Anaerobe 94 (2025) 102982

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clostridioides difficile (including epidemiology)

Clostridioides (Clostridium) difficile in children and adolescents in the community in Cambodia

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ARTICLE INFO

Handling Editor: Sandra Janezic

Keywords:
Students
Siem Reap
Prevalence
Loose stool
Ribotypes
Epidemiological purposes

ABSTRACT

Background: *Clostridioides (Clostridium) difficile* transmission between community and healthcare settings has been increasingly reported. We aimed to identify the prevalence and molecular epidemiology of *C. difficile* colonising adolescents and non-hospitalised children in Cambodia.

Methods: Stool samples were collected from 266 students at the University of Health Sciences (UHS) in Phnom Penh, between July and August 2022, and 246 children ≥1 year old visiting the outpatient department (OPD) at Angkor Hospital for Children in Siem Reap, between January and August 2022. *C. difficile* culture, toxin gene detection and PCR ribotyping were performed.

Results: Overall, *C. difficile* was recovered from 07/266 specimens (2.6 %) from UHS students and 59/246 specimens (23.9 %) from OPD children. The overall prevalence of *C. difficile* in children peaked in April, and of toxigenic strains peaked in August. Children with loose stools were less likely to be colonised by *C. difficile* (COR = 0.05, 95 %CI: 0.008–0.32) than children with normal stools. UHS students were colonised only by toxigenic *C. difficile.* Of the 66 isolates, 36 % (24/66) were toxigenic: *C. difficile* ribotype (RT) 017 was the most predominant, followed by RTs 012, 046, 056, QX709, 001, 014/020 and QX710. Non-toxigenic strains accounted for 35 RTs including 32 novel RTs that had not been isolated previously.

Conclusions: The findings of only toxigenic strains in adolescents and the high prevalence of *C. difficile* in OPD children suggest exposure to *C. difficile* within the community; thus, appropriate interventions may be needed. Genotypic identification of diverse *C. difficile* is important for molecular epidemiological purposes.

1. Introduction

Clostridium (Clostridioides) difficile is known to cause antimicrobial-associated diarrhoea (AAD), one of the most common hospital-associated infections (HAIs) [1]. However, community-associated *C. difficile* infection (CA-CDI) has been increasingly reported in the last decade [2–4], based on symptom development within 48 h of hospitalisation [5] and a lack of an identified transmission path or source within the hospital [6].

Children and adults can be asymptomatic carriers of both toxigenic

This article is part of a special issue entitled: ICDS2024 published in Anaerobe.

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https://doi.org/10.1016/j.anaerobe.2025.102982
Received 9 May 2025; Received in revised form 12 June 2025; Accepted 26 June 2025
Available online 3 July 2025

and non-toxigenic strains of *C. difficile* [7,8]; thus, detection of *C. difficile* is recommended only in individuals with clinical signs of disease such as diarrhoea, ileus or toxic megacolon [9]. With more *C. difficile* testing being performed on non-hospitalised individuals with clinical signs of CDI, more CA-CDI cases are being found. The overall prevalence of CA-CDI has ranged between ~1.3 % and 5.5 % [10,11], and between 39 % and 51 % of total CDI cases [2–4]. Severely complicated CA-CDI and high rates of recurrent CA-CDI (rCA-CDI) have been reported [12,13], and the impact of CDI on the community is worrisome. CA-CDI risk factors include antimicrobial consumption, age ≥65 years, care in an emergency department, cardiac disease, inflammatory bowel disease, white race, chronic kidney disease, close contact with a family member who had been hospitalised in the previous 6 months, low socio-economic status and crowded households [3,11,14,15].

The increasing number of CA-CDI cases has raised concerns over infection prevention and control (IPC) strategies for CDI in both community and hospital settings. *C. difficile* ribotypes (RTs) including 014/020, 181, 002, 012, 056, 046 and many others have been found in both CA-CDI and HA-CDI [3,11], however, some RTs e.g. 005, 020, 014/020, 056, 002 are more likely to be found in CA-CDI [2,3]. Delays in diagnosis and undiagnosed CA-CDI cases present challenges for IPC implementation. In Europe, up to 47 % of CA-CDI in adults were not diagnosed until after up to 10 days of diarrhoea [11].

In South East Asia, few studies on *C. difficile* have been conducted on hospitalised adults and children with diarrhoea [16,17]. In Indonesia, *C. difficile* was recovered from 22 % (74/340) of diarrheal stools [17], with similar proportions of toxigenic and non-toxigenc *C. difficile* (10.9 % vs 10.6 %, respectively). The most common strain was *C. difficile* RT 017 (24.3 % of 74 isolates). In a later study of children with diarrhoea aged ≤16 years in Vietnam, the overall prevalence of *C. difficile* in stool samples was 37.8 % (140/370), and children in the age group 2–12 months had the highest prevalence (52.9 %; 74/140) [16]. A total of 151 isolates of *C. difficile* was recovered and the most common toxigenic strain was RT 012 (11/151). In another study in Hebei Province, close to Beijing in China, *C. difficile* colonisation was 22.8 % (250/1098) in young children aged between 0 and 36 months, and more than half of the strains (55.2 %) were toxigenic [18], likely indicating community-acquired colonisation.

The finding of *C. difficile* in food, livestock, the environment and elsewhere suggests possible sources/reservoirs of *C. difficile* in the community [19–21]. Indeed, a landmark study in Oxfordshire in the UK published in 2013 identified that 45 % of CDI cases in Oxfordshire were genetically distinct from all previous cases, implying that diverse sources of *C. difficile*, in addition to symptomatic patients, played a major part in *C. difficile* transmission [6]. There have been no studies on sources or reservoirs of *C. difficile* in the community in Cambodia, except for one report of *C. difficile* in smoked and dried fish (16 %) [19], however, *C. difficile* has been found in piglets in Thailand [21] and the environment in Vietnam [20], two countries adjacent to Cambodia. The present study aimed to investigate the prevalence and molecular epidemiology of *C. difficile* colonisation in two groups of Cambodians, young children and adolescent university students.

2. Methods

2.1. Study setting and sample collection

A cross-sectional study was conducted at the University of Health Sciences (UHS), Phnom Penh, Cambodia, between July and August 2022. At UHS, the study design and objectives were presented to eight groups of UHS students in their foundation year and year one. After informed consent was given, a stool specimen was requested from each student. Stool specimens were provided by students to a study coordinator at UHS and sent to the laboratory at Calmette Hospital (CH) in Phnom Penh for storage at 2–8 °C. Stool samples from children aged ≥1 year, attending the outpatient department (OPD) of Angkor Hospital for Children (AHC), Siem Reap, between January and August 2022, that had been sent to the laboratory for routine detection of enteric pathogens besides *C. difficile,* were deidentified and stored at −80°C for use in this study.

The Bristol Stool Form Scale (BSFS) was followed for stool consistency assessment: hard stool (types 1 and 2), normal stool (types 3, 4 and 5) and loose stool (types 6 and 7) [22]. All stool samples were finally stored on Transwabs® (Medical Wire & Equipment, England) for transportation at ambient temperature, undertaken in October 2023 from Cambodia to the Queen Elizabeth II Medical Center (QEIIMC) in Nedlands, Western Australia. Previous studies on *C. difficile* in other Asian countries have used this process for transportation successfully [16,17].

2.2. Detection of C. difficile

The detection of *C. difficile* was done as described in the study conducted by Khun et al. [23]. Toxigenic culture of *C. difficile* was performed using ChromID® *C. difficile* agar (bioMérieux, Marcy l'Etoile, France) for direct culture and Robertson's cooked meat medium (RCM) (PathWest Laboratory Medicine Excel Media, Mount Claremont, Western Australia, Australia) containing 250 mg/L cycloserine, 5 mg/L gentamicin, 8 mg/L cefoxitin, for enrichment culture. Alcohol shock with an equal volume of RCM broth and 95 % ethanol (1:1 mL) was done for 1 h and the mixture was plated on cycloserine cefoxitin fructose agar (CCFA) (PathWest Laboratory Medicine Excel Media). All agar plates were incubated anaerobically in an A35 anaerobic chamber (Don Whitley Scientific, Ltd., Shipley, West Yorkshire, United Kingdom) at 35°C for 48 h, in an atmosphere of 80 % N_2, 10 % H_2 and 10 % CO_2 with a relative humidity of 75 %. Black putative *C. difficile* colonies on ChromID and yellow ground-glass-looking colonies on CCFA were subcultured onto horse blood agar (BA) plates, incubated in the same conditions mentioned above. The odour of horse dung and chartreuse fluorescence under UV light were used for the presumptive identification of *C. difficile* on BA.

2.3. Toxin gene detection and ribotyping

From 48 to 72 h cultures on BA, heat DNA extraction was performed using 5 % Chelex-100 (Sigma-Aldrich, Castle Hill, NSW, Australia). The toxin A gene was detected in duplex PCR, using NK2/NK3 primers to detect *tcdA1* and novel BEtcdA1/BEtcdA2 primers to detect *tcdA3* [24,25]. Monoplex PCR was used to detect the toxin B gene (*tcdB*) with NK104/NK105 primers, and binary toxin genes (*cdtA* and *cdtB*) with cdtApos/cdtArev and cdtBpos/cdtBrev primers, respectively, as described in previous studies [26,27]. PCR ribotyping targeting the 16S-23S rRNA intergenic spacer region was performed as previously described [28] with some modifications. After cleaning with a MinElute PCR purification kit (Qiagen, Venlo, Limburg, The Netherlands), PCR ribotyping products were visualised on the QIAxcel capillary electrophoresis platform (Qiagen, Venlo, Limburg, The Netherlands). With BioNumerics software package v.7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium), the band profile of each isolate was compared to a collection of international reference strains in the laboratory. Strains that did not match any reference strain in the library were given the prefix "QX". Completely novel strains were identified as such.

2.4. Statistical analysis

At UHS, demographic information including sex, age, residency, living with a sibling <1 year of age, living close to livestock i.e. in the same compound, frequent OPD visits (at least once a week), clinical information including diarrhoea, abdominal pain, fever, other clinical conditions and antimicrobial and other medication consumption at the time of sample collection, within the last 1 week and the last 4 weeks of sample collection, were recorded. At AHC, only sex, age, stool

consistency and residency information were recorded.

A logistic regression model was used to analyse factors associated with *C. difficile* carriage. A *P*-value <0.05 was the cut-off for statistical significance. All statistical analyses were conducted in the IBM SPSS Statistics package version 26.0.0.0.

2.5. Ethical issues

Ethics approval was granted by the National Ethics Committee for Health Research in Cambodia (248NECHR) and by the Human Research Ethics Office of Curtin University (HRE2022-0027). Stool samples from AHC were deidentified residual samples from the diagnostic laboratory and were thus not subject to formal ethical review.

3. Results

3.1. Children

A total of 252 samples was received from children attending the OPD at AHC between January and August 2022. Samples from children aged <1 year were excluded from the study and 246 samples remained. The number of female and male participants was approximately balanced (126 vs 120, respectively).

By both direct and enrichment cultures, *C. difficile* was recovered from 23.9 % (59/246) of samples (Fig. 1**A**). Hard stools had the highest proportion (50 %, 5/10) of *C. difficile*, while only two of 42 loose stools (taking the shape of the container) tested positive for *C. difficile*. Univariate analysis indicated that children with loose stools had a

Table 1
Univariate logistic regression analysis of socio-demographic factors associated with *C. difficile* carriage in children from the outpatient department of Angkor Hospital for Children.

Description	Number		COR (95 %CI) Univariate analysis	*P* value
	Without *C. difficile* (N = 187) n	With *C. difficile* (N = 59) n		
Sex (female)	96	30	1.02 (0.56–1.83)	0.948
Age (IQR, 3–8 yrs)	112	37	0.99 (0.91–1.08)	0.928
Stool consistency (loose)	40	2	**0.05 (0.008–0.32)**	**0.002**

COR: Crude Odds Ratio; IQR: Interquartile range.

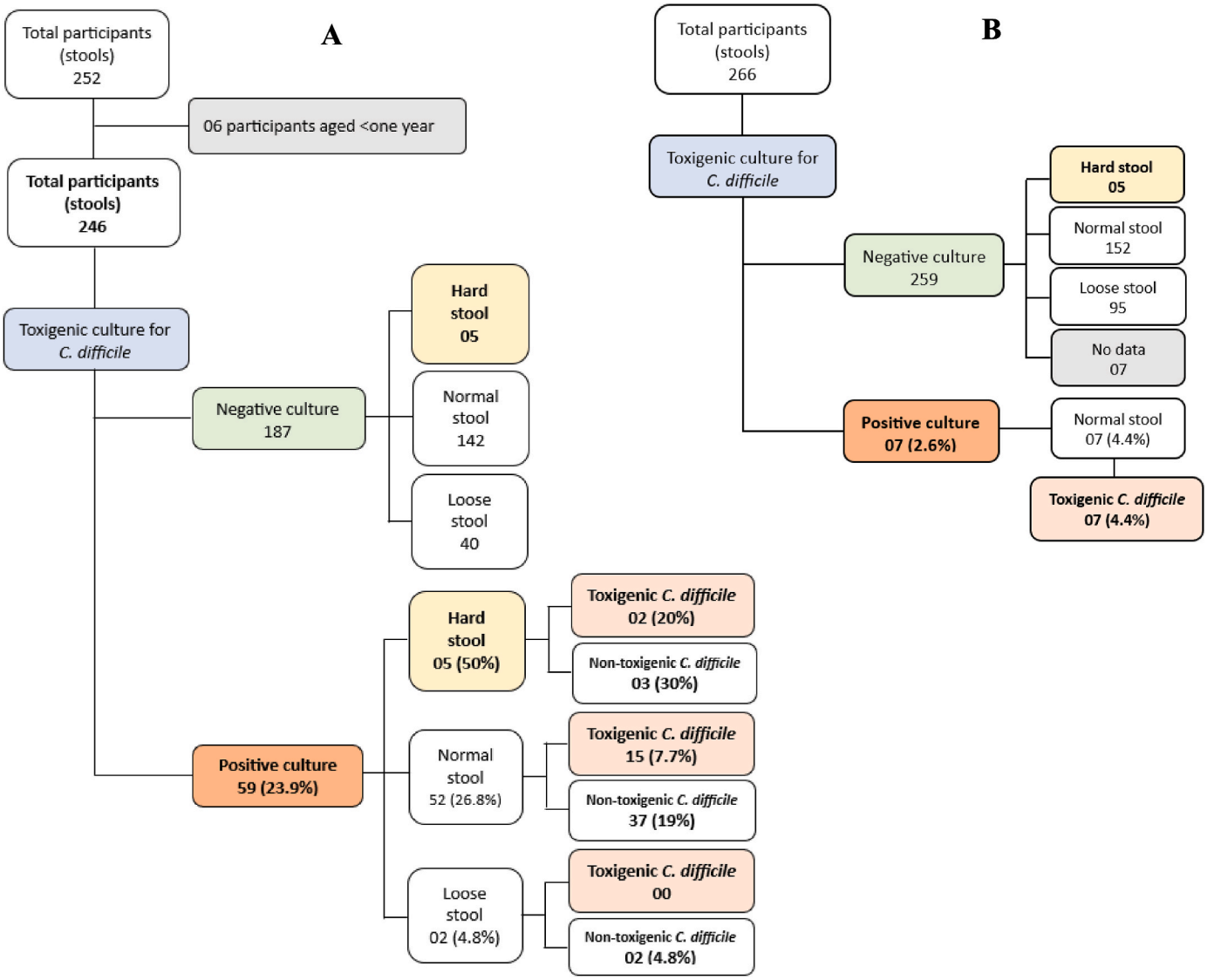


Fig. 1. *Participants in the study:* ***A***) Children from the outpatient department of Angkor Hospital for Children, **B**) Adolescents from the University of Health Sciences.

significantly lower chance (Crude Odds Ratio [COR] = 0.05, 95 %CI: 0.008–0.32) of being colonised/infected by *C. difficile* (Table 1). All children in the study were residents of 16 regions of Cambodia; the prevalence ranged between 14 % and 38 % if considering only the four regions with a minimum of 10 participants (Fig. 2).

The prevalence by month of sample collection ranged between 6 %, the lowest in May, and 36 %, the highest in April (Fig. 3A). Although the number of positive cases varied with no trend by month, the number of toxigenic *C. difficile* increased steadily from January (0 %, 0/1) to August (57 %, 8/14) (Fig. 3A). The age of children ranged between 1 and 15 years (IQR, 3–8 years). The overall prevalences across the age groups were not significantly different ($p = 0.909$) (Fig. 3B).

3.2. Adolescents

Between July–August 2022, 266 adolescent UHS students, aged between 17 and 23 years, were recruited. Female students outnumbered male students (177 vs 87, respectively). All of them were living in the capital city, Phnom Penh, at the time of sample collection, so their residency was not included in the analyses. None of them had chronic obstructive pulmonary diseases, heart failure, chronic renal disease, haematological malignancy, cancer or stroke, however, 12 students reported that they had diarrhoea at the time of sample collection.

Of the 266 samples, *C. difficile* was recovered from seven (2.6 %), all normal stools (Fig. 1B). No statistical analysis was conducted for this population due to the small number of cases. Among the seven cases, 71 % (5/7) were female, none had diarrhoea, nor was hospitalised >24 h or had recent frequent OPD visits within the last 3 months. Four of them answered that they had antimicrobials within the last 4 weeks, and two had taken antimicrobials within the last 1 week of stool collection.

3.3. C. difficile molecular characteristics and toxin profiles

Of the 59 *C. difficile* isolates from OPD children at AHC, 28.8 % (17/59) were toxigenic; thus, the prevalence of toxigenic *C. difficile* was 6.9 % (17/246) (Fig. 4). All toxigenic strains were isolated only from hard and normal stools; hard stools had the highest proportion of toxigenic *C. difficile* (20 %, 2/10) (Fig. 1A). The lowest prevalence of toxigenic *C. difficile* (3 %, 3/95) was seen in the 2–5 years age group, though this was not significantly different from other age groups ($p = 0.315$) (Fig. 3B).

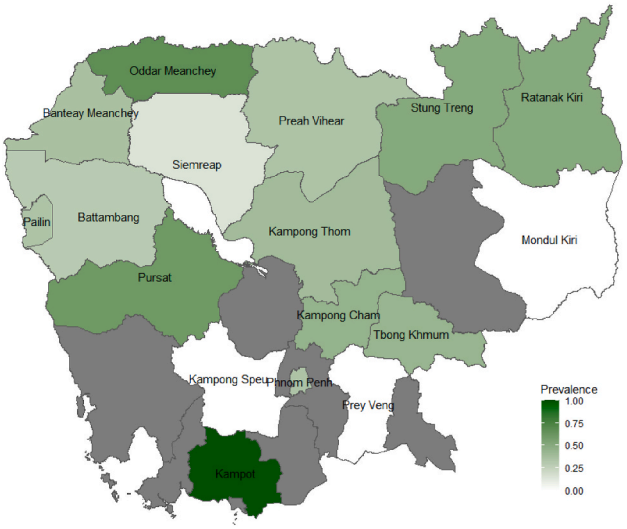
C. difficile RT 017 represented 13.5 % (8/59) of isolates and was the most predominant strain, followed by RTs 056, 012, 046, 014/020, 001 and QX710, in OPD children at AHC. Most toxigenic strains (55 %, 10/18) possessed both toxin genes, *tcdA* and *tcdB*. The most predominant non-toxigenic strain was *C. difficile* RT QX107, followed by RTs QX002, QX011, QX021, QX552, 010, 038 and another 28 RTs all with one isolate each. All *C. difficile* isolates recovered from UHS students were toxigenic and the prevalence was 2.6 % (7/266), including three isolates of RT 012, two isolates of QX709 and one isolate each of RTs 056 and 017 (Fig. 4).

Overall, a total of 66 isolates from the two populations was classified into nine known *C. difficile* RTs (35 %, 23/66) and 34 unknown RTs (65 %, 43/66) including both strains with "QX" prefix and unique singletons. The known *C. difficile* RTs included RTs 017, 012, 046, 056, 014/020, 001, 010, 009 and 038, ranging from the most to the least predominant (Fig. 4).

4. Discussion

4.1. Prevalence and risk factors

This study aimed to investigate *C. difficile* in non-hospitalised individuals; adolescent university students and young children visiting an OPD. With no attempt to make a clinical diagnosis of CDI for the study participants, *C. difficile*-positive cases in this study were presumably asymptomatic carriers of either toxigenic or non-toxigenic strains. Interestingly, toxigenic *C. difficile* was found only in children with hard and normal stools (20 %, 2/10 and 8 %, 15/194, respectively), and in adolescents with normal stools (4.4 %, 7/159) (Fig. 1). These cases are likely to be *C. difficile* asymptomatic carriers, possibly transient only, and a longitudinal study is required for confirmation. The prevalences of toxigenic *C. difficile* asymptomatic carriers in the current study were not significantly different to those in Europe in children (6.9 % vs 6.8 %, respectively) [8] and adults (2.6 % vs 1.3 %–3.1 %, respectively) [11,29]; nor to those in Asia, children (6.9 % vs 8.8 %, respectively) and adults (2.6 % vs 3.6 %, respectively) [30]. Individuals who carry *C. difficile* asymptomatically are at higher risk of disease development at



Region of residence	Prevalence by region	RT017	RT012	RT046	RT056	RT 014/020	Others
Banteay Meanchey	7/20 (35%)	1					6
Battambang	4/14 (29%)	1	1				2
Kampong Cham	4/9 (44%)					1	4
Kampong Speu	0/1						
Kampong Thom	5/13 (38%)	1		1			3
Kampot	1/1 (100%)						1
Mondul Kiri	0/1						
Oddar Meanchey	4/6 (67%)			1			3
Pailin	1/3 (33%)	1					
Phnom Penh	1/3 (33%)	1					
Preah Vihear	2/6 (33%)						2
Prey Veng	0/2						
Pursat	3/5 (60%)				1		2
Ratanak Kiri	1/2 (50%)						1
Siemreap	22/151 (15%)	1	1		2	1	17
Stung Treng	1/2 (50%)						1
Tbong Khmum	3/7 (43%)	1					2

Regions in grey had no data.

Fig. 2. *C. difficile* in children attending the outpatient department, Angkor Hospital for Children, by region of residence.

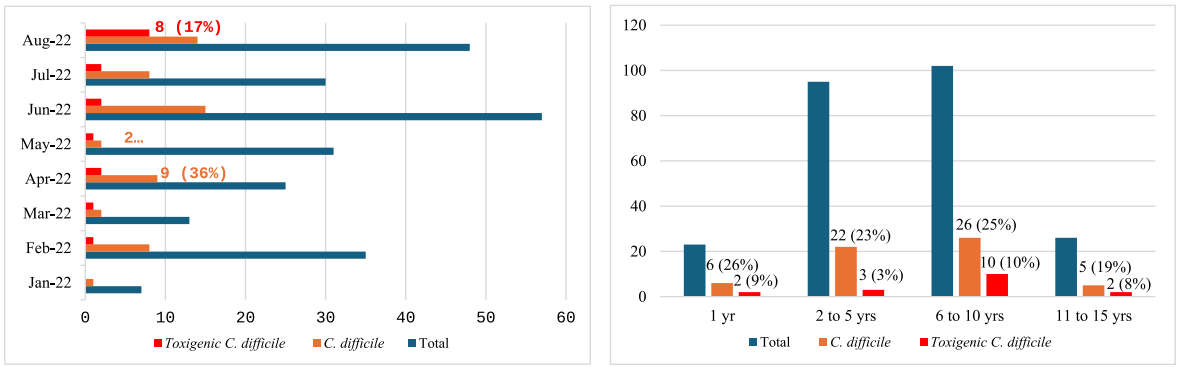


Fig. 3. *Children participating from the outpatient department of Angkor Hospital for Children*: **A).** Prevalence of *C. difficile* by month of sample collection. **B).** Prevalence of *C. difficile* by age group.

a later stage and are also a source of *C. difficile* for other individuals in the same setting [31]. Since information on hospitalisation and the clinical condition of the children carrying *C. difficile* was not available, we cannot assume that these *C. difficile* strains originated from sources/reservoirs in their community or previous hospital visits. In contrast, *C. difficile* found in all adolescent participants was more likely to have originated from the community since they had no recent hospital contact. The finding of *C. difficile* in dried and smoked fish in Cambodia is a good example of a source of *C. difficile* in the community in this country [19], however, there have been no other reports. Although the prevalence of toxigenic strains of *C. difficile* in adolescents in the current study (2.6 %) was significantly lower than in diarrheic adults in Vietnam (9.8 %) [23] and Thailand (9.2 %) [32], our study confirms the existence of asymptomatic carriers of community-associated *C. difficile* in Cambodia and suggests further investigations into the impact of asymptomatic carriage on *C. difficile* transmission as well as CDI management in the country are warranted.

The high prevalence of non-toxigenic strains of *C. difficile* in the current study correlated well with another recent study in Cambodia [33], as well as studies in neighbouring Vietnam [16,23] and Thailand [32]. Notably, non-toxigenic strains in the current study were isolated only from children, supporting the theory that children are more frequently asymptomatic carriers of *C. difficile* compared to adults and that children plausibly contribute to the transmission of *C. difficile* in community and healthcare settings. The findings of only toxigenic *C. difficile* in healthy and non-hospitalised adolescents suggest an effect of the gut microbiota of these Cambodians in inhibiting non-toxigenic strains [34]. Investigations into the role of silent circulation of *C. difficile* in CDI epidemiology could be helpful. Also, the presence of *C. difficile* in almost every region of Cambodia in this study (Fig. 2) correlates well with the findings in the two previous studies conducted in hospitalised patients, including adults [33] and children [7], in Cambodia. Though the prevalences by province among the three studies have some dissimilarities, they confirm the presence of local *C. difficile* in the country, rather than imported strains, and further investigations are required to find sources/reservoirs in the country for IPC purposes.

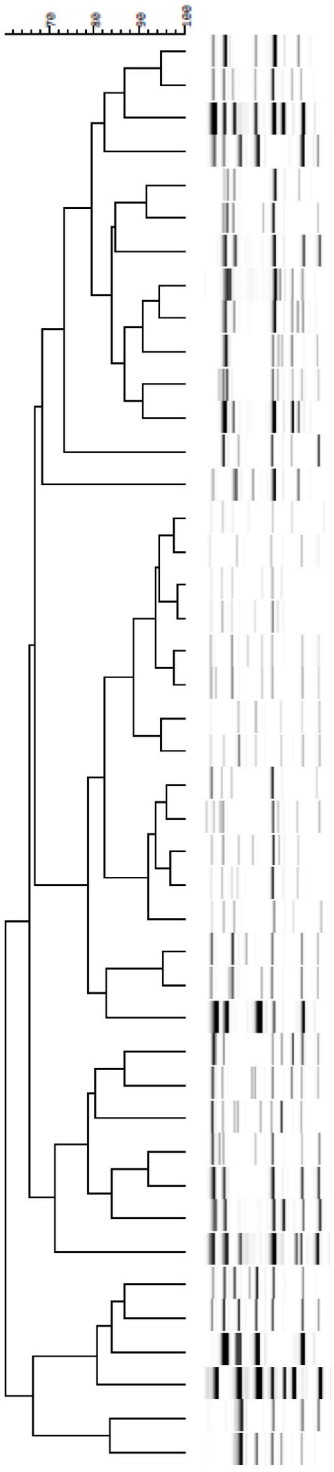
The study was conducted between January and August 2022. The overall prevalence of *C. difficile* in children peaked in April, the end of the dry season, and the prevalence of toxigenic *C. difficile* peaked in August, the rainy season in Cambodia. These findings did not suggest a seasonality that has been found in some countries with four distinct seasons [35]; however, they were similar to the seasonal increase of *C. difficile* prevalence seen in Bangladesh, where the prevalence of toxigenic *C. difficile* peaked between June and October [36]. Multiple contributing factors in tropical countries could be hypothesised. In low-income countries in particular, hygiene practices could be more limited during the rainy season compared to the dry season, especially for those with low socioeconomic status. Other seasonal infectious diseases in the country could have an impact [37]; for example, influenza and melioidosis peak during the rainy season from June to November [37,38] and rotavirus peaks between November and May [39]. Different patterns of antimicrobial consumption and the consumption of different antimicrobial types in response to these seasonal infectious diseases could contribute to host susceptibility to infection/colonisation with either toxigenic or non-toxigenic *C. difficile*. Multi-year, longitudinal investigations of *C. difficile* prevalence in Cambodia are recommended to accurately characterise seasonality.

Based on stool consistency, children with loose stools were at significantly lower risk of carrying *C. difficile* (COR = 0.05, 95 %CI: 0.008–0.32), and none of the loose stools collected from adolescents contained *C. difficile*, suggesting possible competitive roles of other factors including pathogens in loose stool. Similar results were found in young children in other Asian countries with similar hygiene conditions to Cambodia, like India, Nepal and Bangladesh [36]. However, the Bristol Stool Form Scale (BSFS) was followed for stool consistency assessment [22] and only provided a suggestion of possible *C. difficile* carriage in individuals. It will be important for future studies to test all stools for *C. difficile* in patients with CDI-associated clinical signs to avoid under- or overestimation of true CDI cases [9]. Together with the high prevalence (38.5 %) of *C. difficile* found in an earlier study in hospitalised children in Cambodia [7], the findings in children in the community in the current study suggest that the implementation of routine detection of *C. difficile* in children, more importantly in those with underlying diseases, requires strict adherence to the guidelines and interpretation with caution [9].

4.2. Molecular epidemiology

The high diversity of RTs in the current study is suggestive of multiple sources/reservoirs of *C. difficile* in Cambodia that require investigation. Many studies in Southeast Asia reported similar findings, where novel strains usually represented >50 % of the total isolates, whereas known strains previously identified in other continents including Europe, North America and Australia, were usually found in smaller proportions or not at all e.g. RTs 027, 002 [11,16,17,32,33]; thus, those novel strains could be local strains, possibly in evolutionary clade 4, originating in Asia. If this is true, these findings represent more evidence that Asia is the home of evolutionary clade 4 of *C. difficile*.

C. difficile RT 017 has been the most frequently found in Asia in both asymptomatic carriers and symptomatic CDI [17,33] and this strain also belongs to evolutionary clade 4. The existence of other toxigenic strains in the current study in Cambodia and other studies in Asia, including RTs 012, 046, 014/020, 056, 001 belonging to evolutionary clade 1, suggests the movement of *C. difficile* from geographical locations where



Ribotype	Toxin profile	Number (%)	Location
Unique singleton 1	A-B-CDT-	1 (2%)	AHC
QX 117	A-B-CDT-	1 (2%)	AHC
QX 077	A-B-CDT-	1 (2%)	AHC
QX 541	A-B-CDT-	1 (2%)	AHC
QX 675	A-B-CDT-	1 (2%)	AHC
QX 711	A-B-CDT-	1 (2%)	AHC
QX 552	A-B-CDT-	2 (3%)	AHC
QX 714	A-B-CDT-	1 (2%)	AHC
QX 574	A-B-CDT-	1 (2%)	AHC
Unique singleton 2	A-B-CDT-	1 (2%)	AHC
QX 509	A-B-CDT-	1 (2%)	AHC
QX 464	A-B-CDT-	1 (2%)	AHC
038	A-B-CDT-	1 (2%)	AHC
QX 713	A-B-CDT-	1 (2%)	AHC
010	A-B-CDT-	1 (2%)	AHC
Unique singleton 3	A-B-CDT-	1 (2%)	AHC
Unique singleton 4	A-B-CDT-	1 (2%)	AHC
QX 659	A-B-CDT-	1 (2%)	AHC
QX 021	A-B-CDT-	2 (3%)	AHC
Unique singleton 5	A-B-CDT-	2 (2%)	AHC
017	**A-B+CDT-**	**9 (14%)**	AHC, UHS
QX 710	**A-B+CDT-**	**1 (2%)**	AHC
QX 514	A-B-CDT-	1 (2%)	AHC
QX 716	A-B-CDT-	1 (2%)	AHC
Unique singleton 6	A-B-CDT-	1 (2%)	AHC
Unique singleton 7	A-B-CDT-	1 (2%)	AHC
046	**A+B+CDT-**	**4 (6%)**	AHC
QX 371	A-B-CDT-	1 (2%)	AHC
QX 011	A-B-CDT-	2 (3%)	AHC
QX 107	A-B-CDT-	3 (5%)	AHC
QX 709	**A+B+CDT-**	**2 (3%)**	UHS
056	**A+B+CDT-**	**4 (6%)**	AHC, UHS
012	**A+B+CDT-**	**5 (8%)**	AHC, UHS
QX 002	A-B-CDT-	2 (3%)	AHC
001	**A+B+CDT-**	**1 (2%)**	AHC
014/020	**A+B+CDT-**	**1 (2%)**	AHC
Unique singleton 8	A-B-CDT-	1 (2%)	AHC
009	A-B-CDT-	1 (2%)	AHC
Unique singleton 9	A-B-CDT-	1 (2%)	AHC
QX 453	A-B-CDT-	1 (2%)	AHC
Unique singleton 10	A-B-CDT-	1 (2%)	AHC
Unique singleton 11	A-B-CDT-	1 (2%)	AHC
QX 516	A-B-CDT-	1 (2%)	AHC
Total		**66 (100%)**	

UHS: The University of Health Sciences, AHC: Angkor Hospital for Children

Fig. 4. Dendrogram of *C. difficile* PCR ribotyping banding patterns and toxin profiles.

strains in evolutionary clade 1 originate [40].

Previous studies from Cambodia found *C. difficile* RTs 046, 017 and 012 to predominate in hospitalised adults [33] and children [7], respectively. *C. difficile* RT 014/020 in the current study, also found in previous studies in Cambodia and elsewhere, is more commonly associated with community cases [2,3]. The overlapping of these predominant RTs in both community and hospital settings suggests possible bidirectional transmission between hospital and community settings in Cambodia.

Several limitations were observed. The study collected samples from healthy adolescents aged between 17 and 23 years. While this may not represent the true prevalence of *C. difficile* in adults in the country, it

provides information on the epidemiology of CDI in this age group. Clinical information on children participating in the study was not available, impacting the interpretation of risk factors of CDI; however, the findings of the seasonal variation of *C. difficile* prevalence could highlight a trend of infection during the first 8 months of the year, and interventions could also be applied accordingly. The putative identification of *C. difficile* relying on the colonial morphology and horse dung odour on BA was confirmed by ribotyping; thus, though PCR for the *tpi* gene or matix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) could enhance the identification of non-toxigenic strains, this method was not incorporated in the current study.

4.3. Conclusions

The asymptomatic carriage of either toxigenic or non-toxigenic *C. difficile* in healthy adolescents and non-hospitalised children in Cambodia points to sources of *C. difficile* in the community that might impact transmission into hospital settings. Multiple sources of *C. difficile* in the community are suggested by the high diversity of RTs. Individuals with loose stools were less likely to be infected with *C. difficile*, suggesting significant roles of other enteric pathogens in Cambodia, and further investigations are needed. The investigations into sources/reservoirs of *C. difficile* in the community and the reasons contributing to asymptomatic carriage could be beneficial for future CDI management in the country. Genotypic identification of local *C. difficile* strains remains warranted.

CRediT authorship contribution statement

Lengsea Eng: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paul Turner:** Writing – review & editing, Project administration, Conceptualization. **Kefyalew Addis Alene:** Writing – review & editing, Supervision, Project administration, Formal analysis. **Deirdre A. Collins:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Su-Chen Lim:** Validation. **Pisey Tan:** Project administration. **Sona Soeng:** Project administration. **Dylorng Hun:** Project administration. **Sotera Yohn:** Project administration. **Sarim Vong:** Project administration. **Archie C.A. Clements:** Writing – review & editing, Visualization, Project administration, Conceptualization. **Thomas V. Riley:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Source of funding

L. Eng is the recipient of an Australian Government Research Training Program Scholarship.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the laboratory team of AHC and CH for assisting in collecting and storing the specimens before sending them to Perth, Western Australia.

T.V.R has received a grant from Roche Diagnostics outside the present work.

Data availability

Data will be made available on request.

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