Moreover, the abuse of antimicrobials and paucity of education regarding appropriate usage remain important driving factors in the evolution of CDI worldwide. The combination of underreporting of true CDI rates, along with continued misuse of antimicrobial agents, poses an alarming threat for regions like Indochina. *C. difficile* ribotype (RT) 027 has caused outbreaks in North America and European countries, however, *C. difficile* RT 017 commonly occurs in Asia. Toxin A-negative/toxin B-positive (A^-B^+) strains of RT 017 have circulated widely and caused outbreaks throughout the world and, in southeast Asia, this strain is endemic.

INTRODUCTION

Clostridium (*Clostridioides*) *difficile* commonly causes toxin-mediated intestinal disease in humans after hospitalization and/or antimicrobial treatment. The outcomes of infection range from asymptomatic colonization to severe conditions such as pseudomembranous colitis (PMC), bowel perforation, toxic megacolon, sepsis, septic shock, and death.[1] Hardy spores are usually the infectious form of *C. difficile* and are commonly found in a variety of environments both in hospital and community settings. These include surfaces in hospitals and long-term care facilities,[2] soil and water, and the intestinal tracts of humans and food production animals such as pigs and cattle, often with higher numbers in younger animals.[3,4] Spores can persist in the environment for many months and cannot be eradicated with regular disinfectants.[2]

Toxins A and B produced by *C. difficile* play a major role in its pathogenicity. The main risk factor for *Clostridium* (*Clostridioides*) *difficile* infection (CDI) is antimicrobial exposure,[5] and antimicrobial use plays an important role in the development of CDI-related diseases. To date, CDI has occurred most commonly in high-income countries, where the management of antimicrobial use can be achieved. The regulation of antimicrobials in both medical and agricultural settings is extremely challenging in parts of Asia;[6] specifically the Indochina region has poor regulation of antimicrobial usage in both humans and animals.[7–10] Furthermore, the level of awareness of CDI among Asian physicians remains limited.[11] Hence, the prevalence of CDI in both humans and animals in Asia is assumed to be relatively high.

This review describes the epidemiology of CDI in southeast Asia, with a focus on Indochina, a region within that lies between India and China, and includes Cambodia, Lao PDR, and Vietnam. Reports of cases of CDI or CDI outbreaks in this region have been limited. Geographically, neighboring Indochina are Thailand, Myanmar, and two southern Chinese provinces (Yunnan and Guangxi), as illustrated in Figure 1. Other than from Thailand, there have been few published studies from these countries on the incidence or prevalence of *C. difficile* in hospital or community environments.[12–15] Thus, additional information from these southeast Asian countries surrounding Indochina has been included in the review for comparative purposes.

HISTORICAL BACKGROUND OF CDI

The first case of the gastrointestinal disease most likely caused by *C. difficile* was reported at the Johns Hopkins Hospital in Baltimore in 1893 by Finney who described PMC in a 22-year-old patient with hemorrhagic diarrhea after surgery and a diphtheritic membrane in the large bowel at autopsy.[16] *Clostridium* (*Clostridioides*) *difficile* was first identified from the meconium and feces of healthy neonates, and named *Bacillus difficilis*, by Hall and O'Toole in 1935.[17] Shortly after it was renamed *C. difficile* by Prevot[18] and, initially, was of little interest as a pathogen. Indeed, in 1940, Snyder described *C. difficile* as normal intestinal flora, based on his finding that *C. difficile* comprised 15.4% of the flora of infants from 2 weeks to 1 year of age.[19] *Clostridium* (*Clostridioides*) *difficile* was ignored for some 30 years when the toxins were shown to cause a number of biological effects in the early 1970s,[20] as well as a cytopathic effect (CPE) in cultured cells (although the investigators thought the CPE was viral rather than a bacterial toxin).[21] Finally, *C. difficile* was shown to be the cause of PMC in a series of papers published in 1978.[22–25]

For the next 20 years, *C. difficile* was not often thought of as a major infectious diseases issue; for example, the prevalence of toxigenic *C. difficile* in diarrheal stool samples in

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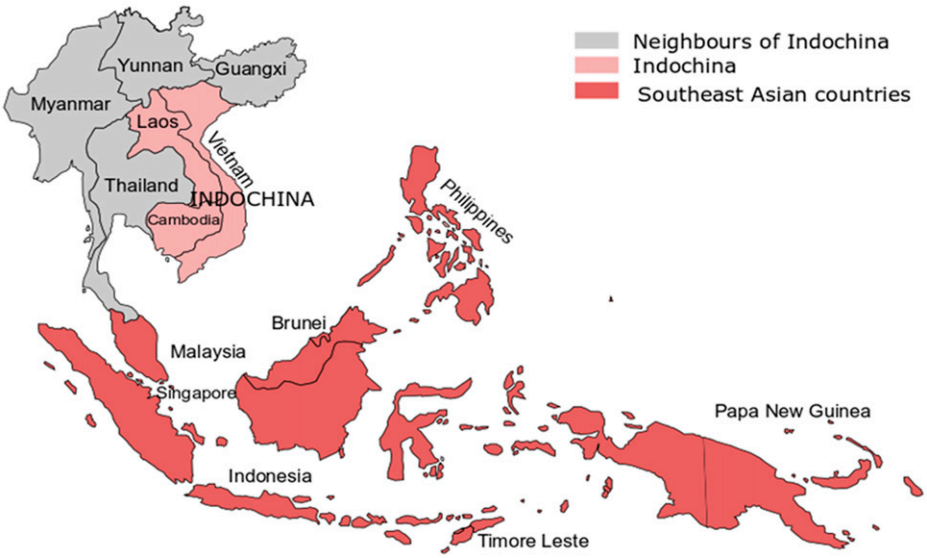

FIGURE 1. Indochina includes Cambodia, Laos, and Vietnam. The closest neighbors of Indochina are Myanmar, Thailand, and two Chinese provinces (Yunnan and Guangxi). Southeast Asian countries include all countries on this map, except two Chinese provinces. This figure appears in color at www.ajtmh.org.

Sweden from 1980 to 1982 was only 1.8%.[26] However, between 1980 and 1984, *C. difficile* accounted for approximately 45% of nosocomial diarrhea in the United States, with the incidence rate of nosocomial gastroenteritis a relatively low 1.3 per 10,000 discharges.[27] During the 1980s and 1990s, a significant correlation between an increase of the use of antimicrobials, such as third-generation cephalosporins, and an increase of CDI cases was demonstrated.[28,29] In Australia, the prevalence of *C. difficile* in stool samples rose when third-generation cephalosporins were increasingly used from 1983 to 1992.[28] The incidence of community-acquired *C. difficile* infection (CA-CDI) in the United States was 7.7 cases per 100,000 person-years from 1988 to 1990[30] and, in 1989, the prevalence of *C. difficile* associated hospital-acquired infection in the United States was 21%.[31] However, from 1996 to 2003, the prevalence of discharges with CDI in the United States almost doubled from 31/100,000 population to 61/100,000 population.[32] Similar increases were seen elsewhere in the world. Between 1994 and 2004, the incidence rate of CDI in the United Kingdom increased from below one case per 100,000 persons to 22 cases per 100,000 persons.[33] Polymerase chain reaction (PCR) for *C. difficile* toxin genes became popular for *C. difficile* detection in clinical samples from the early 1990s,[34,35] and this generated more interest in CDI, however, it also came with the problem of overdiagnosis that remains today.

A significant change in CDI epidemiology occurred at the beginning of the current millennium.[36] *Clostridium* (*Clostridioides*) *difficile* infection associated with the *C. difficile* ribotype (RT) 027 strain was first reported as a cause of major outbreaks in South-Eastern Canada in the early 2000s.[37] This strain later (or perhaps concurrently) caused outbreaks in the North East of the United States[38] spreading throughout North America and crossing the Atlantic to the United Kingdom and then to other European countries where numerous outbreaks were recorded[39–42] The prevalence of *C. difficile* RT 027 infection in many countries was high, particularly in the United Kingdom; RT 027 accounted for 41.3% and 27.5% of all toxigenic *C. difficile* strains in England and France, respectively.[40,42] Most countries invested in improved laboratory diagnostics and infection prevention and control[43–46] and a hospital-based survey across 34 European countries finished in 2008 (but published in 2011) recorded a decline in the prevalence of *C. difficile* RT 027 to as low as 5%, while an increased prevalence was seen for RTs 014/020, 001, and 078.[41] *Clostridium* (*Clostridioides*) *difficile* RT 078 had been reported as an emerging strain in The Netherlands around the same time.[47] Interestingly, small clusters of *C. difficile* RT 027 infections occurred in several countries outside North America and Europe such as Australia and Singapore between 2008 and 2009, but the strain did not appear to establish in these countries.[48,49] A report of a RT 027 outbreak in Japan was incorrect as it was neither an outbreak nor a "hypervirulent" strain, rather a historical RT 027 strain.[50] Over the past two decades, CDI in North America, Europe, and Australia has been better understood, while CDI data from Asia is still limited.

CLOSTRIDIUM DIFFICILE INFECTION IN SOUTHEAST ASIA

In the Asian region in general, the number of publications about *C. difficile* and CDI has increased substantially over the last 10–15 years; however, given this is where nearly 60% of the world's population live, a lack of data remains an important problem. A recent publication pertaining to the Asia-Pacific region confirmed that A^-B^+ strains of *C. difficile*, predominantly RT 017 (16.4%), and A^+B^+ strains of RT 014/020 (10.9%) were the most common strains of *C. difficile* causing CDI from 2014 to 2015. Binary toxin-positive (CDT^+) strains of *C. difficile* were rare (5.3%).[51]

The prevalence of CDI in southeast Asia appears variable, most likely due to a lack of awareness or a lack of testing (Table 1).[52] In the Philippines, for example, an ELISA method detected *C. difficile* in 43.6% of diarrheal stool samples from

TABLE 1
Summary of the prevalence of *Clostridium difficile* infection in southeast Asia

Country	Prevalence % (n) or incidence	Year	Toxigenic *C. difficile*	Testing method	Reference
Indonesia	20.6% (70/340)	2014–2015	10.9% (37/340)	EIA, PCR ribotyping, and toxin profiling	57
Malaysia	22.9% (100/437)	2015–2016	10.3% (45/437)	EIA, PCR ribotyping, and toxin profiling	62
Singapore	10.7/10,000 patient-days	2011–2012	92.4% (61/66)	EIA, PCR ribotyping	69
Thailand	23% (100/422)	2015	9.2% (39/422)	BD MAX CDiff assay, PCR ribotyping, and toxin gene profiling	75
China	14.1% (138/978)	2013–2016	14.1% (138/978)	MLST, PCR ribotyping, and toxin gene profiling	84
Vietnam	24.9% (95/382)	2013–2015	24.9% (95/382)	Nested PCR, and *slpA* sequence typing	12
Cambodia	3.75% (3/80)	2005	3.75% (3/80)	Toxin A-EIA	14
Lao PDR	8.6% (6/70)	2013	60% (3/5)	EIA, PCR ribotyping, and toxin gene profiling	15

EIA = enzyme immunoassay; PCR = polymerase chain reaction.

patients with colitis. At the time, this prevalence was the highest reported in southeast Asian countries,[53] and suggested that CDI was common and might be overlooked in settings where colitis due to *Entamoeba histolytica* and other intestinal parasites was common.[53] In addition, metronidazole therapy for parasitic infections would likely have had some impact on CDI. The following sections contain information from several other southeast Asian countries for comparative purposes.

Indonesia. Publications on CDI in Indonesia are not common. In a study on the etiology of pediatric diarrheal diseases in Jakarta published in 2002, 1.3% (2/154) of samples were positive for toxin A using the Premier *C. difficile* Toxin A Enzyme Immunoassay (EIA) (Meridian Diagnostic Inc., USA).[54] As this EIA detected toxin A only, the true prevalence of *C. difficile* was likely to have been higher. Later in 2014, a rapid membrane EIA (C. Diff Quik Chek Complete, Techlab) was used in a small cross-sectional study of elderly patients with hepatocellular carcinoma in Banten. The prevalence of nosocomial CDI in those patients was 25% (4/16 patients) and stool samples were positive for both glutamate dehydrogenase (GDH) and toxins A/B.[55] A more recent study combined multiple methods, including *C. difficile* selective agar or chromogenic agar culture, MALDI-ToF mass spectrometry, EIA (Quik Chek Complete) or Serazym® *C. difficile* toxin A+B immunoassay, multiplex PCR, and PCR ribotyping.[56] The prevalence of symptomatic CDI was 14.7% (25/170).[56] A total of 14 different RTs was identified among *C. difficile* isolates with SLO160 and RT 017 dominating (45.5%). Furthermore, toxin profiling showed $tcdA^+/tcdB^+$ accounted for 63.6% of *C. difficile* strains; the prevalence of RT 017 ($tcdA^-/tcdB^+$) was 18.2%.[56]

Between July 2014 and February 2015 in four hospitals in Central Java, diarrheal stool samples were tested with an EIA for GDH and toxins A/B.[57] The prevalence of samples positive for GDH alone and for both GDH and toxins A/B was 20.6% (70/340) and 5.6% (19/340), respectively.[57] After culture, 74 *C. difficile* strains were isolated and the prevalence of toxigenic *C. difficile* strains was 10.9% (37/340). The most common toxigenic *C. difficile* strains were RT 017 (A^-B^+) (24.3%), QX134 (A^-B^+) (4.1%), RT 053 (A^+B^+) (4.1%), and QX215 (A^+B^+) (4.1%), however, there were no CDT^+ strains.[57] In a recent small study, 1/31 diarrheal patients (3%) who received at least 2 days of antimicrobial therapy was positive for both GDH and toxins A/B by a rapid lateral flow immunoassay (IMMUNOQUICK Tox A/B).[58] There was no further investigation in this study.

Malaysia. Like Indonesia, few reports of CDI in Malaysia have been published. The earliest of these, in 1998, was a series of seven *C. difficile*-associated diarrhea (CDAD) cases with comorbidities.[59] *Clostridium* (*Clostridioides*) *difficile* isolates from these cases were phenotypically identified by colonial morphology on *C. difficile* agar, Gram staining, chartreuse-green fluorescence under long-wavelength ultraviolet light and fatty acid profiles. Additionally, *C. difficile* toxins were detected by tissue culture assay.[59] In a 2008 study from northeastern Malaysia, an immunochromatographic kit (Remel Xpect, Oxoid, United Kingdom) and cell cytotoxicity assays were used to test 175 stool samples from antimicrobial-associated diarrhea (AAD) inpatients. The prevalence of *C. difficile* toxins was 13.7% and CDI was most common in ethnic Malays;[60] molecular typing was not performed. In 2015, a molecular component was added to a study conducted on inpatients in a hospital in Kota Bharu and showed the prevalence of CDAD in inpatients was 13%. The predominant toxigenic strains of *C. difficile* were RT 043 and RT 017 (both 14%, 3/22 strains).[61] In a more recent report from 2018, across four hospitals in Kuala Lumpur and Kota Bharu, the prevalence of toxigenic strains from diarrheal stool samples was 10.3%.[62] Of the toxigenic strains, RTs 017 ($A^-B^+CDT^-$) and 043 ($A^+B^+CDT^-$) accounted for 20% (20 isolates) and 10% (10), respectively. Furthermore, the remaining four toxigenic *C. difficile* strains producing both toxins A and B were RT 053, QX 026, and RT 014/020.[62] No binary toxin-producing strains were reported.[62]

Singapore. Singapore has the most advanced medical technology and research institutions in southeast Asia.[63] The earliest finding of *C. difficile* in Singapore was in an etiological study of diarrhea in which 4,508 diarrheal stool samples were examined over a 50-month period from 1985 to 1989. *Clostridium* (*Clostridioides*) *difficile* was recovered from 9.6% (35/365) of stool samples that were requested for *C. difficile* investigation.[64]

According to Lim et al., between 2001 and 2006, CDAD incidence in a 1,200-bed general hospital in Singapore dramatically increased from 1.49 cases per 10,000 patient-days to 6.64 cases per 10,000 patient-days, with a simultaneous increase in toxin detection in stool samples.[65] The incidence of CDI in a larger hospital was 5.38 cases per 10,000 patient-days from 2002 to 2003, and CDI was more common in ethnic Malays and patients aged above 50 years. Of 118 *C. difficile* isolates, 14 (11.8%) produced toxin B only and were presumably RT 017.[66] Based on several publications and the Network for Antibiotic Resistance Surveillance in Singapore, the incidence of CDI in inpatients gradually dropped until 2010.[49,67,68] The detection of *C. difficile* RT 027 in Singapore was reported in 2008.[49] Since then, molecular and ribotyping techniques have been widely

implemented and, consequently, more data on CDI in Singapore hospitals has been available.

From December 2011 to May 2012, the incidence of CDI significantly increased to 10.7 cases per 10,000 patient-days. *Clostridium* (*Clostridioides*) *difficile* RTs 053 (21.3%) and 012 (18%) were common, followed by RTs 014, 020, 017, 002, 087, and 064-like that ranged in prevalence from 1.6% to 9.8%; however, 31.1% of RTs remained unclassified.[69] Between 2013 and 2014, nucleic acid tests for the *C. difficile* toxin B gene and immunochromatography kits were combined to identify the causes of community-acquired diarrhea in adult patients admitted to the hospital for acute gastroenteritis. Of 100 samples examined, only 2% contained toxigenic *C. difficile* strains.[70]

Thailand. Thailand has a longer history of *C. difficile* research than other countries in southeast Asia. Siriraj Hospital, which is the oldest and largest hospital in Thailand, was responsible for conducting several of these studies. The first CDAD research in Thailand was carried out at Siriraj Hospital, Ramathibodi Hospital, and a children's hospital in 1990 using culture on selective media (CCFA, Oxoid) and a cytotoxin assay. The prevalence of cytotoxin-positive samples in patients aged $<$ 60 years was 52.5% (106 of 203 samples), but the proportion of *C. difficile* in diarrheal stool samples from all age groups was only 4.8% (13/269) by culture.[71] Cytotoxin positivity increased to 61% in samples from patients who received antimicrobial treatment, compared with 51% in samples from patients without antimicrobial treatment.[71] A later study in Siriraj Hospital reported that the prevalence of toxin A-positive *C. difficile* in patients with clindamycin (10.7%) or beta-lactams (10%) treatment was higher than the control group (1.4%).[72] Between 2000 and 2001, 574 fecal samples from in-patients with suspected AAD in Siriraj hospital and other hospitals in Thailand were analyzed.[73] The prevalence of *C. difficile* culture-positivity was 18.6% (107/574 stool samples).[73] Of *C. difficile* isolates, the proportions of detection of *tcdA* and *tcdB* by PCR and toxin A/B by EIA were 44.9% and 46.7%, respectively.[73] A combination of EIA for toxins A and B and direct stool PCR for *tcdB* more than doubled the rate of detection compared with when EIA alone was used.[74]

In Siriraj Hospital in 2015, the prevalence of toxigenic *C. difficile* in samples was 9.2% (39/422).[75] Toxin profiles of toxigenic *C. difficile* strains, A^+B^+ and A^-B^+, were 69.2% (27/39) and 30.8% (12/39), respectively. By PCR ribotyping, RTs 014/020, 010, 017, 039, and 009 (53.3%) were predominant RTs among 38 RTs, noting that RTs 009, 010, and 039 are nontoxigenic.[75] In a 2019 study which covered over 13 provinces in Thailand, 51% (74/145) of all *C. difficile* isolates were toxigenic. Of those toxigenic strains, the most common were RTs 017 ($A^-B^+CDT^-$) and 014/020 ($A^+B^+CDT^-$), which accounted for 19% (28/145) and 7% (10/145), respectively.[76]

The first publication on CDI among immunosuppressed patients was in 1998.[77] Based on an ELISA method, toxin A was present in 36.7% (11/30) of stool samples from pediatric patients with febrile neutropenia at Siriraj Hospital. Chemotherapy and cephalosporins were the risk factors for *C. difficile* colonization in neutropenic malignancy pediatric patients.[77] At King Chulalongkorn Memorial Hospital from 2002 to 2005, toxin A-positive stool samples from hospitalized patients with CDAD were analyzed.[78] Approximately 90% of positive samples were from patients with comorbidities, mostly immunosuppression, such as those with hematologic malignancies, solid tumors, diabetes, HIV infection, cirrhosis, and chronic renal failure. All patients received antimicrobial therapy within 60 days of being diagnosed with CDAD, and oral metronidazole was commonly prescribed for treatment. Furthermore, the study also concluded chemotherapy and gastric anti-acid agents were risk factors for CDAD.[78]

CLOSTRIDIUM DIFFICILE INFECTION IN INDOCHINA AND ITS NEIGHBORS

Cambodia. There have been no studies specifically looking at *C. difficile* in Cambodia. In 2005, there was an investigation of chronic diarrhea in Cambodian patients with HIV/AIDS that identified toxin A-producing *C. difficile* in 3.75% (3/80) of stool samples with an immunoassay.[14] Chronic diarrhea was defined as $>$ 3 loose stools per day for at least 30 days. Of *C. difficile* positive stool samples, 5% (2/40) were from chronic diarrhea patients, and 2.5% (1/40) were from nonchronic diarrhea patients.[14] The study was limited to toxin A identification only and groups of HIV/AIDS patients with chronic and nonchronic diarrhea and suffered from a lack of clarity in defining CDI. There was no molecular typing and therefore no information on the strains of *C. difficile* circulating in Cambodia.

Lao PDR. Like Cambodia, very little has been published about CDI in Lao PDR. In 2013, a small survey was undertaken on diarrheal stool samples being routinely examined. ChromID *C. difficile* agar (BioMerieux, France) was used for anaerobic culture, latex agglutination (*C. difficile* latex test kit, Oxoid, United Kingdom) for identification of isolates, and ribotyping and toxin profiling were performed at a reference laboratory in Australia.[15] By culture and latex agglutination, five of 70 fecal samples were positive for *C. difficile*. Of the five isolates, three were positive for *tcdB* and two were positive for *tcdA* by PCR. The five isolates were identified as five different RTs: 014 ($A^+B^+CDT^-$), 020 ($A^+B^+CDT^-$), 017 ($A^-B^+CDT^-$), QX107 ($A^-B^-CDT^-$), and QX574 ($A^-B^-CDT^-$). They were from patients aged 1 to 46 years most of whom had diarrheal symptoms.[15] However, two cases whose stool samples were positive for toxin B-producing *C. difficile* were concurrently infected with *Salmonella* species, and/or *Burkholderia pseudomallei*, and had a history of antimicrobial exposure.[15]

Vietnam. Vietnam has conducted more studies on the epidemiology of CDI than the other two countries in Indochina, although some have been reported in Vietnamese. Diarrhea was usually defined as $\geq$ 3 loose stools per day based on the definition of the WHO.[79] In one investigation of diarrhea, 9% (45/479) of stool samples from inpatients were positive for toxigenic *C. difficile,* using the Luminex xTAG Gastrointestinal Pathogen Panel assay.[13] Of the toxigenic strains, the prevalence of toxin A-positive *C. difficile* strains was twice as high as toxin B-positive *C. difficile* strains. The prevalence of nontoxigenic or binary toxin-positive *C. difficile* strains was not reported, and there was no further analysis, such as molecular typing.

A study of CDI among patients with AAD in northern Vietnam used culture on cycloserine–cefoxitin–mannitol agar, nested PCR, PCR ribotyping, and surface-layer protein A

(*slpA*) typing. Antimicrobial-associated diarrhea was defined as loose stools and a history of antimicrobial exposure within 4 weeks. The prevalence of CDI among patients with AAD was 24.9% (95/382), and stool samples from seven fatal cases were positive for toxigenic *C. difficile*.[12] Of the toxigenic isolates, toxin A- and B-producing *C. difficile* strains were found in 8.1% (31/382) of all diarrheal stool samples and 10.2% (39/382) were only toxin B-producing strains. *Clostridium* (*Clostridioides*) *difficile* RTs 017 A^-B^+ and trf A^-B^+ (53.5%) were common among the eight RTs identified by PCR ribotyping. By *slpA* typing, the most common strain was fr-01, followed by kr-03.1 and og39-01.[12]

From 2013 to 2015, *C. difficile* RT 017 was the most common (9/30) of six RTs, including trf, og39, cc835, 001 and cr (using Japanese nomenclature).[80] Research in Bach Mai Hospital from 2013 to 2017 identified 107 toxigenic *C. difficile* strains from 101 adult patients with diarrhea.[81–83] The definition of CDI included detection of toxigenic *C. difficile* and diarrhea $\geq$ 3 times per day. Various acceptable methods were used including GDH EIA (Nissui, Tokyo, Japan), multiplex PCR for *tcdA, tcdB*, and triosephosphate isomerase (*tpi*) genes, and PCR ribotyping. The PCR RTs were aligned with other RTs in the database of the Department of Bacteriology II, National Institute of Infectious Diseases, Japan.[82] The prevalence of CDI patients positive for toxins A^+B^+ and A^-B^+ was 49.5% and 44.6%, respectively. Six cases (5.9%) had two types of toxigenic *C. difficile* strains (A^+B^+ and A^-B^+).[82,83] Among eight PCR RTs, RTs trf, 017, and cc835 were 24.5%, 23.5%, and 22.6% prevalent, respectively,[82,83] RTs 027 and 078 were not detected.

Thus, although studies of CDI in Vietnam are still limited, there has been some information published on the prevalence of CDI in diarrheal patients together with ribotyping and toxin gene data.

China. China is immediately north of the Indochina region and two provinces, Yunnan and Guangxi, share borders with Indochina. There have no published reports on CDI in Guangxi, however, some work has been done in Yunnan. Yunnan province shares its border with Laos and Vietnam. From 2013 to 2016, a retrospective study of community-acquired CDI in Yunnan used a variety of techniques, including PCR (Tiangen, Beijing), PCR ribotyping, toxin gene profiling, and MLST. By PCR, the prevalence of *tcdA* and/or *tcdB* in 978 fecal samples was 14.11% (138 samples).[84] However, from those 138 fecal samples, only 55 *C. difficile* strains were cultured, 87.3% of which (48/55) were toxigenic. Nine RTs were identified; RTs 001, 009, 010, 012, 046, 085, 140, 207, and 220, and the remaining isolates were unclassified.[84] Strains ST3, ST35, and ST54 were the most common among 15 ST types, by MLST.[84] Although there is only one available study, it provides a broad picture of CDI in Yunnan province.

SUMMARY OF *C. DIFFICILE* RIBOTYPING DATA FROM THE INDOCHINA REGION

The most common *C. difficile* RTs seen in southeast Asia were RTs 017, 014/020, 043, and 053. Other RTs, 009, 010, and 012 (009 and 010 being nontoxigenic) were reported in Indonesia, Malaysia, Singapore, Thailand, and Yunnan province, as shown in Figure 2. Although *C. difficile* RT 027 strains have caused many outbreaks in high-income countries,[85] and were introduced in Singapore in 2008,[49] they have not established nor caused epidemics in southeast Asia.

These findings were supported by a large surveillance study that found *C. difficile* RTs 017, 018/QX239, 014/020, and 002 were the most common RTs among 79 RTs in the Asia-Pacific region. Of the toxigenic strains, toxin A-negative and toxin B-positive *C. difficile* strains mostly belonged to RT 017.[51] *Clostridium* (*Clostridioides*) *difficile* RT 017 A^-B^+ strains have circulated in southeast Asia for many years and caused epidemics in other countries in the world.[86,87] There is still controversy about the origin of RT 017 strains in southeast Asia. It was thought that these strains had arisen in Asia due to their high prevalence in the region, belonged to clade 4 and had spread to other parts of the world causing outbreaks.[88] However, based on whole genome sequencing (WGS) and single-nucleotide polymorphism analysis,[87] Cairns et al. suggested that *C. difficile* RT 017 originated in North America and then spread to Europe, Asia, and Australia. A more recent analysis has again supported the theory of an origin in Asia.[89]

C. DIFFICILE IN ANIMALS IN SOUTHEAST ASIA

Studies on *C. difficile* in animals in southeast Asia are minimal. In 2015/2016, a molecular epidemiology study of the prevalence of *C. difficile* in piglets from Thailand and Malaysia was performed using culture on ChromID agar and selective enrichment culture, followed by toxin profiling, PCR ribotyping, and cgSNV analysis following WGS. The prevalence of *C. difficile* in the gastrointestinal tracts of piglets from Malaysia was 91.6%, while the prevalence of *C. difficile* in Thai piglets was 35.1%.[90]

On average, approximately 90% of environmental samples (soils and water) from pig farms were positive for *C. difficile. Clostridium* (*Clostridioides*) *difficile* RT 038 was the most prevalent RT among six RTs recovered. All *C. difficile* strains from both piglets and the environment were nontoxigenic.[90] *Clostridium* (*Clostridioides*) *difficile* RT 038 strains from piglets in Thailand and Malaysia belonged to clade 1 ST48 by in silico MLST. In addition, RT 038 strains from humans selected from Thailand and Indonesia for comparison differed by a relatively small 30 cgSNVs compared with animal strains.[90]

In a recent animal study from Yunnan province, two toxigenic *C. difficile* strains (RT 126, $A^+B^+CDT^+$ and ICDC 094, $A^+B^+CDT^-$) were recovered from 200 stool samples from adult sheep.[91] In another animal study in Japan, 57.5% of fecal samples from neonatal piglets were positive for *C. difficile*. Among *C. difficile* isolates, 61% were toxin A and B^- producing strains and 42.6% were binary toxin-producing.[92] *Clostridium* (*Clostridioides*) *difficile* RT 078 was the third-most prevalent strain among 14 RTs and was genetically related to European RT 078 strains, which caused outbreaks in humans and pigs in Europe. The ancestors of these pigs most likely originated in Europe and North America and were brought to Japan and other Asian countries for breeding.[92]

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *C. DIFFICILE*

While there is little data on the antimicrobial susceptibility of *C. difficile* in Indochina specifically, there are some good

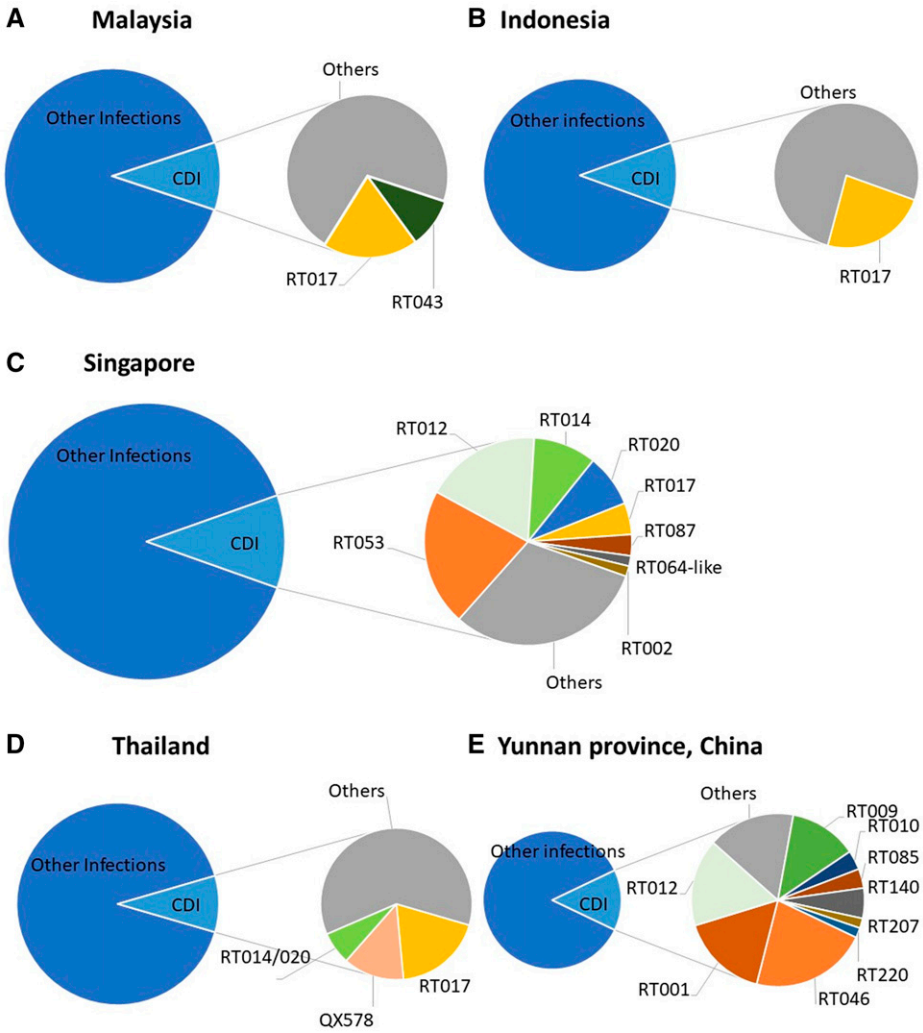

FIGURE 2. (**A**) Malaysia: $N = 100$ isolates, from July 2015 to July 2016; over 32 ribotypes.[62] (**B**) Indonesia: $N = 74$ isolates, from July 2014 to February 2015; 26 ribotypes.[57] (**C**) Singapore: $N = 61$ isolates, from December 2011 to May 2012; 8 ribotypes.[69] (**D**) Thailand: For the prevalence of CDI ($N = 105$ isolates, April–June 2015; 38 ribotypes),[75] for ribotyping analysis ($N = 145$ isolates, from 2006 to 2018; 40 ribotypes).[76] (**E**) Yunnan province, China: $N = 55$ isolates, from 2013–2016; 9 ribotypes.[84] This figure appears in color at www.ajtmh.org.

data from the southeast Asian region in general, and particularly from Thailand. In a 2015 Thai study, 53 *C. difficile* human strains, isolated from 2006 to 2008, were tested using the gradient strip method against metronidazole (100% susceptible), vancomycin (98.2%), moxifloxacin (54.8%), tigecycline (100%), and daptomycin (100%).[93] In a bigger/longer study of 100 strains isolated from 2006 to 2015 used an agar dilution method, the antimicrobial susceptibility of *C. difficile* remained stable for antimicrobials such as metronidazole and vancomycin used for CDI treatment. However, susceptibility decreased for clindamycin (4% susceptible), moxifloxacin (61%), tetracycline (82%), and chloramphenicol (88%). For the 22 *C. difficile* RT 017 strains, the most common RT, the proportions susceptible were clindamycin (14%), moxifloxacin (23%), tetracycline (55%), and chloramphenicol (100%).[76]

In Singapore, all *C. difficile* isolates were susceptible to metronidazole,[64,66] however, a vancomycin-resistant *C. difficile* strain was reported in 2007.[66] After *C. difficile* RT 027 emerged in Singapore, multidrug resistance in RT 027 was observed; resistant to clindamycin and levofloxacin, but susceptible to erythromycin and moxifloxacin.[49] Last, reports from Laos and Indonesia were examined; five *C. difficile* isolates from Laos were susceptible to moxifloxacin, metronidazole, and vancomycin, but not clindamycin[15] whereas, in Indonesia, 24.2% of *C. difficile* isolates, mainly RT 017, were resistant to moxifloxacin.[56] The gradient strip method was commonly used in these studies.

In a recent report from 12 Asia-Pacific countries, all *C. difficile* strains, which were tested using agar dilution, were susceptible to metronidazole, vancomycin, fidaxomicin, and amoxicillin-clavulanate, whereas most were resistant to clindamycin, erythromycin, and moxifloxacin. The highest rates of resistance to at least three antimicrobials were found in RT 017 (66.1%), RT 018 (92.7%), RT 369 (100%), and RT QX239 (100%).[94]

ONE HEALTH

"One Health" focuses on the relationships between the health of humans, animals, and the environment, and encompasses food safety, the control of zoonotic diseases

and combatting antimicrobial resistance.[95] Physicians in many parts of the world think of CDI as a hospital-acquired infection only and neglect community-acquired infections, however, a link between some toxigenic *C. difficile* RTs seen in humans and those in animals and the environment has been observed.[96–100] There is emerging evidence suggesting sources of CDI from the community and environment play an important role in transmission. In a United Kingdom study based on WGS and core-genome single nucleotide variant differences, there were only 35% (333/957) of all isolates that were genetically associated with previous cases, while 45% (428/957) of isolates were genetically different from previous cases.[98]

Clostridium (*Clostridioides*) *difficile* RT 078 and RT 014 are considered to be zoonotic strains as they and RT 027 have been detected in food, animals, vegetables, households, and seafood.[99] Based on the comparison of WGS and MLST of *C. difficile* strains from human and animal samples, 42% of *C. difficile* RT 014 strains from human samples in Australia have a clonal relationship with at least one porcine strain.[100] Australian piglets predominantly carry *C. difficile* RT 014, which also commonly causes CDI in humans in many parts of the world.[100] Furthermore, based on outbreaks of *C. difficile* RT 078 infection in humans in Europe and the presence of this strain in pigs and cattle, *C. difficile* RT 078 has been suggested as a potentially zoonotic pathogen.[96] Furthermore, some southeast Asian countries where the prevalence of both toxigenic *C. difficile* in hospitalized patients is high are popular for medical tourism. Therefore, there is a risk of *C. difficile* transmission to many parts of the world.[86]

Apart from *C. difficile* RT 078, other binary toxin-positive *C. difficile* strains were also thought to be zoonotic. Rupnik reported the prevalence of binary toxin-positive *C. difficile* strains in animals, including horses, piglets, and cattle, ranged from 23% to 100%. Those binary toxin-positive strains were probably spread from animals to humans as the prevalence of HA-CDI and CA-CDI caused by binary toxin-positive strains in humans has increased since the 1990s.[101] In addition to this, some studies in Scotland and the United States reported the presence of *C. difficile* RTs 017, 027, and 078 in food such as salads, uncooked meat, and several types of sausage.[102,103]

In terms of a One Health perspective, the most important requirement is to closely monitor production animals and antimicrobial use in these animals, particularly for infection prophylaxis.[86]

CONCLUSION

Very little is known about the epidemiology of *C. difficile* in humans, animals, and the environment in southeast Asia. A lack of diagnostics and awareness of physicians are probably the main factors that restrict reports on CDI from this region. In addition, inappropriate use of antimicrobials in animals and humans is a significant concern in southeast Asia. Consequently, this may increase the prevalence of CDI. Although RTs vary from one country to another in southeast Asia, toxin A-negative/toxin B-positive strains of *C. difficile* RT 017 are endemic throughout the region and have caused outbreaks in other parts of the world. Virulent strains of *C. difficile* such as RT 078 have been detected from animals that were brought from Europe to northern Asia, however, these strains do not appear to have caused CDI in humans or to be circulating in animals in southeast Asia. One exception is Taiwan where *C. difficile* RT 078 strains have been found in piglets and caused CDI in humans.[104] Improvement in laboratory capacity and surveillance in the region is essential to reduce morbidity and mortality from CDI. More important, regulation of antimicrobial use should be improved to decrease both multidrug resistance and CDI.

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