Scientific Research Publishing

Advances in Microbiology, 2025, 15(9), 484-495
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Detection of *Clostridium difficile* TcdA and TcdB Genes in Paediatric Diarrhoeal Stools by PCR in Abidjan

Yao Gilles Privat Kouamé[1], Britoh Alice Mlan[2,3], Mian N. Arsher Cablan[1,4], Befiassou Perpetue Ani[3], Serge Joseph Lathro[1,4], Arthur Dreyfus Boka[1], Flore Sandrine Zaba[4], N'goran Eric Koffi[5], Denise Affoué Blassonny Guina[1,4], Touré Aissata Epse Kourouma[1], Aimé N'djomon[1], Yesse Zinzendorf Nanga[1,4], N'douba Adele Kacou[2]

[1]National Public Health Laboratory (LNSP), Abidjan, Côte d'Ivoire
[2]Department of Bacteriology and Virology, Teaching and Research Unit of Medical Sciences, Abidjan, Côte d'Ivoire
[3]Institut Pasteur of Côte d'Ivoire, Abidjan, Côte d'Ivoire
[4]Department of Bacteriology-Virology, Teaching and Research Unit of Pharmaceutical and Biological Sciences, Abidjan, Côte d'Ivoire
[5]Paediatric Department, Treichville University Hospital, Abidjan, Côte d'Ivoire
Email: *kouameyaop@gmail.com, alicemlanpat@gmail.com, cablanmianarsher@yahoo.fr, aniperptue@yahoo.fr, lathrosergefr@yahoo.fr, arthurdreyfus@gmail.com, sandyzaba@yahoo.fr, koffingoraneric@gmail.com, bdenise01@gmail.com, sakinalamtou@yahoo.fr, n.aimso@yahoo.fr, dorfange@gmail.com, knadele@yahoo.fr

How to cite this paper: Kouamé, Y.G.P., Mlan, B.A., Cablan, M.N.A., Ani, B.P., Lathro, S.J., Boka, A.D., Zaba, F.S., Koffi, N.E., Guina, D.A.B., Kourouma, T.A.E., N'djomon, A., Nanga, Y.Z., and Kacou, N.A. (2025) Detection of *Clostridium difficile* TcdA and TcdB Genes in Paediatric Diarrhoeal Stools by PCR in Abidjan. *Advances in Microbiology*, **15**, 484-495.
https://doi.org/10.4236/aim.2025.159031

Received: July 23, 2025
Accepted: September 2, 2025
Published: September 5, 2025

Copyright © 2025 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Background: Diagnosis of *Clostridium difficile* infection (CDI) is a major challenge in controlling the spread of virulent strains of C. difficile. However, testing for *C. difficile* is not part of the standard stool culture, despite the high infant diarrhoeal mortality rate in low-income countries. Hence the interest of the present study is to optimise the diagnosis of CDI using molecular biology. **Objective**: To detect the tcdA and tcdB genes of *C. difficile* in diarrhoeal stools and to identify the risk factors associated with CDI. **Method**: This prospective cross-sectional study was conducted from April to August 2021. It consisted of collecting diarrhoeal stools from children in the paediatrics department of Treichville University Hospital. Detection of the *C. difficile* tcdA and tcdB genes was performed by molecular biology. **Results**: PCR results revealed the tcdB gene in 8.3% of cases. This was the A^-/B^+ variant. The risk factors studied (sex, age, mode of patient admission and previous antibiotic therapy) did not allow us to establish a correlation between the risk of CDI and these factors after statistical analysis of the data collected. However, analysis of previous antibiotic treatment revealed that only flucloxacillin and metronidazole were associated with the detection of TcdB genes. **Conclusion**: This experimental work revealed the circulation of the virulence gene (TcdB) in strains of C. difficile isolated from the stools of children with diarrhoea.

Keywords

Clostridium difficile, Children, Diarrhoea, TcdA and TcdB Genes

1. Introduction

Clostridium difficile (*C. difficile*) is an anaerobic spore-forming Gram-positive bacillus that can be found in the environment and in the gastrointestinal tract of animals and humans [1]. The pathogenicity of *C. difficile* is attributed to the production of two protein toxins, designated A (enterotoxin) and B (cytotoxin), encoded by the tcdA and tcdB genes respectively. They are located at the pathogenicity locus (PaLoc) and both cause disease by significantly disrupting the integrity of the colonic mucosa [2]. The clinical picture of *C. difficile*-associated disease can range from asymptomatic carriage in the gastrointestinal tract to mild diarrhoea or even potentially fatal pseudomembranous colitis [3]. According to several authors, these symptoms have increased in recent decades, with incidence rates estimated in some studies in the United States at 147.2 cases per 100,000 people, and around 29,300 associated deaths [4] and in the Netherlands at 390 to 730 cases per 100,000 people/year [5]. However, diagnosis of CDI remains difficult [6]. Initial strategies to detect *C. difficile* consisted of anaerobic culture of stool samples, which was however, time-consuming despite its good sensitivity and specificity [7]. The development of the cell culture cytotoxicity test has bypassed the standard stool culture but presents technical constraints [8] [9]. Rapid detection tests (RDTs), consisting of common antigenic tests, have largely replaced culture, and cytotoxicity tests present limitations in the interpretation of results [10]. Today, PCR is an appropriate diagnostic tool for DCI because of its rapid turnaround time, good sensitivity and high specificity. This specificity is characterised by the fact that PCR targets the genes (tcdA and tcdB) for *C. difficile* toxins A and B [11]. This fact has led to increasing interest in studies in developing countries, where diarrhoeal diseases remain one of the major causes of morbidity and mortality in children, and where the prevalence of CDI remains poorly understood [12]. This observation prompted the study of paediatric patients in order to gain a better understanding of the role of CDI in childhood diarrhoea. The general aim of this work was to optimise the diagnosis of *C. difficile* using molecular biology techniques. More specifically, the objectives were to detect the tcdA and tcdB genes for *C. difficile* toxins A and B in diarrhoeal stools and to identify the risk factors associated with *C. difficile* infection.

2. Method

- **Ethical considerations**

Confidentiality and anonymity were respected during the study, with the use of a single survey form in which patients' identities were replaced by codes. In addition, samples were taken with the written and informed consent of the parents of

eligible patients. This study also required the authorisation of the Medical Director of Health (DMS) at the University Hospital Centre (CHU) in Treichville.

- **Scope, duration and type of study**

The pre-analytical phase of this study, either the selection of patients and the collection of diarrhoeal stools from eligible patients, was carried out at the Treichville University Hospital, in the paediatric medical department. Once the stools had been collected, molecular analysis was carried out in the molecular biology unit of the Institut Pasteur de Côte d'Ivoire (IPCI), using primer pairs specific for the *Clostridium difficile* TcdA and TcdB genes and compared with positive controls (Monica®). The study lasted six months from April to August 2021 and was a cross-sectional study.

- **Population study**

The study population was children presenting with diarrhoea. In practice, stool collection was preceded by a questionnaire session after parental consent had been obtained. Stool samples were taken in coproculture jars. The study population consisted of 36 patients whose reason for consultation and hospitalisation was diarrhoea, and who were on antibiotic treatment for even 30 days after stopping it. Patients from the community and hospitalised children were included in the study. At the time of the survey, patients classified as being of community origin were children with diarrhoea who had come directly from the community and whose reason for consultation and hospitalisation was diarrhoea. On the other hand, patients hospitalised in our context were children admitted to the paediatric ward for other illnesses and who subsequently developed diarrhoea. Because of its strong impact on the occurrence of CDI, patients' history of antibiotic treatment was defined as a risk factor and monitored during the survey.

- **Molecular detection of the TcdA and TcdB genes in *Clostridium difficile*.**

  - **Extraction of the tcdA and tcdB genes**

Double-stranded DNA was isolated from anonymised stool samples obtained from patients in the medical paediatrics department of Treichville University Hospital, Abidjan. The extraction of genomic DNA from stools, was performed according to the protocol of the "Quick-DNA™ Fecal/Soil Microbe Miniprep" kit from ZYMO Research. This is a kit designed for the simple and rapid isolation of inhibitor-free, PCR-grade DNA from a variety of faecal (including human, bird, rat, mouse, cattle, etc.) and soil samples. In our context, the kit successfully isolated DNA from Gram-positive bacteria in faecal samples.

  - **Amplification method for tcdA and tcdB genes**

Target sequences were amplified, from previously isolated DNA (10 ng) added to a final reaction volume of 50 µl. The reaction mix contained in addition to 1 x buffer; 0.01 µmoL Taq polymerase (New England Biolabs, Ipswich, MA), 2 mmol/L $MgCl_2$ (Life Technologies, Carlsbad, CA), 8 µmol/L DMSO, 2 mmol/L each dNTP (New England Biolabs), 0.1 U/µL Taq polymerase (New England Biolabs), 0.3 µmol/L each primer (tcdA, tcdB). To optimise PCR, DMSO was added to the mix, which improved template denaturation by facilitating the breaking of

hydrogen bonds connecting the 2 strands of the DNA molecule. The primer sequences used were :

tcdA: F-5'-CTGGAGAATCTATTTGTAG-3', R-5'-GCAGTTGATACTAATTCAAC-3'; tcdB F-5'-GCATGATAAGGCAACTTCAGTGGTA-3', R-5-'AGTTCCTCCTGCTCCATCAAATG-3'. After an initial 5-minute heating step at 94˚C, the samples were amplified for 40 cycles of 30 seconds at 94˚C, 30 seconds at 65˚C, 53˚C for 30 seconds (decreasing by 0.5˚C per cycle and held constant thereafter), and 72˚C for 1 minute; the samples were then held at 72˚C for 5 minutes for a final extension step. Target sequences were amplified using a conventional thermal cycler (Bio-Rad). PCR products, either. Amplicons, were analysed using 1% agarose gel electrophoresis with ethidium bromide and imaged using the Gel Doc Imager.

- **Statistical analysis**

The statistical analysis was carried out using Epi/Info 6.2 (CDC) software to compare the independence of the means of the qualitative variables using the chi-square test (χ^2). Fisher's exact probability test was also used with this software to test the independence of the quantitative variables studied.

3. Results

- **Epidemiological characteristics (Table 1)**

Out of a study population of 36 patients who agreed to take part in the survey, the majority were male (n = 21) with a proportion of 58% and a sex ratio (M/F) = 1.4. Apart from gender, the age group (2 - 12 months) was the most important, with 19 patients, or 53%. The minimum and maximum ages were 2 and 180 months respectively. The mean age was 30 months and the median age 12 months. Significantly, most of the patients (n = 22) eligible for the study came from the community or (61%) (Table 1). One of the risk factors most consistently associated with the occurrence of CDI was antibiotic therapy. The class of antibiotics most commonly used by the respondents was the beta-lactam class, in particular cefixime, the combination of amoxicillin and clavulanic acid, and fucloxacillin. The use of antibiotics such as metronidazoles and 5-nitrofurans was also observed during this study.

Table 1. Distribution of the TcdB gene according to socio-demographic risk factors and history of antibiotic treatment.

Socio-demographic characteristics	TcdB gene		P value
	Positive (n = 3)	Negative (n = 33)	
Gender			
Male (n = 21)	2 (5%)	19 (53%)	0.06
Female (n = 15)	1 (3%)	14 (39%)	
Age			
2 - 12 years (n = 19)	(2) 6%	(17) 47%	0.60

Continued

13 - 24 year olds (n = 9)	**(1) 3%**	**(8) 22%**	**0.60**
24 - 180 years (n = 8)	0(00)	(8)22%	
How patients are admitted			
Community (n = 22)	1 (3%)	21 (58%)	0.52
Hospitalized (n = 14)	2 (6%)	12 (33%)	
Prevalence of toxin B according to antibiotic use			
With previous history Antibiotic (n = 24)	2 (6%)	22 (61%)	0.65
No history of antibiotics (n = 12)	1 (3%)	11 (30%)	

- **Molecular detection of genes** (tcdB and tcdA) **for *Clostridium difficile* toxins A and B** (Table 2)

At the end of the survey, only the TcdB gene for *Clostridium difficile* toxin B was detected in 03 patients (8.3%) (Table 1). No toxin A gene (TcdA gene) was detected in the stools of children presenting with diarrhoea.

Identifying the risk factors for CDI is a major challenge in reducing the incidence of these infections. The main risk factor for developing CDI is antibiotics, which alter the intestinal flora. However, in our context, with a prevalence of 8.3% for toxin B, children with no history of antibiotic treatment accounted for a third (1/3) of positive cases. The other risk factors studied, either sex, age and mode of admission of patients, did not reveal any of the aforementioned factors in the risk of CDI after statistical analysis of the data collected.

Table 2. Distribution of the presence of the TcdB gene according to antibiotic use.

Antibiotic	Frequency	Presence of TcdB gene	p value
5-Nitro-furan	2	0	
Metronidazole	7	1	
Amoxicillin + clavulanic acid	8	0	0.65
Fucloxacillin	3	1	
Cefixime	4	0	

The TcdB gene was mainly detected in 02 children with a medical history of metronidazole and flucloxacillin (Table 2).

4. Discussion

Following molecular detection of virulence genes (tcdA and tcdB) in *C. difficile* strains isolated from diarrhoeal stools, only the tcdB gene was identified in three children. The strains identified were A/B^{-+} variants. It should be remembered that according to the results of several previous studies on the genetics of *C. difficile,* the strains (A/B^{-+}) corresponded to a toxigenic variant of *C. difficile.* They were

characterised by deletions, insertions or polymorphic restriction sites in one or more sequences of the Paloc gene which no longer produced detectable toxin A [13] [14]. Among the many studies that have reported on these variants, mention should be made of work on the characterisation of the strain (A/B^{-+}) of *C. difficile* responsible for nosocomial outbreaks [15], the virulence of strains (A/B^{-+}) of *C. difficile* [16] emergence of *C. difficile* strains (A/B^{-+}): epidemiological and *clinical* considerations [17] characterisation of polymorphisms in the *C. difficile* A and B toxin genes [13] [15]-[17]. *On the* other hand, it has to be said that the identification of strains (A/B^{++}) was more frequent in most studies. While in the past, due to the mechanism of synergistic action, all toxigenic strains associated with the disease were considered to produce both toxin A and B, to date, the virulence of strains (A/B^{-+}) has been widely reported in several studies [16]-[18]. Today, it is well documented that the (A/B^{-+}) variants are responsible for the same spectrum of diseases associated with the (A/B^{++}) strain [16] [19]. This may be due to the concordant data from several in vitro studies indicating that toxin B is a highly potent cytotoxin capable of inducing toxicity 1000 times greater than that of toxin A in experimental colonic implants [19]-[21]. In addition, four studies in hamster and mouse infection models indicated that the tcdB gene was capable of inducing pathological phenotypes in the absence of tcdA [22]. At this level, it is also important to note that the genes (tcdA and tcdB) are normally located on the same Paloc [23]. Consistent with the notion that the tcdB gene is independently capable of causing disease, a considerable number of clinical isolates of *C. difficile* expressing only the tcdB gene have been reported. For example, the virulence of the PCR 017 ribotype pathogenicity, clearly identified in several epidemic situations [17] [24]. In addition, several multicentre studies have highlighted the clinical virulence of isolates (A/B^{-+}) in epidemics of *C.difficile* diarrhoea, sporadic cases of infection and cases of pseudomembranous colitis (PMC) caused by the *C. difficile* variant (A/B^{-+}) [25]-[27]. Far from being an isolated study, the results (8.6%) of the present study are consistent with other studies of variants (A/B^{-+}) in several countries with varying prevalence [28]. In France, rates of 3% (A/B^{-+}) have been reported in 25 different Paris hospitals [29]. Subsequently, in 2012, rates of 1.26% (1/80) (A^{-}/B^{+}) were reported in Serbia [30]. Recently, in a comparative multicentre study of 593 controls and 608 patients with diarrhoea in communities in Germany, Ghana, Tanzania and Indonesia, 18% (6/33) of isolates from Indonesia were reported (A^{-}/B^{+}) [28]. In Iran, out of 538 *C. difficile* isolates, 169 (31.41%) were A/B^{-+} [31]. From 2006 to 2018, in Thailand, rates of 32% were reported in a multicentre study where 46 of 145 *C.* difficile isolates from approximately 13 provinces were A/B^{-+} [32]. Higher rates of (A^{-}/B^{+}) of 39% were described in a Japanese study [33]. In addition, an Israeli study documented *C. difficile* rates (A/B^{-+}) of 56% [34]. A study carried out in Argentina showed that strains (A/B^{-+}) completely replaced strains (A/B^{++}) over a four-year period, with isolates (A/B^{-+}) rising from 12.5% in 2000 to 97.9% in 2003 [34].

Interestingly, despite the increasing incidence of *C. difficile* infections worldwide and the high mortality rate associated with diarrhoea in children, statistical

analysis (p = 0.06) of the results of the present study failed to establish a correlation between the occurrence of CDI and gender. In fact, the distribution of positive cases was as follows: 6% for males and 3% for females. There appears to be heterogeneity in the distribution of the tcdB gene independently of sex. Sex cannot therefore be considered a major risk factor in the development of CDI. From 2003 to 2014, a similar study focusing on age and sex differences and based on trends in *C.* difficile-related hospitalisations in Madrid (Spain) over a 12-year period was unable to establish a relationship in favour of a sex-related risk factor [35]. However, this study reported a gradient of infection rates across age groups as in our study. In our study, two positive cases were observed in children under 12 months of age, which represented half of the samples. For the 13 to 24 months and 25 to 180 months age groups, the positive cases were respectively one case out of nine (9) and zero cases out of eight (8) samples. It was found that the age variable did not influence the risk of CDI in the paediatric population (p = 0.65). It should be noted that the paediatric population, unlike adults, is generally characterised by a high asymptomatic carriage (20 - 70%) of *C. difficile* [36]. Some authors explain this by the fact that in infants, the absence of toxin receptors on the surface of their colonocytes inhibits the internalisation of the toxins that cause colonic lesions [37] [38]. Like age in the present study, statistical analysis of the mode of admission indicated that this was not a risk factor in the occurrence of CDI. In fact, the positive results for patients from the community were 3%, compared with 6% for hospitalised patients. In 2016, at the end of his work on *C. difficile* infection, Wiep Kaas Smits explained that due to exposure to a hospital environment, comorbidities and/or antibiotic therapy, hospitalised patients generally had a much higher rate of colonisation than people from the community [39]. He also reported that an increase in the rate of asymptomatic colonisation by toxigenic isolates in patients at the time of hospital admission was observed without, however, inducing a real impact on the occurrence of CDI [39]. This lack of trend indicates that the mode of admission was not a risk factor in the occurrence of CDI. These results are consistent with the data from the meta-analysis by Zacharioudakis *et al.* 2015 in PubMed and EMBASE, on the role of mode of admission in CDI [40].

In addition to the mode of admission, one of the risk factors most regularly associated with the occurrence of CDI was antibiotic therapy [41]. However, in our study, we were unable to establish a statistical correlation between virulence gene detection and antibiotic therapy. Among the diarrhoea patients selected, the positive cases were as follows: 6% positive cases in patients on antibiotic therapy compared with 3% negative cases in children without antibiotics. The class of antibiotics used by the patients consisted mainly of beta-lactams. Antibiotics such as metronidazole and nitroimidazole were also used. In this group, it should be noted that detection of the TcdB gene was observed mainly in two children with a medical history of metronidazole and fucloxacillin. During the course of the survey, the presence of traditional medicines in the medical histories preceding the admission

of certain patients was also noted. This lack of relationship between the occurrence of CDI in children and antibiotic therapy could be explained by the very low exposure to antibiotics compared to the elderly [42]. Other authors, referring to paediatric immunity, have even indicated that the titres of *C. difficile* toxin found in the stools of healthy infants are similar to those found in adults with *C. difficile-associated* diarrhoea [43] [44]. The mechanism of protection of infants against *C. difficile is* not clearly established, but a number of theories have been proposed, including the absence of toxin receptors on the surface of intestinal cells, as well as the protective action of breast milk and the defence provided by other neonatal intestinal flora [43] [45]. In an experimental study on a neonatal rabbit ileum model, the researchers found that there were no binding sites for toxin A on the cell surface. Furthermore, even with maximum concentrations of toxin A applied to the ileum cells of neonatal and young rabbits, the effects were minimal compared with the severe mucosal damage caused in adult rabbits [45].

5. Limitations

There were a number of limitations to the study. Due to the rapid discharge of patients, we were unable to follow up all patients with toxigenic *Clostridium difficile in* their faeces. Despite the help of the supervisors, the lack of financial support for the study was also a major limitation in the acquisition of the reagents and small equipment needed for the work. The small sample size did not sufficiently reflect the prevalence of CDI in the paediatric population. Although widely recognised as the most appropriate current standard approach, the development of a conventional PCR for the detection of TcdA and TcdB was much more time-consuming and remains very difficult.

6. Conclusions

Molecular analysis of the faeces of children with diarrhoea detected a single *C. difficile* toxin gene, toxin B (TcdB), with a prevalence of 8.3%. The toxin A gene, TcdA, was not detected in the stools. The *C. difficile* strains obtained were described as A/B^{-+} variants. Positive cases were slightly more prevalent in males (6%) than in females (3%). The age group of children was predominantly between 2 and 12 months, with 53% of patients. Community patients accounted for 58% of cases, compared with 42% of hospital patients. The antibiotics associated with the detection of TcdB genes were flucloxacillin and metronidazole. Finally, with the circulation of virulent strains, it is important to monitor the carriage of *C. difficile in* order to improve the management of CDI.

State of knowledge on the subject

- The incidence of morbidity and mortality from clostridium difficile infections in childhood diarrhoeal syndromes is well known in Western countries;
- The limitations of culture-based diagnostic methods and rapid diagnostic tests are well known;
- The role of antibiotics as a risk factor in the occurrence of clostridium difficile

infections is also regularly reported.
- Molecular biology is the appropriate diagnostic tool for clostridium difficile infections.

Our Study's Contribution to Knowledge

- The incidence rate of clostridium difficile infections in the study population is now known: 8.3%.
- The variant of clostridium difficile reported in diarrhoeal syndromes in this study is the strain (A^-/B^+).
- The antibiotics associated with the detection of TcdB genes were flucloxacillin and metronidazole.

Authors' Contributions

Each author has contributed by reading and correcting the article. Each has undertaken to contribute financially as soon as the article is accepted. All authors have read and approved the final version of the manuscript.

Acknowledgements

We would like to thank all the authors who contributed to the reading and correction of this article, both in terms of content and form, as well as for the analysis of the data using epi-info. We would also like to thank all the staff of the medical paediatrics department of the CHU of Treichville and the technical staff of the Institut Pasteur de Côte d'Ivoire for having supervised the 6-month survey.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Czepiel, J., Dróżdż, M., Pituch, H., Kuijper, E.J., Perucki, W., Mielimonka, A., *et al.* (2019) Clostridium difficile Infection: Review. *European Journal of Clinical Microbiology & Infectious Diseases*, **38**, 1211-1221. https://doi.org/10.1007/s10096-019-03539-6

[2] Dingle, K.E., Griffiths, D., Didelot, X., Evans, J., Vaughan, A., Kachrimanidou, M., *et al.* (2011) Clinical Clostridium Difficile: Clonality and Pathogenicity Locus Diversity. *PLOS ONE*, **6**, e19993. https://doi.org/10.1371/journal.pone.0019993

[3] (2022) Clostridioides (Clostridium) Difficile Colitis: Practice Essentials, Background, Pathophysiology.

[4] Sartelli, M., Malangoni, M.A., Abu-Zidan, F.M., Griffiths, E.A., Di Bella, S., McFarland, L.V., *et al.* (2015) WSES Guidelines for Management of Clostridium Difficile Infection in Surgical Patients. *World Journal of Emergency Surgery*, **10**, Article 38.

[5] Lessa, F.C., Mu, Y., Bamberg, W.M., Beldavs, Z.G., Dumyati, G.K., Dunn, J.R., *et al.* (2015) Burden of *Clostridium difficile* Infection in the United States. *New England Journal of Medicine*, **372**, 825-834. https://doi.org/10.1056/nejmoa1408913

[6] Bagdasarian, N., Rao, K. and Malani, P.N. (2015) Diagnosis and Treatment of *Clos-*

*tridium difficile*in Adults: A Systematic Review. *JAMA*, **313**, 398-408. https://doi.org/10.1001/jama.2014.17103

[7] Luna, R.A., Boyanton, B.L., Mehta, S., Courtney, E.M., Webb, C.R., Revell, P.A., *et al.* (2011) Rapid Stool-Based Diagnosis of *Clostridium difficile* Infection by Real-Time PCR in a Children's Hospital. *Journal of Clinical Microbiology*, **49**, 851-857. https://doi.org/10.1128/jcm.01983-10

[8] Tenover, F.C., Baron, E.J., Peterson, L.R. and Persing, D.H. (2011) Laboratory Diagnosis of *Clostridium difficile* Infection: Can Molecular Amplification Methods Move Us Out of Uncertainty? *The Journal of Molecular Diagnostics*, **13**, 573-582. https://doi.org/10.1016/j.jmoldx.2011.06.001

[9] Wilcox, M.H. (2010) Point-Counterpoint: What Is the Current Role of Algorithmic Approaches for Diagnosis of Clostridium Difficile Infection? *Journal of Clinical Microbiology*, **48**, 4347-4353. https://doi.org/10.1128/JCM.02028-10

[10] Becker, S.L., Chatigre, J.K., Coulibaly, J.T., Mertens, P., Bonfoh, B., Herrmann, M., *et al.* (2015) Molecular and Culture-Based Diagnosis of *Clostridium difficile* Isolates from Côte d'ivoire after Prolonged Storage at Disrupted Cold Chain Conditions. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, **109**, 660-668. https://doi.org/10.1093/trstmh/trv071

[11] Ngamskulrungroj, P., Sanmee, S., Putsathit, P., Piewngam, P., Elliott, B., Riley, T.V., *et al.* (2015) Correction: Molecular Epidemiology of *Clostridium difficile* Infection in a Large Teaching Hospital in Thailand. *PLOS ONE*, **10**, e0134771. https://doi.org/10.1371/journal.pone.0134771

[12] Shoaei, P., Shojaei, H., Khorvash, F., Hosseini, S.M., Ataei, B., Tavakoli, H., *et al.* (2019) Molecular Epidemiology of Clostridium Difficile Infection in Iranian Hospitals. *Antimicrobial Resistance & Infection Control*, **8**, Article No. 12. https://doi.org/10.1186/s13756-018-0454-6

[13] Rupnik, M. and Janezic, S. (2016) An Update on *Clostridium difficile* Toxinotyping. *Journal of Clinical Microbiology*, **54**, 13-18. https://doi.org/10.1128/jcm.02083-15

[14] Eckert, C., Emirian, A., Le Monnier, A., Cathala, L., De Montclos, H., Goret, J., *et al.* (2015) Prevalence and Pathogenicity of Binary Toxin-Positive *Clostridium difficile* Strains That Do Not Produce Toxins a and B. *New Microbes and New Infections*, **3**, 12-17. https://doi.org/10.1016/j.nmni.2014.10.003

[15] Alfa, M.J., Kabani, A., Lyerly, D., Moncrief, S., Neville, L.M., Al-Barrak, A., *et al.* (2000) Characterization of a Toxin A-Negative, Toxin B-Positive Strain of *Clostridium difficile* Responsible for a Nosocomial Outbreak of *Clostridium difficile*-Associated Diarrhea. *Journal of Clinical Microbiology*, **38**, 2706-2714. https://doi.org/10.1128/jcm.38.7.2706-2714.2000

[16] Drudy, D., Fanning, S. and Kyne, L. (2007) Toxin A-Negative, Toxin B-Positive *Clostridium difficile*. *International Journal of Infectious Diseases*, **11**, 5-10. https://doi.org/10.1016/j.ijid.2006.04.003

[17] King, A.M., Mackin, K.E. and Lyras, D. (2015) Emergence of Toxin A-Negative, Toxin B-Positive *Clostridium difficile* Strains: Epidemiological and Clinical Considerations. *Future Microbiology*, **10**, 1-4. https://doi.org/10.2217/fmb.14.115

[18] Rupnik, M. (2001) How to Detect *Clostridium difficile* Variant Strains in a Routine Laboratory. *Clinical Microbiology and Infection*, **7**, 417-420. https://doi.org/10.1046/j.1198-743x.2001.00290.x

[19] Skinner, A.M., Phillips, S.T., Merrigan, M.M., O'Leary, K.J., Sambol, S.P., Siddiqui, F., *et al.* (2020) The Relative Role of Toxins a and B in the Virulence of *Clotridioides difficile*. *Journal of Clinical Medicine*, **10**, Article 96. https://doi.org/10.3390/jcm10010096

[20] Vedantam, G., Clark, A., Chu, M., McQuade, R., Mallozzi, M. and Viswanathan, V.K. (2012) *Clostridium difficile* Infection. *Gut Microbes*, **3**, 121-134. https://doi.org/10.4161/gmic.19399

[21] Di Bella, S., Ascenzi, P., Siarakas, S., Petrosillo, N. and Di Masi, A. (2016) *Clostridium difficile* Toxins a and B: Insights into Pathogenic Properties and Extraintestinal Effects. *Toxins*, **8**, Article 134. https://doi.org/10.3390/toxins8050134

[22] Ferreira, C.E., Nakano, V., Durigon, E.L. and Avila-Campos, M.J. (2003) Prevalence of Clostridium Spp. and *Clostridium difficile* in Children with Acute Diarrhea in São Paulo City, Brazil. *Memórias do Instituto Oswaldo Cruz*, **98**, 451-454. https://doi.org/10.1590/s0074-02762003000400003

[23] López-Ureña, D., Orozco-Aguilar, J., Chaves-Madrigal, Y., Ramírez-Mata, A., Villalobos-Jimenez, A., Ost, S., *et al.* (2019) Toxin B Variants from *Clostridium difficile* Strains VPI 10463 and NAP1/027 Share Similar Substrate Profile and Cellular Intoxication Kinetics but Use Different Host Cell Entry Factors. *Toxins*, **11**, Article 348. https://doi.org/10.3390/toxins11060348

[24] Lyras, D., O'Connor, J.R., Howarth, P.M., Sambol, S.P., Carter, G.P., Phumoonna, T., *et al.* (2009) Toxin B Is Essential for Virulence of *Clostridium difficile*. *Nature*, **458**, 1176-1179. https://doi.org/10.1038/nature07822

[25] Janezic, S., Dingle, K., Alvin, J., Accetto, T., Didelot, X., Crook, D.W., *et al.* (2020) Comparative Genomics of *Clostridioides difficile* Toxinotypes Identifies Module-Based Toxin Gene Evolution. *Microbial Genomics*, **6**, e000449. https://doi.org/10.1099/mgen.0.000449

[26] Vitucci, J.C., Pulse, M., Tabor-Simecka, L. and Simecka, J. (2020) Epidemic Ribotypes of *Clostridium* (Now *Clostridioides*) *difficile* Are Likely to Be More Virulent than Non-Epidemic Ribotypes in Animal Models. *BMC Microbiology*, **20**, Article No. 27. https://doi.org/10.1186/s12866-020-1710-5

[27] McDonald, L.C., Gerding, D.N., Johnson, S., Bakken, J.S., Carroll, K.C., Coffin, S.E., *et al.* (2018) Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clinical Infectious Diseases*, **66**, e1-e48. https://doi.org/10.1093/cid/cix1085

[28] Norén, T. (2010) *Clostridium difficile* and the Disease It Causes. In: Mullany, P. and Roberts, A., Eds., *Methods in Molecular Biology*, Humana Press, 9-35. https://doi.org/10.1007/978-1-60327-365-7_2

[29] Collins, D.A., Sohn, K.M., Wu, Y., Ouchi, K., Ishii, Y., Elliott, B., *et al.* (2019) *Clostridioides difficile* Infection in the Asia-Pacific Region. *Emerging Microbes & Infections*, **9**, 42-52. https://doi.org/10.1080/22221751.2019.1702480

[30] Seugendo, M., Janssen, I., Lang, V., Hasibuan, I., Bohne, W., Cooper, P., *et al.* (2018) Prevalence and Strain Characterization of *Clostridioides* (*Clostridium*) *difficile* in Representative Regions of Germany, Ghana, Tanzania and Indonesia—A Comparative Multi-Center Cross-Sectional Study. *Frontiers in Microbiology*, **9**, Article ID: 1843. https://doi.org/10.3389/fmicb.2018.01843

[31] Barbut, F., Lalande, V., Burghoffer, B., Thien, H.V., Grimprel, E. and Petit, J. (2002) Prevalence and Genetic Characterization of Toxin a Variant Strains of *Clostridium difficile* among Adults and Children with Diarrhea in France. *Journal of Clinical Microbiology*, **40**, 2079-2083. https://doi.org/10.1128/jcm.40.6.2079-2083.2002

[32] Stojanovic, P., Kocic, B., Stojanovic, M., Miljkovic-Selimovic, B., Tasic, S., Miladinovic-Tasic, N., *et al.* (2012) Clinical Importance and Representation of Toxigenic and Non-Toxigenic Clostridium Difficile Cultivated from Stool Samples of Hospital-

ized Patients. *Brazilian Journal of Microbiology*, **43**, 215-223. https://doi.org/10.1590/s1517-83822012000100023

[33] Mohammadbeigi, M., Safayi Delouyi, Z., Mohammadzadeh, N., Ala'almohadesin, A., Taheri, K., Edalati, E., *et al.* (2019) Prevalence and Antimicrobial Susceptibility Pattern of Toxigenic *Clostridium difficile* Strains Isolated in Iran. *Turkish Journal of Medical Sciences*, **49**, 384-391. https://doi.org/10.3906/sag-1808-11

[34] Imwattana, K., Wangroongsarb, P. and Riley, T.V. (2019) High Prevalence and Diversity of *tcdA*-Negative and *tcdB*-Positive, and Non-Toxigenic, *Clostridium difficile* in Thailand. *Anaerobe*, **57**, 4-10. https://doi.org/10.1016/j.anaerobe.2019.03.008

[35] Komatsu, M., Kato, H., Aihara, M., Shimakawa, K., Iwasaki, M., Nagasaka, Y., *et al.* (2003) High Frequency of Antibiotic-Associated Diarrhea Due to Toxin A-Negative, Toxin B-Positive *Clostridium difficile* in a Hospital in Japan and Risk Factors for Infection. *European Journal of Clinical Microbiology & Infectious Diseases*, **22**, 525-529. https://doi.org/10.1007/s10096-003-0992-5

[36] Esteban-Vasallo, M.D., Naval Pellicer, S., Domínguez-Berjón, M.F., Cantero Caballero, M., Asensio, Á., Saravia, G., *et al.* (2016) Age and Gender Differences in *Clostridium difficile*-Related Hospitalization Trends in Madrid (Spain) over a 12-Year Period. *European Journal of Clinical Microbiology & Infectious Diseases*, **35**, 1037-1044. https://doi.org/10.1007/s10096-016-2635-7

[37] Eckert, C., Lalande, V. and Barbut, F. (2011) Diagnostic des infections à *Clostridium difficile*. *Journal des Anti-Infectieux*, **13**, 67-73. https://doi.org/10.1016/j.antinf.2011.03.004

[38] Antonara, S. and Leber, A.L. (2016) Diagnosis of *Clostridium difficile* Infections in Children. *Journal of Clinical Microbiology*, **54**, 1425-1433. https://doi.org/10.1128/jcm.03014-15

[39] Nibbering, B., Gerding, D.N., Kuijper, E.J., Zwittink, R.D. and Smits, W.K. (2021) Host Immune Responses to Clostridioides Difficile: Toxins and Beyond. *Frontiers in Microbiology*, **12**, Article ID: 804949. https://doi.org/10.3389/fmicb.2021.804949

[40] Smits, W.K., Lyras, D., Lacy, D.B., Wilcox, M.H. and Kuijper, E.J. (2016) *Clostridium difficile* Infection. *Nature Reviews Disease Primers*, **2**, Article No. 16020. https://doi.org/10.1038/nrdp.2016.20

[41] Zacharioudakis, I.M., Zervou, F.N., Pliakos, E.E., Ziakas, P.D. and Mylonakis, E. (2015) Colonization with Toxinogenic C. Difficile Upon Hospital Admission, and Risk of Infection: A Systematic Review and Meta-Analysis. *American Journal of Gastroenterology*, **110**, 381-390. https://doi.org/10.1038/ajg.2015.22

[42] Hensgens, M.P.M., Goorhuis, A., Dekkers, O.M. and Kuijper, E.J. (2011) Time Interval of Increased Risk for *Clostridium difficile* Infection after Exposure to Antibiotics. *Journal of Antimicrobial Chemotherapy*, **67**, 742-748. https://doi.org/10.1093/jac/dkr508

[43] Lalande, V., Barbut, F. and Petit, J. (2004) Diagnostic bactériologique des infections liées à *Clostridium difficile*. *Revue Française des Laboratoires*, **2004**, 57-63. https://doi.org/10.1016/s0338-9898(04)80026-x

[44] Crobach, M.J.T., Vernon, J.J., Loo, V.G., Kong, L.Y., Péchiné, S., Wilcox, M.H., *et al.* (2018) Understanding *Clostridium difficile* Colonization. *Clinical Microbiology Reviews*, **31**, e00021-17. https://doi.org/10.1128/cmr.00021-17

[45] Upton, D.A. (2014) *Clostridium difficile* in Paediatric Populations. *Paediatrics & Child Health*, **19**, 43-48. https://doi.org/10.1093/pch/19.1.43