6-1-2023

Environmental contamination with clostridioides (Clostridium) difficile in Vietnam

Peng A. Khun

Long D. Phi

Huong T. T. Bui

Nguyen T. Bui

Quyen T. H. Vu

See next page for additional authors

10.1093/jambio/lxad118

Khun, P. A., Phi, L. D., Bui, H. T. T., Bui, N. T., Vu, Q. T. H., Trinh, L. D., . . . Riley, T. V. (2023). Environmental contamination with clostridioides (Clostridium) difficile in Vietnam. Journal of Applied Microbiology, 134(6), article lxad118. https://doi.org/10.1093/jambio/lxad118

Journal of Applied Microbiology, 2023, 134, 1–10
https://doi.org/10.1093/jambio/lxad118
Advance access publication date: 9 June 2023
Research Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Environmental contamination with *Clostridioides* (*Clostridium*) *difficile* in Vietnam

Peng An Khun [1], Long Duc Phi [2], Huong Thi Thu Bui [3], Nguyen Thi Bui [4], Quyen Thi Huyen Vu [4], Luong Duy Trinh [4], Deirdre A. Collins [5], Thomas V. Riley [1,5,6,7,*]

[1]School of Biomedical Sciences, The University of Western Australia, Nedlands 6009, Western Australia, Australia
[2]Thai Binh University of Medicine and Pharmacy, Thai Binh 410000, Vietnam
[3]Thai Binh General Hospital, Thai Binh 410000, Vietnam
[4]Ninh Binh Women and Children's Hospital, Ninh Binh 430000, Vietnam
[5]School of Medical and Health Sciences, Edith Cowan University, Joondalup 6027, Western Australia, Australia
[6]Medical, Molecular and Forensic Sciences, Murdoch University, Murdoch 6150, Western Australia, Australia
[7]Department of Microbiology, PathWest Laboratory Medicine, Nedlands 6009, Western Australia, Australia

*Corresponding author. The University of Western Australia, School of Biomedical Sciences, Queen Elizabeth II Medical Centre, Nedlands 6009, Western Australia, Australia. E-mail: thomas.riley@uwa.edu.au

Abstract

Aims: To investigate the prevalence, molecular type, and antimicrobial susceptibility of *Clostridioides difficile* in the environment in Vietnam, where little is known about *C. difficile*.

Methods and results: Samples of pig faeces, soils from pig farms, potatoes, and the hospital environment were cultured for *C. difficile*. Isolates were identified and typed by polymerase chain reaction (PCR) ribotyping. The overall prevalence of *C. difficile* contamination was 24.5% (68/278). *Clostridioides difficile* was detected mainly in soils from pig farms and hospital soils, with 70%–100% prevalence. *Clostridioides difficile* was isolated from 3.4% of pig faecal samples and 5% of potato surfaces. The four most prevalent ribotypes (RTs) were RTs 001, 009, 038, and QX574. All isolates were susceptible to metronidazole, fidaxomicin, vancomycin, and amoxicillin/clavulanate, while resistance to erythromycin, tetracycline, and moxifloxacin was common in toxigenic strains. *Clostridioides difficile* RTs 001 $A^+B^+CDT^-$ and 038 $A^-B^-CDT^-$ were predominantly multidrug resistant.

Conclusions: Environmental sources of *C. difficile* are important to consider in the epidemiology of *C. difficile* infection in Vietnam, however, contaminated soils are likely to be the most important source of *C. difficile*. This poses additional challenges to controlling infections in healthcare settings.

Significance and impact of study

Clostridioides difficile was commonly detected in soils, and rates of multidrug resistance in *C. difficile* RTs 001 (toxigenic) and 038 (non-toxigenic) were high; findings that suggest these sources of *C. difficile* in the community may be important in the epidemiology of *C. difficile* infection in Vietnam.

Keywords: *Clostridioides* (*Clostridium*) *difficile*, environment, animals, root vegetables, sources/reservoirs, Vietnam

Introduction

Clostridioides (*Clostridium*) *difficile* (Lawson et al. 2016) is a spore-forming anaerobic bacterium that commonly causes hospital-acquired gastrointestinal infection and antimicrobial-associated diarrhoea. *Clostridioides difficile* is responsible for infections ranging from mild to severe diarrhoea, colitis, pseudomembranous colitis, toxic megacolon, and septic shock, often leading to death (Rupnik et al. 2009). Transmission of *C. difficile* is mainly via the faecal-oral route. *Clostridioides difficile* spores persist in the environment for months to years and cannot be destroyed with the usual cleaning/disinfecting agents. These spores are regularly detected in the environment of hospitals, long-term care facilities (Vonberg et al. 2008), soil, water, the gastrointestinal tracts of humans and animals (more commonly in younger animals; Knight et al. 2013, 2015), on root vegetables (Lim et al. 2018), and in processed food (Bakri et al. 2009).

The incidence of *C. difficile* infection (CDI) in the community has increased since the mid-2000s (Wozniak et al. 2015, Guh et al. 2020), and it is postulated that community-associated CDI (CA-CDI) arises due to exposure to *C. difficile* spores in community environments, including in livestock, farming, and slaughterhouse environments, soils, vegetables, fruits, water, and processed food (Songer et al. 2009, Tsai et al. 2016, Wu et al. 2016, Knight and Riley 2019, Tkalec et al. 2020). The reported prevalence of *C. difficile* contamination in foods ranges from 8% to 42% (Candel-Perez et al. 2019), with the highest prevalence to date in North America where *C. difficile* was isolated from various retail meat products (37/88, 42%), both uncooked and ready-to-eat, purchased from three national-chain grocery stores in the Tucson area of Arizona, USA (Songer et al. 2009). An even higher prevalence of *C. difficile* (59%) was found in Australian lawn samples with the prevalence in new lawns higher than in old lawns (Moono et al. 2017).

Received: March 17, 2023. **Revised:** May 16, 2023. **Accepted:** June 8, 2023

There have been few reports on *C. difficile* in Vietnam. Based on an xTAG gastrointestinal panel assay, the prevalence of *C. difficile* in diarrheic stool samples from hospitalized patients in southern Vietnam was 9% (45/479) between 2009 and 2014 (Duong et al. 2016). The prevalence of CDI increased to 24.9% (95/382) in patients with antimicrobial-associated diarrhoea from 2013 to 2015 (Duong 2017). Between 2013 and 2017, the four most prevalent *C. difficile* strains identified using *slpA* typing, *trf*, 017, *cc835*, and *og39*, accounted for ∼90% of all *C. difficile* isolates (Duong 2017, Giang 2020). About one-third were resistant to moxifloxacin, rifampicin, and ampicillin, however, >90% of the isolates were resistant to clindamycin (Giang 2020). In a more recent study, undertaken from 2020 to 2021, the prevalence of *C. difficile* in stool samples of Vietnamese children with diarrhoea was 37.8% (140/370), however, toxigenic *C. difficile* comprised only 16.6% (25/151) of isolates. Of all isolates, ribotypes (RTs) 010, 012, 017, 046, QX011, QX107, QX230, and QX463 were predominant (Khun et al. 2022).

Even less is known about *C. difficile* circulating in the Vietnamese community, healthcare environments, and food, and this study focuses on some aspects of *C. difficile* in these settings. The objectives of the study were to identify sources/reservoirs of *C. difficile* in Vietnam and to evaluate the relationship between environmental and human *C. difficile* strains by molecular typing and antimicrobial susceptibility.

Methods and materials

Study setting

This study was undertaken in two northern Vietnamese provinces within 100 km of the capital city Hanoi; Ninh Binh, and Thai Binh. Samples were collected from healthcare facilities and in the surrounding community within 40 km of the hospitals in each province. The buildings of Ninh Binh Women and Children's Hospital (NBWCH) were constructed during the 1960s and had been unoccupied for a year, while Thai Binh Paediatric Hospital (TBPH) was a relatively new 450-bed facility constructed in 2015. NBWCH was one of the study sites in an earlier investigation of the relationship of *C. difficile* in the hospital environment and in children in Ninh Binh, which was conducted in 2021 prior to the hospital closure (Khun et al. 2022). *Clostridioides difficile* spores can survive in the environment for many months and cannot be killed with regular disinfectant agents (Vonberg et al. 2008), so sample collection was continued at NBWCH to maintain consistency and to see how much *C. difficile* was still present. Other samples were collected in one district of each province; the Thai Thuy district in Thai Binh province, which was 25 km from TBPH and the Kim Son district in Ninh Binh province, which was 40 km from NBWCH.

Specimens

From December 2021 to February 2022, a convenience sample of stool specimens was collected from 12-month-old female pigs kept by families at their homes in Ninh Binh, and soil was collected from these premises. Pig stool samples and soil were collected also from a commercial pig farm in the Thai Binh area with ∼600 pigs. A tongue depressor was used to collect these samples. Faecal samples were placed in sterile stool containers. For soil samples in the pig environment, ∼10 g of soil was picked up with a tongue depressor and placed in a sterile Ziplock-like bag.

Potatoes were purchased from five different vendors in five local markets in the Kim Son district and near NBWCH in Ninh Binh City. In both healthcare facilities, swab samples were collected from surfaces of hospital beds, floors in inpatient wards, toilets, door handles, keyboards, and telephones. Soil samples were collected at/from edges of pathways leading to the hospital, gardens, and playgrounds at both healthcare facilities in a similar manner as described above (Table 1). For potatoes and surface sampling, an alcohol wipe was used to swab a potato or surface and then placed in a sterile Ziplock-like bag. An ∼100 cm^2 surface was wiped for 15–20 sec and the alcohol was allowed to evaporate before placing it in the Ziplock-like bag.

Clostridioides difficile isolation and identification

Robertson's cooked-meat broth (CMB; PathWest Media, Western Australia, Australia) containing gentamicin (5 μg mL^{-1}), cycloserine (250 μg mL^{-1}), cefoxitin (8 μg mL^{-1}), and taurocholate (1 g L^{-1}) was used for selective enrichment of *C. difficile* as described by Bowman and Riley (1988) but with the addition of taurocholate. The alcohol wipes, or 1–2 g of pig stool or soil sample, were inoculated into CMBs, which were incubated at 37°C for 7 days. Alcohol shock on the CMBs was performed as previously described (Khun et al. 2022), the alcohol/broth suspension centrifuged and the supernatant discarded. The deposit was collected with a Transwab (MWE, Corsham, Wiltshire, UK) containing Amies medium without charcoal. All Transwabs were transported from Vietnam to The University of Western Australia (UWA), Perth, Western Australia, Australia, at ambient temperature, taking about 7 days (Khun et al. 2022). This is a process that our laboratory has used successfully for several studies in Asian countries (Collins et al. 2017, Riley et al. 2018, Khun et al. 2022).

In Australia, Transwabs were stored at 4°C before further investigations, and then plated onto ChromID *C. difficile* agar (bioMérieux, Marcy L'Etiole, France) and inoculated into another CMB (Putsathit et al. 2015) and/or 9 mL brain–heart infusion broth (BHIB) containing cycloserine (250 μg mL^{-1}), cefoxitin (8 μg mL^{-1}), and taurocholate (1 g L^{-1}; Khun et al. 2022). ChromID plates, and BHIB with loose lids, were incubated in an A35 anaerobic chamber (Don Whitley Scientific Ltd, Shipley, West Yorkshire, UK) at 35°C for 2 and 7 days, respectively. Both enrichment broths were alcohol shocked and the resultant suspension was plated onto cycloserine cefoxitin fructose agar (CCFA). Suspected *C. difficile* colonies were subcultured onto blood agar and anaerobically incubated for 48 h before identification by characteristics such as morphology, typical odour, and chartreuse fluorescence under long-wave (365 nm) UV light.

Molecular typing

PCR ribotyping and toxin gene profiling for *tcdA*, *tcdB*, *cdtA*, and *cdtB* were performed as previously described (Knight et al. 2013, Khun et al. 2022). The MinElute PCR Purification Kit (Qiagen) was used to concentrate PCR products and a QIAxcel capillary electrophoresis platform with QIAxcel ScreenGel software (v.1.6.0.10, Qiagen GmbH, Hilden, Germany) was used to separate and identify PCR products. All PCR banding patterns were aligned with a reference library of RTs,

Table 1. The distribution of *C. difficile* in Ninh Binh and Thai Binh provinces in Vietnam.

Location	Sites and/or types of sample	Ninh Binh *n*	Thai Binh *n*	Samples	With *C. difficile* *n* (% Positive)	With toxigenic *C. difficile*
Community	Potatoes	20	0	20	1 (5%)	0
	Pig faeces	20	39	59	2 (3.4%)	1 (1.7%)
	Pig environment soils	10	39	49	36 (73.5%)	8 (16.3%)
	Total	50	78	128	39 (30.5%)	9 (7.0%)
Healthcare facilities	Overall hospital soils	10	20	30	25 (83.3%)	6 (20%)
	Hospital pathway soils	5	0	5	5 (100%)	1 (20%)
	Hospital playground soils	5	10	15	13 (86.7%)	5 (33.3%)
	Hospital garden soils	0	10	10	7 (70%)	0
	Door handles	10	15	25	0	0
	Ward floor surfaces	10	15	25	0	0
	Keyboards and telephones	5	8	13	1 (7.7%)	0
	Toilet floors	10	22	32	1 (3.1%)	1 (3.2%)
	Hospital beds	5	20	25	2 (8%)	1 (4%)
	Total	50	100	150	29 (19.3%)	8 (5.3%)
Provinces	Ninh Binh province	100		100	24 (24%)	17 (17%)
	Thai Binh province		178	178	44 (24.7%)	0
	Total	100	178	278	68 (24.5%)	17 (6.1%)

using the Bionumerics software package (V. 7.6.3, Applied Maths, Sint-Martens-Latem, Belgium). Strains that did not match the reference collection but had been isolated at least once previously by our laboratory were given the prefix 'QX' (Imwattana et al. 2019). New strains in this study that had not been seen before were termed 'Novel'.

Antimicrobial susceptibility

Agar dilution susceptibility testing was performed to determine the minimum inhibitory concentrations (MICs) of a selection of antimicrobials for all *C. difficile* isolates, based on the guidelines of the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The recommendations of O'Connor et al. (2008), Freeman et al. (2015), CLSI (Weinstein et al. 2020), and EUCAST (2021) determined breakpoints for the following antimicrobial agents: rifaximin (RFX), erythromycin (ERY), fidaxomicin (FDX), metronidazole (MTZ), amoxicillin/clavulanic acid (AMC), vancomycin (VAN), moxifloxacin (MXF), clindamycin (CLI), and tetracycline (TET). The following recommended control strains were used: *C. difficile* ATCC 70057, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eubacterium lentum* ATCC 43055.

Statistical analysis

Microsoft Excel 365 and IBM SPSS software package version 28.0.1.0 (142) for Windows were used for data entry and statistical analysis. Fisher's exact test or Pearson's Chi-squared test were used where appropriate to compare proportions, with a P-value ≤ 0.05 considered significant.

Results

Clostridioides difficile in the environmental samples in vietnam

From December 2021 to February 2022, a total of 278 samples (150 from healthcare facilities and 128 from communities) were collected from Ninh Binh and Thai Binh provinces in northern Vietnam (Table 1). The overall prevalence of *C. difficile* in these samples was 24.5% (68/278; 95% CI: 19.4%–29.5%). Of those 68 samples containing *C. difficile* by culture, the proportions of *C. difficile* positivity in community and healthcare facility samples were 30.5% (39/128; 95% CI: 22.5%–38.4%), and 19.3% (29/150; 95% CI: 13%–25.7%), respectively.

Clostridioides difficile was commonly found in soil samples from various sources in communities and hospitals, and prevalence of *C. difficile* ranged from 70% to 100% (Table 1). The proportions of *C. difficile* in swine faecal samples and on potatoes were 3.4% (2/59; 95% CI: 0%–8%) and 5% (1/20; 95% CI: 0%–14.6%), respectively. For healthcare facilities, *C. difficile* was found on one toilet floor surface in NBWCH (3.1%, 1/32, 95% CI: 0%–9.2%). There were two *C. difficile* positive samples from hospital beds (8%, 2/25, 95% CI: 1%–26%), one from each hospital, and one from a telephone from NBWCH, however, *C. difficile* was not found on door handles or hospital floors. Soils from pig farms (73.5%, 36/49, 95% CI: 61.1%–85.8%), and overall hospital soils (83.3%, 25/30, 95% CI: 70%–96.7%); hospital pathway soils (100%, 5/5); hospital playground soils (86.7%, 13/15, 95% CI: 69.5%–100%); and hospital garden soils (70%, 7/10, 95% CI: 41.6%–98.4%) were heavily contaminated with *C. difficile*. There was no statistically significant difference in isolation rates between soils from pig farms and hospital soils ($P > 0.31$).

Direct culture on ChromID detected 64 of the 68 positive samples and CMB enrichment and CCFA detected 58 of 68 samples. Of 58 positive samples, 4 extra positives were recovered with culture in CMB, which direct culture did not detect. CMB enrichment broth and direct culture recovered *C. difficile* from two pig stool samples, while BHIB enrichment broth recovered only one of them. There was no significant difference between the recovery rate from direct culture and the enrichment broth method ($P > 0.3$). Five isolates in samples of soils from pig farms produced white colonies on ChromID plates. Of the 68 positive samples, 40 had a single strain in

each sample and 28 samples of soils contained up to six different strains.

Toxin gene profiling

In total, there were 120 *C. difficile* isolates: 94 (78.3%; 95% CI: 71%–85.7%) were non-toxigenic and 26 (21.7%; 95% CI: 14.3%–29%) toxigenic strains. Most *C. difficile* isolates, both toxigenic and non-toxigenic, were from soil samples from pig environments and hospitals (detailed in Table 1). Toxigenic *C. difficile* (26) comprised 23 strains with the toxin gene profile $A^+B^+CDT^-$ (88.5%, 95% CI: 76.2%–100%), two strains $A^-B^+CDT^-$ (7.7%) and one strain $A^+B^+CDT^+$ (3.8%). Of the toxigenic isolates, most (23, 88.5%, 95% CI: 76.2%–100%) were from pig environment (50%, 13/26, 95% CI: 30.8%–69.2%) and healthcare (38.5%, 10/26, 95% CI: 19.8%–57.2%) soil samples. The overall prevalence of toxigenic *C. difficile* in samples of soils from pig farms (16.3%, 8/49, 95% CI: 6%–26.7%) was not significantly different from that of hospital soil samples (20%, 6/30, 95% CI: 5.7%–34.3%; $P > 0.87$).

The prevalence of toxigenic and non-toxigenic *C. difficile*-positive samples in Ninh Binh was 17% (17/100, 95% CI: 9.6%–24.4%) and 7% (7/100, 95% CI: 2%–12%), respectively, while in Thai Binh, toxigenic *C. difficile* was not detected and the prevalence of nontoxigenic *C. difficile* was 24.7% (44/178, 95% CI: 18.4%–31.1%; $P < 0.001$).

Molecular epidemiology

Based on the reference library and other strains in our laboratory collection, the 120 *C. difficile* isolates were assigned to 53 different RTs, 32 previously identified and 21 novel strains. Soils from pig farms contained 36 different RTs, followed by hospital playground soils with 22 and hospital pathway soils with 9. In Thai Binh, RTs 009, 038, and QX574 were the most common in the environment at 8.3% (10/120, 95% CI: 3.4%–13.3%), 7.5% (9/120, 95% CI: 2.8%–12.2%), and 6.7% (8/120, 95% CI: 2.2%–11.1%), respectively, while RTs 001 (5.8%, 7/120, 95% CI: 1.6%–10%), QX514 (3.3%, 4/120, 95% CI: 0.1%–6.6%), QX389 (3.3%, 4/120, 95% CI: 0.1%–6.6%), QX011(3.3%, 4/120, 95% CI: 0.1%–6.6%), QX107 (2.5%, 3/120, 95% CI: 0%–5.3%), and 012 (2.5%, 3/120, 95% CI: 0%–5.3%) were often detected in Ninh Binh environmental samples (Table 2). All toxigenic *C. difficile* strains in this study, RTs 001, 012, 046, 017, 014/020, 369, 126, QX013, QX032, and QX070, were found only in Ninh Binh. The most prevalent toxigenic *C. difficile* RT, RT 001, was commonly found in pig stool, soils from pig farms, hospital beds, and toilet floor surfaces. The 21 novel RTs, which did not match any RTs in our collection were found in 33 isolates, mainly from soils (97%; Table 2). Four strains had the toxin gene profile $A^+B^+CDT^-$ while the others were non-toxigenic. Of the five non-toxigenic white colony *C. difficile* strains isolated, one was identified as QX 400 and the other four strains were novel.

Antimicrobial susceptibility of Vietnamese *C. difficile* isolates

All 120 isolates were susceptible to metronidazole (MIC_{50}/MIC_{90}, 0.125/0.06 μg mL^{-1}), fidaxomicin (MIC_{50}/MIC_{90}, 0.03/0.06 μg mL^{-1}), vancomycin (MIC_{50}/MIC_{90}, 1/1 μg mL^{-1}), and amoxicillin/clavulanic acid (MIC_{50}/MIC_{90}, 0.5/0.5 μg mL^{-1}; Tables 3 and 4). There was slightly decreased susceptibility to rifaximin (96.7% susceptibility; MIC_{50}/MIC_{90}, 0.015/2 μg mL^{-1}) and moxifloxacin (95%; MIC_{50}/MIC_{90}, 2/2 μg mL^{-1}), moderately decreased susceptibility to tetracycline (58.3%; MIC_{50}/MIC_{90}, 0.25/16 μg mL^{-1}) and erythromycin (64.2%; MIC_{50}/MIC_{90}, 1/>256 μg mL^{-1}), and marked decrease in susceptibility to clindamycin (19.2%; MIC_{50}/MIC_{90},8/>32 μg mL^{-1}).

Phenotypic resistance to a single antimicrobial agent was found in 30.8% (37/120; 95% CI: 22.6%–39.1%) of the 120 isolates, more commonly in pig environment soil (33.8%; 23/68; 95% CI: 22.6%–45.1%) and hospital soil (26.7%; 12/45; 95% CI: 13.8%–39.6%) isolates. Among the 120 *C. difficile* isolates, 83 (69.2%, 95% CI: 60.9%–77.4%) were resistant to at least one class of antimicrobials, while 46 (38.3%, 95% CI: 29.6%–47%) displayed resistance to at least two classes, and 34 (28.3%, 95% CI: 20.3%–36.4%) were multidrug-resistant, defined as resistance to three classes of antimicrobials. In addition, there were six isolates (5%) resistant to four antimicrobial classes. Multidrug resistance commonly included resistance to tetracycline, erythromycin, and clindamycin. There was a significant difference in antimicrobial resistance (AMR) between toxigenic and non-toxigenic strains for erythromycin (toxigenic, 69.2% [18/26, 95% CI: 51.5%–87%] vs non-toxigenic, 26.6% [25/94, 95% CI:17.7%–35.5%], $P < 0.001$), tetracycline (toxigenic, 69.2% [18/26, 95% CI: 51.5%–87%] vs non-toxigenic, 38.3% [36/94, 95% CI: 28.5%–48.1%], $P = 0.005$), and moxifloxacin (toxigenic, 19.2% [5/26, 95% CI: 4.1%–34.4%] vs non-toxigenic, 1% [1/94, 95% CI:0%–3.1%], $P = 0.002$). Among the four most prevalent *C. difficile* RTs (001, 009, 038, and QX574), RTs 009 and QX574 were resistant to only one antimicrobial, either tetracycline or clindamycin, 60% (6/10, 95% CI: 29.6%–90.4%) and 33.3% (3/9, 95% CI: 2.5%–64.1%), respectively. However, all RT 001 and 038 strains were resistant to at least one antimicrobial and 77.8% (7/9, 95% CI: 50.6%–100%) of RT 038 and 85.7% (6/7, 95% CI: 59.8%–100%) of RT 001 were resistant to at least three antimicrobials. The six isolates, which were resistant to four classes of antimicrobials, macrolides, tetracyclines, quinolones and/or rifamycins were from RTs 001, 046, 126, 369, and QX463. Five isolates of *C. difficile* producing white colonies on ChromID *C. difficile* agar were susceptible to all antimicrobials tested.

Discussion

CA-CDI cases likely arise from exposure to sources/reservoirs of *C. difficile* in the community, rather than healthcare facilities (Chitnis et al. 2013). *Clostridioides difficile* (most likely as spores) can be found in lawns, on root vegetables and in the gastrointestinal tracts of young animals and children (Chitnis et al. 2013, Knight and Riley 2013, Lim et al. 2018, 2022, Perumalsamy et al. 2019). Food made from the internal organs of animals, especially pigs, is popular in Asia, particularly in Vietnam, and pigs are known to have high prevalence of *C. difficile* carriage and infection (Squire and Riley 2013, Knight et al. 2016, Tsai et al. 2016). Furthermore, pig manure is commonly used to fertilize agricultural land in Vietnam (Vu et al. 2007); this could contribute to widespread community contamination, and subsequent contamination of root vegetables.

The overall prevalence of *C. difficile* in environmental samples in communities and healthcare facilities in Vietnam was 24.5% (68/278; Table 1). The proportion of *C. difficile* positivity in the community was 30.5% (39/128), lower than a

Table 2. Distribution of 53 *C. difficile* RTs and toxin gene profiles for 120 isolates from environmental samples collected in Ninh Binh (NB) and Thai Binh (TB) provinces from December 2021 to March 2022.

Toxin profiles	RTs	Isolates, *n* (%)					Provinces, *n* (%)		Isolates total *n* = 120
		Soils (*n* = 113)		Surfaces (*n* = 4)	Pig faeces (*n* = 2)	Potatoes (*n* = 1)	NB (*n* = 60)	TB (*n* = 60)	
		Community (*n* = 65)	Hospital (*n* = 48)						
$A^+B^+CDT^+$	126	1 (1.5%)	0	0	0	0	1 (1.7%)	0	1 (0.8%)
$A^+B^+CDT^-$	QX 013	2 (3.1%)	0	0	0	0	2 (3.3%)	0	2 (1.6%)
	QX 032	1 (1.5%)		0	0	0	1 (1.7%)	0	1 (0.8%)
	QX 070	0	1 (2.1%)	0	0	0	1 (1.7%)	0	1 (0.8%)
	001	4 (6.2%)	0	2 (50%)	1 (50%)	0	7 (11.7%)	0	7 (5.8%)
	012	0	3 (6.3%)	0	0	0	3 (5%)	0	3 (2.5%)
	014/020	1 (1.5%)	1 (2.1%)	0	0	0	2 (3.3%)	0	2 (1.6%)
	046	0	2 (4.2%)	0	0	0	2 (3.3%)	0	2 (1.6%)
	Novel	3 (4.6%)	2 (4.2%)	0	0	0	5 (8.3%)	0	5 (4.2%)
$A^-B^+CDT^-$	017	0	1 (2.1%)	0	0	0	1 (1.7%)	0	1 (0.8%)
	369	1 (1.5%)	0	0	0	0	1 (1.7%)	0	1 (0.8%)
$A^-B^-CDT^-$	QX 011	1 (1.5%)	3 (6.3%)	0	0	0	4 (6.7%)	0	4 (3.3%)
	QX 107	0	4 (8.3%)	0	0	0	3 (5%)	1 (1.7%)	4 (3.3%)
	QX 138	1(1.5%)	1 (2.1%)	0	0	0	1 (1.7%)	1 (1.7%)	2 (1.6%)
	QX 201	0	1 (2.1%)	0	0	0	0	1 (1.7%)	1 (0.8%)
	QX 371	0	1 (2.1%)	0	0	0	1 (1.7%)	0	1 (0.8%)
	QX 389	2 (3.1%)	2 (4.2%)	0	0	0	4 (6.7%)	0	4 (3.3%)
	QX 400	1 (1.5%)	0	0	0	0	0	1 (1.7%)	1 (0.8%)
	QX 463	0	1 (2.1%)	0	0	0	1 (1.7%)	0	1 (0.8%)
	QX 509	0	1 (2.1%)	0	0	0	0	1 (1.7%)	1 (0.8%)
	QX 510	1 (1.5%)	0	0	0	0	1 (1.7%)	0	1 (0.8%)
	QX 514	2 (3.1%)	2 (4.2%)	0	0	0	4 (6.7%)	0	4 (3.3%)
	QX 552	1 (1.5%)	1 (2.1%)	0	0	0	0	2 (3.3%)	2 (1.6%)
	QX 571	1 (1.5%)	0	0	0	0	0	1 (1.7%)	1 (0.8%)
	QX 573	1 (1.5%)	0	0	0	0	1 (1.7%)	0	1 (0.8%)
	QX 574	2 (3.1%)	5 (10.4%)	2 (50%)	0	0	1 (1.7%)	8 (13.3%)	9 (7.5%)
	QX 588	0	1 (2.1%)	0	0	0	0	1 (1.7%)	1 (0.8%)
	QX 637	2 (3.1%)	0	0	0	0	1 (1.7%)	1 (1.7%)	2 (1.6%)
	QX 671	0	3 (6.3%)	0	0	0	1 (1.7%)	2 (3.3%)	3 (2.5%)
	QX 674	1 (1.5%)	1 (2.1%)	0	0	0	0	2 (3.3%)	2 (1.6%)
	009	10 (15.4%)	0	0	0	0	0	10 (16.7%)	10 (8.3%)
	038	5 (7.7%)	3 (6.3%)	0	1 (50%)	0	0	9 (15%)	9 (7.5%)
	039	0	2 (4.2%)	0	0	0	2 (3.3%)	0	2 (1.6%)
	Novel	22 (33.9%)	5 (10.4%)	0	0	1 (100%)	9 (15%)	19 (31.7)	28 (23.3%)

Table 3. Antimicrobial susceptibility of 113 *C. difficile* isolates from soil samples by toxin gene profile and source of soil.

Antimicrobial agent	Toxigenic strains ($n = 23$)			Non-toxigenic strains ($n = 90$)			Pig environment soils ($n = 68$)			Hospital soils ($n = 45$)		
	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM
AMC	0.25–1	0.5/0.5	0.451	0.25–1	0.5/0.5	0.453	0.25–1	0.5/0.5	0.452	0.25–1	0.5/0.5	0.47
VAN	1–2	1/1	1	0.5–2	1/1	1	0.5–2	1/1	0.99	1–2	1/1	1.016
MXF	1–16	2/2	2.188	1–32	2/2	2.221	1–16	2/2	2.08	1–32	2/2	2.063
CLI	1–>32	>32/>32	9.575	0.125–>32	8/>32	8.094	0.5–>32	8/>32	6.137	0.125–>32	16/>32	11.06
RFX	0.015–>32	0.015/4	0.065	0.015–>32	0.015/4	0.56	0.015–>32	0.015/2	0.05	0.015–>32	0.015/4	0.081
FDX	0.03–0.06	0.03/0.06	0.031	0.008–0.25	0.03/0.06	0.034	0.008–0.25	0.03/0.06	0.034	0.015–0.125	0.03/0.06	0.035
MTZ	0.125–0.25	0.125/0.25	0.148	0.06–0.25	0.125/0.25	0.151	0.06–0.25	0.125/0.25	0.155	0.125–0.25	0.125/0.25	0.144
ERY	0.5–>256	>256/>256	8.641	0.125–>256	0.5/>256	5.079	0.25–>256	1/>256	3.262	0.125–>256	1/>256	8
TET	0.125–32	16/32	2.188	0.25–32	0.25/16	1.53	0.125–32	0.25/16	1.189	0.25–32	0.25/32	1.939

AMC, amoxicillin/clavulanate; VAN, vancomycin; MXF, moxifloxacin; CLI, clindamycin; RFX, rifaximin; FDX, fidaxomicin; MTZ, metronidazole; ERY, erythromycin; TET, tetracycline; GM, Geometric mean; MIC, minimum inhibitory concentration ($\mu g\ mL^{-1}$); MIC_{50}, MIC inhibiting of 50% of organisms; and MIC_{90}, MIC inhibiting of 90% of organisms.

Table 4. Antimicrobial susceptibility of the four most prevalent strains of *C. difficile* ($n = 35$).

Antimicrobial agent	RT 009 ($n = 10$)			RT 038 ($n = 9$)			QX 574 ($n = 9$)			RT 001 ($n = 7$)		
	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM	MIC range	MIC_{50}/MIC_{90}	GM
AMC	0.5–0.5	0.5/0.5	0.5	0.25–0.5	0.5/0.5	0.463	0.25–0.5	0.5/05	0.397	0.25–0.5	0.25/0.5	0.336
VAN	1–1	1/1	1	1–1	1/1	1	1–1	1/1	1	1–2	1/1	1
MXF	2–2	2/2	2	2–2	2/2	2	2–2	2/2	2	2–32	2/32	6.563
CLI	0.5–8	4/8	7.464	16–>32	>32/>32	29.63	2–8	4/8	4.32	2–>32	>32/>32	21.53
RFX	0.015–0.015	0.015/0.015	0.015	0.015–0.015	0.015/0.015	0.015	0.015–0.015	0.015/0.015	0.015	0.008–0.015	0.015/0.015	0.013
FDX	0.015–0.06	0.03/0.06	0.026	0.03–0.125	0.06/0.06	0.048	0.03–0.06	0.03/0.06	0.035	0.015–0.03	0.015/0.015	0.017
MTZ	0.06–0.25	0.125/0.25	0.133	0.125–0.25	0.25/0.25	0.214	0.125/0.25	0.125/0.25	0.157	0.125–0.25	0.125/0.125	0.138
ERY	0.25–1	1/1	0.871	>256–>256	>256/>256	256	0.125–0.5	0.5/0.5	0.34	0.5–>256	>256/>256	105
TET	0.25–0.25	8/16	2.297	8–16	16/16	13.72	0.25–0.25	0.25/0.25	0.25	8–32	32/32	21.53

AMC, amoxicillin/clavulanate; VAN, vancomycin; MXF, moxifloxacin; CLI, clindamycin; RFX, rifaximin; FDX, fidaxomicin; MTZ, metronidazole; ERY, erythromycin; TET, tetracycline; GM, Geometric mean; MIC, minimum inhibitory concentration ($\mu g\ mL^{-1}$); MIC_{50}, MIC inhibiting of 50% of organisms; MIC_{90}, MIC inhibiting of 90% of organisms.

study in Thailand and Malaysia (89%, 8/9 and 93%, 13/14, respectively; Putsathit et al. 2019), however, the Thai and Malaysian sample numbers ($n = 23$) were low and collected only from pig farms. In addition, in the studies in Thailand and Malaysia, no isolates from the pig farm environment (soil or water) were toxigenic (Putsathit et al. 2019), similar to our findings in Thai Binh province. However, the prevalence of toxigenic *C. difficile* isolates from pig environment soils and all community-environmental samples in Ninh Binh province was 18% (9/50).

The prevalence of *C. difficile* in stool samples from pigs in Asia ranged from 7.8% in China (Zhang et al. 2019) to 49% in Taiwan (Wu et al. 2016). Most *C. difficile* were toxigenic and binary toxin-positive; the most prevalent toxigenic RTs being *C. difficile* RTs 078 and 126 (Wu et al. 2016, Zhang et al. 2019). The prevalence of *C. difficile* in pigs decreases with increased age to <10% in pigs aged >70 days (Hawken et al. 2013) and even lower in breeding boars and sows (Norman et al. 2009). The prevalence of *C. difficile* in pig faecal samples in the current study was 3.4% (2/59), much lower than our earlier findings in Thailand (35.1%, 58/165) and Malaysia (91.5%, 54/59; Putsathit et al. 2019), and the studies in Taiwan (49%, 100/204; Wu et al. 2016). However, the low prevalence of *C. difficile* in the pigs in our study may be because they were at least 1 year old.

Clostridioides difficile contamination of vegetables has been reported from various parts of the world, and in Slovenia was as high as 60%, especially in potatoes with *C. difficile* RTs 001, 010, 014/020, and 053 commonly detected (Tkalec et al. 2020). In Australia, the prevalence of *C. difficile* in root vegetables in Western Australia varied from 5% to 55.6% with the highest prevalence also in potatoes (55.6%; Lim et al. 2018). Our prevalence of *C. difficile* from potatoes was the same as the low end of the Western Australian root vegetable study (5%), although the number of samples was small ($n = 20$) and, interestingly, the *C. difficile* strain isolated was novel, i.e. not present in our reference collection.

Clostridioides difficile spore contamination in the environment of healthcare facilities is considered an important source of hospital-acquired CDI transmission (Rutala and Weber 2013). In the current study, the prevalence of *C. difficile* in soil samples from hospital pathways, playgrounds, and gardens was 100% (5/5), 86.7% (13/15), and 70% (7/10), respectively. This was higher than similar studies in Australia, where the overall prevalence of *C. difficile* in hospital soils was 60.4% (96/159; range 52%–76.2%; Perumalsamy et al. 2019). There were 48 *C. difficile* isolates from 30 hospital ground samples and slightly less than a quarter (22.9%) were toxigenic, not significantly different from the Australian study (29.5%; Perumalsamy et al. 2019).

Recently, the prevalence of *C. difficile* on room floors in a large hospital in the USA was ∼50% (Srinivasa et al. 2019). In Australia, the prevalence of *C. difficile* on hospital floors, and shoes of medical staff, visitors, and patients, was 29.7% (89/300) and 32% (96/300), respectively (Lim et al. 2022). In our study, the prevalence was low, compared to the US and Australian studies as *C. difficile* was detected on 7.7% (1/13) of telephones, 3.1% (1/32) of toilet floors, and 8% (2/25) of hospital beds. Furthermore, we did not isolate *C. difficile* from door handles and ward floor surfaces. However, NBWCH had been unoccupied for a year, and there were no known CDI cases at TBPH, a relatively new hospital, at the time of sampling.

There were four predominant strains of *C. difficile* in the present study: *C. difficile* RTs 009 (8.3%, 10/120), 038 (7.5%, 9/120), QX574 (7.5%, 9/120; all non-toxigenic), and the toxigenic RT 001 (5.8%, 7/120). *Clostridioides difficile* RTs 001 $A^+B^+CDT^-$ and 038 $A^-B^-CDT^-$ were isolated from pig faeces in Ninh Binh and Thai Binh provinces. In the Thai and Malaysian studies, RT 038 was the most prevalent *C. difficile* RT found in pig rectal swab samples and pig farm soils (Putsathit et al. 2019). *Clostridioides difficile* RT 126 was the only CDT^+ strain isolated in this study and belongs to clade 5 (Knight and Riley 2016). *Clostridioides difficile* RT 126 and the closely related RT 078 have been found in calves aged <7 days in Shandong province in China (Zhang et al. 2020). In Taiwan and Japan, RT 078-related strains (RTs 126 and 127) are common in pigs and are considered to have high potential for zoonotic transmission to humans (Tsai et al. 2016, Wu et al. 2016, Usui et al. 2017). Our *C. difficile* RT 126 isolate was detected only in a soil sample from a pig farm and there have not yet been reports of CDI in humans caused by RT 126 in Vietnam. Five *C. difficile* isolates that produced white colonies on ChromID *C. difficile* agar were detected in samples from soils from pig farms. *Clostridioides difficile* isolates derived from white colonies lack a β-glucosidase gene and do not have the ability to hydrolyse esculetin in ChromID agar and thus produces a white not black colony (Imwattana et al. 2023).

Based on the findings of our earlier paediatric study and the present study, ∼50% (15/32) of identified RTs were found in both the environmental and children's stool samples (Khun et al. 2022). *Clostridioides difficile* RTs 012, 017, 046, QX 107, and QX 463 were found only in healthcare facilities, while RTs 009, QX 573, and QX 671 were found only in communities. *Clostridioides difficile* RTs 038, QX 011, QX 138, QX 514, QX 552, QX 574, and QX 674 were found in both healthcare facilities and communities. The antimicrobial susceptibility profiles of *C. difficile* isolates from environmental and children stool samples were not significantly different suggesting, possibly, a close association between the environment and children. Additionally, children are likely to bring *C. difficile* from the community into the hospital either as contaminants of clothing or shoes or as ingested *C. difficile* spores. Lim et al. (2022) used whole genome sequencing (WGS) to confirm *C. difficile* spores in hospitals can be introduced from the community.

In the current study, several other strains were identified as having zoonotic transmission potential via environmental contamination in Vietnam. Both *C. difficile* RTs 001 and QX574 were found in pig farm soils and pig faeces, as well as on hospital beds, hospital telephones, and/or ward floors. In our earlier paediatric study in Vietnam, *C. difficile* QX574 was isolated from a patient with diarrhoea in TBPH. *Clostridioides difficile* RT 001 was not detected in either NBWCH and TBPH (Khun et al. 2022), but toxigenic *C. difficile* RTs 001, 012, 014/020, 046, 017, QX032, and 070 were identified in hospital environmental samples. These RTs have previously been found in Vietnamese investigations of CDI. In a study conducted from 2013 to 2015, four common *C. difficile* strains, including *trf* (RT 369), RT 017, *cc835* (RT 012), and *og39* (RT 046), caused antibiotic-associated CDI in adults in Hanoi (Duong 2017). In another study of CDI in adults, *C. difficile* strains *trf* (RT 369), RT 017, *cc835* (RT 012), and *og39* (RT 046) accounted for 86.2% of isolates and the rest belonged to RT 014, *ozk*, *cr*, and RT 001 (Giang 2020). *Clostrid-*

ioides difficile RT 001 in adults with CDI accounted for 2% of diarrhoeal stool isolates (Duong 2017, Giang 2020). According to Zhou et al. (2021), the transmission of *C. difficile* was confirmed between animals, the environment, and humans using WGS. The most prevalent strains were RTs 001, 046 and 596, and RTs 012, 017, and 046 were among the strains circulating in the hospital environment and caused CDI (Zhou et al. 2021). Several samples, pig environment soils, pig stool, toilets, and hospital beds, were positive for toxigenic RT 001, suggesting this was a zoonotic strain in Vietnam. Although *C. difficile* RT 001 was not reported in diarrhoeal stool samples of children at NBWCH, RTs 012, 017, and 046 were detected (Khun et al. 2022). Interestingly, these RTs were found in the hospital environment in the current study, so toxigenic *C. difficile* RTs 012, 017, and 046 clearly circulated in healthcare facilities and might also be hospital acquired.

Antimicrobial susceptibility data have rarely been reported for *C. difficile* in Vietnam (Duong 2017, Giang 2020, Lew et al. 2020). In 12 Asia-Pacific countries, *C. difficile* isolates were susceptible to fidaxomicin, vancomycin, amoxicillin/clavulanate, and metronidazole (Lew et al. 2020), while they were resistant to varying degrees to rifaximin (15.5%), clindamycin (80.7%), erythromycin (55.3%), and moxifloxacin (44.4%). Toxigenic *C. difficile* strains in Vietnam are frequently resistant to rifaximin, moxifloxacin, clindamycin, and erythromycin (Duong 2017, Giang 2020, Lew et al. 2020). In the current study, the susceptibility of all *C. difficile* isolates to fidaxomicin, metronidazole, amoxicillin/clavulanate, and vancomycin was similar to the findings of our earlier paediatric study (Khun et al. 2022). In comparison, resistance prevalence in *C. difficile* isolates in the current environmental and earlier paediatric studies to clindamycin, moxifloxacin, and rifaximin were 65% vs 90.1%, 5% vs 6.5%, and 3.3% vs 3.3%, respectively, not significantly different. The Asia-Pacific study showed 85.7% of Vietnamese isolates were resistant to erythromycin (12/14, MIC_{50}/MIC_{90} = >256/>256 μg mL^{-1}; Lew et al. 2020), while 35.8% of current isolates were (43/120, MIC_{50}/MIC_{90} = 1/>256 μg mL^{-1}).

AMR in *C. difficile* has become a major issue world-wide with the US Centers for Disease Control and Prevention rating *C. difficile* as an urgent AMR public health threat in the USA (CDC 2019). *Clostridioides difficile* has an extensive repertoire of AMR, which includes resistance to lincomycin and clindamycin, aminoglycosides, tetracyclines, macrolides, cephalosporins, penicillins, and fluoroquinolones (O'Grady et al. 2021). In Vietnam, regulation of antimicrobials in human and veterinary medicine is poor and, in the present study, AMR was particularly high for clindamycin, erythromycin, and moxifloxacin. Various resistance mechanisms in *C. difficile* have been described, including chromosomal resistance genes, mobile genetic elements, alterations to metabolic pathways and antimicrobial targets, and biofilms (Spigaglia et al. 2018), with some strains showing multidrug resistance, as seen in our study. The spread of these AMR strains of *C. difficile* presents particular challenges to controlling infections in healthcare settings where it is likely that some resistant strains are being brought into hospitals from the external environment.

Conclusion

This is the first study of *C. difficile* in the environment of Vietnamese communities and hospitals. The prevalence of *C. difficile* in soil samples was high and a wide range of *C. difficile* RTs was identified, the majority of which were non-toxigenic. Children are likely to transmit *C. difficile* from the community to healthcare facilities. *Clostridioides difficile* RT 001 was more likely zoonotic, while RTs 012, 017, and 046 were more healthcare-related transmission. Toxigenic *C. difficile* isolates, including RT 001 and the non-toxigenic RT 038, displayed multidrug resistance. The main limitation of this study was that we assessed a relatively narrow range of samples from production animals and vegetables in the Vietnamese community. Future research should evaluate the regulation of toxigenic strains or multidrug resistance that could become a serious issue in Asia and to further investigate the relationship of *C. difficile* strains in clinical human samples and the environment with WGS. *Clostridioides difficile* RTs 126 and 001 should be closely monitored in terms of the epidemiology of the infections they cause and the development of preventative strategies.

Acknowledgements

We would like to thank Dr Chinh Thi Minh Nguyen (Director of TBGH), Prof. Thanh Thi Hoang (Former Head of Paediatric Department of Thai Binh University of Medicine and Pharmacy), Dr Phuc Huu Phan (Vice-head of Paediatric Intensive Care Unit, Vietnam National Children's Hospital), and Dr Dau Van Pham (Director of NBWCH) for their logistic supports and facilitation and Phuong Thi Le (Cardiovascular Department, TBPH) and Oanh Thi Tran (Intensive care unit, TBPH) collected samples in Thai Binh province and microbiologists and laboratory technicians (Microbiology department, TBGH) for their help with sample processes and technical issues.

Ethics statement

The Vietnam National Children's Hospital and Research Institute for Child Health Ethics Committee (VNCH-RICH-2020–64) and the Human Research Ethics Committee at The University of Western Australia (RA/4/20/5993) approved the study. Animal ethics approval was not required because there was no direct interaction with animals and the pig stool samples were collected from the ground.

Conflict of interest

None declared.

Funding

P.A.K. was funded by a Scholarship for International Research Fees awarded by The University of Western Australia. D.A.C. is the recipient of a National Health and Medical Research Council Early Career Fellowship.

Author contributions

Peng An Khun (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing—original draft, Writing—review and editing), Long Duc Phi (Conceptualization, Investigation, Supervision), Huong Thi Thu Bui, Nguyen Thi Bui, Quyen Thi Huyen Vu, and Luong Duy Trinh (Con-

ceptualization, Investigation), Deirdre A. Collins (Writing—review and editing), Thomas V. Riley (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing—review and editing).

Data availability

The datasets generated during and/or analysed during current study are included in this published article and raw data are available from the corresponding author upon a reasonable request.

References

Bakri MM, Brown DJ, Butcher JP *et al*. *Clostridium difficile* in ready-to-eat salads, Scotland. *Emerg Infect Dis* 2009;**15**:817–8. https://doi.org/10.3201/eid1505.081186.

Bowman RA, Riley TV. Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea. *Eur J Clin Microbiol Infect Dis* 1988;**7**:476–84.

Candel-Perez C, Ros-Berruezo G, Martinez-Gracia C. A review of *Clostridioides* [*Clostridium*] *difficile* occurrence through the food chain. *Food Microbiol* 2019;**77**:118–29. https://doi.org/10.1016/j.fm.2018.08.012.

Centers for Disease Control and Prevention (CDC). *Antibiotic resistance threats in the United States*. Atlanta, GA: Centers for Disease Control and Prevention, 2019.

Chitnis AS, Holzbauer SM, Belflower RM *et al*. Epidemiology of community-associated *Clostridium difficile* infection, 2009 through 2011. *JAMA Intern Med* 2013;**173**:1359–67. https://doi.org/10.1001/jamainternmed.2013.7056.

Collins DA, Gasem MH, Habibie TH *et al*. Prevalence and molecular epidemiology of *Clostridium difficile* infection in Indonesia. *New Microbes New Infect* 2017;**18**:34–37. https://doi.org/10.1016/j.nmni.2017.04.006.

Duong DTT. *Determination of infection rates and some molecular epidemiological characteristics of toxin gene carried Clostridium difficile strains isolated from patients with diarrhea after antibiotic treatment in four hospitals in Hanoi (2013–2015)*. Hanoi: Department of Microbiology, Vietnam University of Science, 2017, 149.

Duong VT, Phat VV, Tuyen HT *et al*. Evaluation of luminex xTAG gastrointestinal pathogen panel assay for detection of multiple diarrheal pathogens in fecal samples in Vietnam. *J Clin Microbiol* 2016;**54**:1094–100. https://doi.org/10.1128/jcm.03321-15.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters, version 11. 2021. Available at: http://www.eucast.org/. (last accessed on 31 May, 2023)

Freeman J, Vernon J, Morris K *et al*. Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes. *Clin Microbiol Infect* 2015;**21**:248.e249–16. https://doi.org/10.1016/j.cmi.2014.09.017.

Giang NTH. *Characteristics of epidemiology, clinical manifestation, and risk factors of Clostridium difficile infection among adult patients with diarrhoea in bach mai hospital from 2013 to 2017*. Department of Epidemiology, NIHE Press: National Institute of Hygiene and Epidemiology, Hanoi. 2020, 196.

Guh AY, Mu Y, Winston LG *et al*. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. *N Engl J Med* 2020;**382**:1320–30. https://doi.org/10.1056/NEJMoa1910215.

Hawken P, Weese JS, Friendship R *et al*. Longitudinal study of *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* associated with pigs from weaning through to the end of processing. *J Food Prot* 2013;**76**:624–30. https://doi.org/10.4315/0362-028X.JFP-12-330.

Imwattana K, Shivaperumal N, Leepattarakit T *et al*. A white colony-producing *Clostridioides difficile* ribotype 020 strain. *J Clin Microbiol* 2023;**61**:1–7.

Imwattana K, Wangroongsarb P, Riley TV. High prevalence and diversity of *tcdA*-negative and *tcdB*-positive, and non-toxigenic, *Clostridium difficile* in Thailand. *Anaerobe* 2019;**57**:4–10. https://doi.org/10.1016/j.anaerobe.2019.03.008.

Khun PA, Phi LD, Pham PT *et al*. *Clostridioides* (*Clostridium*) *difficile* in children with diarrhoea in Vietnam. *Anaerobe* 2022;**74**:102550. https://doi.org/10.1016/j.anaerobe.2022.102550.

Knight DR, Riley TV. Prevalence of gastrointestinal *Clostridium difficile* carriage in Australian sheep and lambs. *Appl Environ Microbiol* 2013;**79**:5689–92. https://doi.org/10.1128/AEM.01888-13.

Knight DR, Riley TV. *Clostridium difficile* clade 5 in Australia: antimicrobial susceptibility profiling of PCR ribotypes of human and animal origin. *J Antimicrob Chemother* 2016;**71**:2213–7. https://doi.org/10.1093/jac/dkw124.

Knight DR, Riley TV. Genomic delineation of zoonotic origins of *Clostridium difficile*. *Front Public Health* 2019;**7**:164. https://doi.org/10.3389/fpubh.2019.00164.

Knight DR, Squire MM, Collins DA *et al*. Genome analysis of *Clostridium difficile* PCR ribotype 014 lineage in Australian pigs and humans reveals a diverse genetic repertoire and signatures of long-range interspecies transmission. *Front Microbiol* 2016;**7**:2138. https://doi.org/10.3389/fmicb.2016.02138.

Knight DR, Squire MM, Riley TV. Nationwide surveillance study of *Clostridium difficile* in Australian neonatal pigs shows high prevalence and heterogeneity of PCR ribotypes. *Appl Environ Microbiol* 2015;**81**:119–23. https://doi.org/10.1128/AEM.03032-14.

Knight DR, Thean S, Putsathit P *et al*. Cross-sectional study reveals high prevalence of *Clostridium difficile* non-PCR ribotype 078 strains in Australian veal calves at slaughter. *Appl Environ Microbiol* 2013;**79**:2630–5. https://doi.org/10.1128/AEM.03951-12.

Lawson PA, Citron DM, Tyrrell KL *et al*. Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prevot 1938. *Anaerobe* 2016;**40**:95–99. https://doi.org/10.1016/j.anaerobe.2016.06.008.

Lew T, Putsathit P, Sohn KM *et al*. Antimicrobial susceptibilities of *Clostridium difficile* isolates from 12 Asia-Pacific countries in 2014 and 2015. *Antimicrob Agents Chemother* 2020;**64**:1–11. https://doi.org/10.1128/AAC.00296-20.

Lim SC, Collins DA, Imwattana K *et al*. Whole-genome sequencing links *Clostridium* (*Clostridioides*) *difficile* in a single hospital to diverse environmental sources in the community. *J Appl Microbiol* 2022;**133**:1156–68. https://doi.org/10.1111/jam.15408.

Lim SC, Foster NF, Elliott B *et al*. High prevalence of *Clostridium difficile* on retail root vegetables, Western Australia. *J Appl Microbiol* 2018;**124**:585–90. https://doi.org/10.1111/jam.13653.

Moono P, Lim SC, Riley TV. High prevalence of toxigenic *Clostridium difficile* in public space lawns in Western Australia. *Sci Rep* 2017;**7**:41196. https://doi.org/10.1038/srep41196.

Norman KN, Harvey RB, Scott HM *et al*. Varied prevalence of *Clostridium difficile* in an integrated swine operation. *Anaerobe* 2009;**15**:256–60. https://doi.org/10.1016/j.anaerobe.2009.09.006.

O'Connor JR, Galang MA, Sambol SP *et al*. Rifampin and rifaximin resistance in clinical isolates of *Clostridium difficile*. *Antimicrob Agents Chemother* 2008;**52**:2813–7. https://doi.org/10.1128/AAC.00342-08.

O'Grady K, Knight DR, Riley TV. Antimicrobial resistance in *Clostridioides difficile*. *Eur J Clin Microbiol Infect Dis* 2021;**40**:2459–78. https://doi.org/10.1007/s10096-021-04311-5.

Perumalsamy S, Putsathit P, Riley TV. High prevalence of *Clostridium difficile* in soil, mulch and lawn samples from the grounds of Western Australian hospitals. *Anaerobe* 2019;**60**:102065. https://doi.org/10.1016/j.anaerobe.2019.06.018.

Putsathit P, Morgan J, Bradford D *et al*. Evaluation of the BD Max Cdiff assay for the detection of toxigenic *Clostridium difficile* in human stool specimens. *Pathology (Phila)* 2015;**47**:165–8. https://doi.org/10.1097/PAT.0000000000000214.

Putsathit P, Neela VK, Joseph NMS *et al*. Molecular epidemiology of *Clostridium difficile* isolated from piglets. *Vet Microbiol* 2019;**237**:108408. https://doi.org/10.1016/j.vetmic.2019.108408.

Riley TV, Collins DA, Karunakaran R *et al*. High prevalence of toxigenic and nontoxigenic *Clostridium difficile* strains in Malaysia. *J Clin Microbiol* 2018;**56**:e00170–00118. https://doi.org/10.1128/JCM.00170-18.

Rupnik M, Wilcox MH, Gerding DN. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009;**7**:526–36. https://doi.org/10.1038/nrmicro2164.

Rutala WA, Weber DJ. Role of the hospital environment in disease transmission, with a focus on *Clostridium difficile*. *Healthcare Infect* 2013;**18**:14–22. https://doi.org/10.1071/hi12057.

Songer JG, Trinh HT, Killgore GE *et al*. *Clostridium difficile* in retail meat products, USA, 2007. *Emerg Infect Dis* 2009;**15**:819–21. https://doi.org/10.3201/eid1505.081071.

Spigaglia P, Mastrantonio P, Barbanti F. Antibiotic resistances of *Clostridium difficile*. *Adv Exp Med Biol* 2018;**1050**:137–59. https://doi.org/10.1007/978-3-319-72799-8_9.

Squire MM, Riley TV. *Clostridium difficile* infection in humans and piglets: a 'One Health' opportunity. *Curr Top Microbiol Immunol* 2013;**365**:299–314. https://doi.org/10.1007/82_2012_237.

Srinivasa VR, Hariri R, Frank LR *et al*. Hospital-associated *Clostridium difficile* infection and reservoirs within the hospital environment. *Am J Infect Control* 2019;**47**:780–5. https://doi.org/10.1016/j.ajic.2018.12.013.

Tkalec V, Jamnikar-Ciglenecki U, Rupnik M *et al*. *Clostridioides difficile* in national food surveillance, Slovenia, 2015 to 2017. *Eurosurveillance* 2020;**25**. https://doi.org/10.2807/1560-7917.ES.2020.25.16.1900479.

Tsai BY, Ko WC, Chen TH *et al*. Zoonotic potential of the *Clostridium difficile* RT078 family in Taiwan. *Anaerobe* 2016;**41**:125–30. https://doi.org/10.1016/j.anaerobe.2016.06.002.

Usui M, Kawakura M, Yoshizawa N *et al*. Survival and prevalence of *Clostridium difficile* in manure compost derived from pigs. *Anaerobe* 2017;**43**:15–20. https://doi.org/10.1016/j.anaerobe.2016.11.004.

Vonberg RP, Kuijper EJ, Wilcox MH *et al*. Infection control measures to limit the spread of *Clostridium difficile*. *Clin Microbiol Infect* 2008;**14**:2–20. https://doi.org/10.1111/j.1469-0691.2008.01992.x.

Vu TKV, Tran MT, Dang TTS. A survey of manure management on pig farms in northern Vietnam. *Livest Sci* 2007;**112**:288–97. https://doi.org/10.1016/j.livsci.2007.09.008.

Weinstein MP, Lewis JS, II, Bobenchik AM *et al*. *Performance Standards for Antimicrobial Susceptibility Testing: Seventh Informational Supplement M100-S30*. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.

Wozniak TM, Rubin G, MacIntyre CR. The emergence of community-acquired *Clostridium difficile* in an Australian hospital. *Healthcare Infect* 2015;**20**:72–77. https://doi.org/10.1071/hi15003.

Wu YC, Lee JJ, Tsai BY *et al*. Potentially hypervirulent *Clostridium difficile* PCR ribotype 078 lineage isolates in pigs and possible implications for humans in Taiwan. *Int J Med Microbiol* 2016;**306**:115–22. https://doi.org/10.1016/j.ijmm.2016.02.002.

Zhang LJ, Yang L, Gu XX *et al*. The first isolation of *Clostridium difficile* RT078/ST11 from pigs in China. *PLoS One* 2019;**14**:e0212965. https://doi.org/10.1371/journal.pone.0212965.

Zhang WZ, Li WG, Liu YQ *et al*. The molecular characters and antibiotic resistance of *Clostridioides difficile* from economic animals in China. *BMC Microbiol* 2020;**20**. https://doi.org/10.1186/s12866-020-01757-z.

Zhou Y, Zhou W, Xiao T *et al*. Comparative genomic and transmission analysis of *Clostridioides difficile* between environmental, animal, and clinical sources in China. *Emerg Microbes Infect* 2021;**10**:2244–55. https://doi.org/10.1080/22221751.2021.2005453.