

Review

***Clostridium difficile*: a problem of concern in developed countries and still a mystery in Latin America**I. T. Balassiano,^{1,2} E. A. Yates,³ R. M. C. P. Domingues⁴ and E. O. Ferreira⁴

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Clostridium difficile-associated disease (CDAD) is caused by a spore-forming bacterium and can result in highly variable disease, ranging from mild diarrhoea to severe clinical manifestations. Infections are most commonly seen in hospital settings and are often associated with on-going antibiotic therapy. Incidences of CDAD have shown a sustained increase worldwide over the last ten years and a hypervirulent *C. difficile* strain, PCR ribotype 027/REA type BI/North American pulsed-field (NAP) type 1 (027/BI/NAP-1), has caused outbreaks in North America and Europe. In contrast, only a few reports of cases in Latin America have been published and the hypervirulent strain 027/BI/NAP-1 has, so far, only been reported in Costa Rica. The potential worldwide spread of this infection calls for epidemiological studies to characterize currently circulating strains and also highlights the need for increased awareness and vigilance among healthcare professionals in currently unaffected areas, such as Latin America. This review attempts to summarize reports of *C. difficile* infection worldwide, especially in Latin America, and aims to provide an introduction to the problems associated with this pathogen for those countries that might face outbreaks of epidemic strains of *C. difficile* for the first time in the near future.

Context

During the decades since the discovery of *Clostridium difficile*, lessons have been learned about the pathophysiology, epidemiology and management of *C. difficile* associated disease (CDAD). However, many challenges still remain. Presently, *C. difficile* is the most common cause of hospital-acquired infectious diarrhoea in the developed world and has re-emerged in recent years with higher incidence rates and a greater severity of condition. Today, *C. difficile* is recognized as the causative agent of a broad spectrum of intestinal diseases, ranging from mild self-limiting diarrhoea to more serious and potentially life-threatening manifestations, such as pseudomembranous colitis (PMC) and toxic megacolon (Carter *et al.*, 2007). In hospitals in North America and Europe, the number of CDAD cases has increased each year since 2000.

In contrast, very little is known about cases of *C. difficile* infection occurring in Latin American countries, since there are only a few published studies. What is known is that diarrhoeal disease is a major cause of morbidity in developing countries and causes 12 600 deaths in children

each day in Latin America. This review summarizes the current information about *C. difficile* pathogenesis and disease, and attempts to put the studies concerning this micro-organism in Latin America into perspective.

Infections associated with *C. difficile*

Previously, it was thought that *C. difficile* could be a commensal micro-organism, especially considering its presence in the faecal flora of new-born infants. Today, it is known that the acquisition of this bacterium occurs frequently in hospitals, where it is commonly isolated from surfaces that people come into contact with and from the hands and clothes of medical staff dealing directly with *C. difficile*-infected patients (Poutanen & Simor, 2004). It has also been shown that hospitalization and antibiotic therapy are directly related to an increased chance of acquiring an infection caused by *C. difficile* (Kyne *et al.*, 2002). How does the presence of an antibiotic contribute to the emergence of disease? Some studies have shown that the colonization of *C. difficile* and establishment of the associated disease is caused by the suppression of the microbial flora during and after

antibiotic therapy. In fact, healthy adults with a balanced flora are usually resistant to *C. difficile* colonization. However, once antibiotic therapy starts, a disturbance in the intestinal niche may occur, facilitating *C. difficile* infection (Poutanen & Simor, 2004). Although all classes of antibiotics can cause this problem, those most likely to do so are broad-spectrum antibiotics, such as clindamycin, penicillins, cephalosporin and fluoroquinolones (Schroeder, 2005; Hookman & Barkin, 2009; O'Connor *et al.*, 2009).

Once the disruption occurs and the individual is exposed to exogenous *C. difficile* spores, the spores start to germinate and the vegetative cells multiply (Schroeder, 2005). It is then that *C. difficile* can penetrate the mucus layer in the intestinal tract by secreting proteases, and can adhere to enterocytes using its repertoire of adhesins, resulting in the first stage of colonization (Denève *et al.*, 2009). The second stage of the infection is established after the bacteria starts to produce its main virulence factors, the TcdA and TcdB toxins. Their importance in the process will be explained in more detail later.

It is important to emphasize that not all colonized individuals develop CDAD, because the pathogenicity of this bacterium is directly related to the expression of its virulence factors and the strength of the host immune system (Poutanen & Simor, 2004; Schroeder, 2005; Hookman & Barkin, 2009).

Many researchers have pointed out that incidences of CDAD among the elderly (over 65 years of age) are up to ten times higher than among younger adults and that attributable mortality increases with age in this population, especially in an ICU setting (Graf *et al.*, 2009; Gravel *et al.*, 2009; Marcon *et al.*, 2006). This is generally thought to reflect a failure in the immune system, known as senescence of the immune response, resulting from comorbidities and normal age-related changes. This phenomenon, which is associated with T-cell alterations, may explain unresponsiveness to *C. difficile* toxins (Leclair *et al.*, 2010; Mitty, 2009).

The clinical manifestations of infectious CDAD can vary from asymptomatic, through mild infectious manifestations, such as *C. difficile*-associated diarrhoea, to severe conditions, such as PMC and toxic megacolon (an acute inflammation of the colon, which causes swelling and possible perforation of the intestinal lining), which are considered the most severe forms of the infection (Loo *et al.*, 2005; Rupnik *et al.*, 2009).

***C. difficile* virulence factors and their relation to disease**

Several virulence factors have been described in *C. difficile*, such as fimbriae (Borriello *et al.*, 1988), flagella (Eveillard *et al.*, 1993), proteolytic enzymes (Borriello *et al.*, 1990) and surface layer proteins (Bianco *et al.*, 2011), all of which contribute to the establishment of disease at different stages during the process of infection.

One of the factors that contribute to the dynamic nature of the infection and the transmission of *C. difficile* is its capacity to produce spores. In fact, the bacterium can persist for months in hospital environments because of this (Poutanen & Simor, 2004; Rupnik *et al.*, 2009). Previously, it has been demonstrated that when *C. difficile* strains are exposed, *in vitro*, to subinhibitory concentrations of non-chloride disinfectants (detergents and hydrogen peroxide, for example) its capacity to sporulate increases (Wilcox & Fawley, 2000; Fawley *et al.*, 2007; Wheeldon *et al.*, 2008).

Another relevant characteristic is the resistance of some *C. difficile* strains to certain antibiotics. Mobile genetic elements, chromosomal markers and mutations that could confer resistance to macrolides, lincosamide, spretogramine, tetracycline, chloranfenicol, rifampicin and fluoroquinolones were also found in some strains. For this reason, these strains are responsible for most of the *C. difficile* outbreaks worldwide (Huang *et al.*, 2009; O'Connor *et al.*, 2009).

Although it is well documented that these virulence factors might contribute to the pathogenicity of *C. difficile*, the major virulence factors are the toxins TcdA and TcdB. These toxins have monoglycosyl transferase activity, mediating the glycosylation of proteins from the Rho family of GTPases (Rho, Rac and Cdc42), which normally bind to guanosine triphosphate (GTP) and are involved in signal translation and the regulation of actin filaments in the host cell (Dillon *et al.*, 1995; Just *et al.*, 1995; McFarland, 2009). As a consequence, the cytoskeleton becomes disorganized, causing roundness and opening the GAP junctions of the epithelium, which are responsible for maintaining tissue integrity (Hall, 1998; O'Connor *et al.*, 2009). Altogether, these events cause a massive secretion of fluids, followed by diarrhoea (McFarland, 2009). *In vitro* experiments using cell cultures have shown that this phenomenon is followed by detachment of the cell monolayer (Hall, 1998).

All virulent strains of *C. difficile* carry a pathogenicity locus named PaLoc (19.6 kb), corresponding to *tcdR*, *tcdB*, *tcdE*, *tcdA* and *tcdC* genes (Cohen *et al.*, 2000; O'Connor *et al.*, 2009). The *tcdA* and *tcdB* genes encode the toxins TcdA and TcdB, respectively. The *tcdE* gene encodes a holin-type protein that helps release TcdA and TcdB toxins from the bacterial cell because these proteins do not possess a signal peptide (Tan *et al.*, 2001). The *tcdR* gene encodes the TcdR protein, which acts as a sigma factor, attaching to RNA polymerase and stimulating *tcdR*, *tcdB*, *tcdE* and *tcdA* gene translation. Conversely, the *tcdC* gene is located downstream of the other genes in the PaLoc and controls them via negative regulation (Voth & Ballard, 2005; O'Connor *et al.*, 2009).

The TcdA and TcdB enterotoxins are the main virulence factors studied in *C. difficile*, and their involvement in disease, such as *C. difficile*-associated diarrhoea, is usually referred to as toxic colitis. Aqueous diarrhoea occurs because both toxins cause the secretion of fluids with vascular permeability. The toxins also activate macrophages and

mastocytes, inducing the production of pro-inflammatory interleukins and TNF- α , leading to the formation of a pseudomembrane. Endoscopy usually reveals the presence of adherent colonies, forming ulcers with inflammation of the mucus membrane and the presence of white–yellow plate features. Histological reports show that pseudomembranes contain neutrophils, fibrin, mucin and necrotic debris (Poutanen & Simor, 2004; Schroeder, 2005). In patients with a different form of colitis, such as that associated with Crohn's disease, typical pseudomembranes are not seen (Hookman & Barkin, 2009).

During the 1990s, several studies identified TcdA-negative/TcdB-positive strains which presented different mutations in the PaLoc, resulting in the production of very low levels of TcdA (Kato *et al.*, 1998, 1999; Sambol *et al.*, 2000; Rupnik *et al.*, 2003). These strains, isolated from patients with CDAD, are clinically relevant and responsible for isolated cases and outbreaks in different countries (Alfa *et al.*, 2000; McDonald *et al.*, 2005; Drudy *et al.*, 2007; Elliott *et al.*, 2011). When it was noticed that infection with the *C. difficile* TcdA⁻/TcdB⁺ strain caused the same disease symptoms as those caused by the TcdA⁺/TcdB⁺ strain, investigations into the pathogenesis of these toxins started all over again. Recently, Lyras *et al.* (2009) created mutants with deletions in one of the toxin genes. Their observations revealed that mutants without the *tcdA* gene were still capable of causing the whole infection process in hamsters, which killed the animals. On the other hand, when mutants without the *tcdB* were tested in the same animal model, these strains were avirulent. These results, together with the clinical data of TcdA⁻/TcdB⁺ strains, which were virulent, suggest that TcdB, and not TcdA, is essential for the virulence of *C. difficile*. It is important, however, to mention that other researchers (Freeman *et al.*, 2010), using a different methodology to delete the toxin genes (ClosTron technology), could not reproduce the results of Lyras *et al.* (2009). Further studies are necessary to define precisely the role of this toxin during infection.

An additional toxin that is produced by some *C. difficile* strains and is considered to be an important virulence factor was reported by Popoff *et al.* (1988). The toxin was named binary toxin or CDT and was found to be an A–B two-component protein type that was found in 1–16 % of infected patients (Geric *et al.*, 2004; Gonçalves *et al.*, 2004; Barbut *et al.*, 2007; McFarland, 2009). CDT has no relation to TcdA or TcdB and is encoded by a specific chromosomal region, separate from PaLoc, designated CdtLoc. This locus houses the *cdtA* gene, which encodes the enzymic component CdtA, and *cdtB*, which encodes CdtB, a target-cell binding component. CdtLoc also encodes a regulator gene, *cdtR* (McFarland, 2009; Rupnik *et al.*, 2009). CdtB binds to a cell receptor (not yet identified), causing CdtA internalization. Once in the cytoplasm of the target cell, CdtA catalyses the ADP-ribosylation of actin monomers, resulting in disorganization of the cytoskeleton (Carter *et al.*, 2007).

The epidemiology of *C. difficile*

Infections associated with *C. difficile* were once considered to be sporadic, but nowadays this micro-organism has been isolated in most parts of the world. The United States and Canada have had big problems with CDAD, especially after 2002, when an increase in cases of toxic megacolon related to severe infection, resulting in high rates of mortality among elderly people was reported (Dallal *et al.*, 2002; Pépin *et al.*, 2004). However, groups that were once considered to be not at high risk, such as pregnant women and community health individuals, have now been shown to be at risk from exposure to infections associated with *C. difficile* (Hookman & Barkin, 2009).

Recently, molecular biology typing tools have allowed researchers to identify the aetiological agents associated with these epidemics. At the same time, a supposedly novel pathogen was identified and named *C. difficile* PCR ribotype 027/REA type BI/North American pulsed-field (NAP) type 1 (027/BI/NAP-1). At the time of writing, 027/BI/NAP-1 was considered to be epidemic and well distributed worldwide. Besides Canada and all states of the USA, the presence of this strain has been widely documented in Europe, including the UK, France, Germany, Italy, Denmark, Ireland, Holland, Austria, Poland, Switzerland, Norway, Belgium, Finland and Spain; in Asia, including Japan, Korea and Hong Kong; and in Australia, where a few cases have been reported (Kato *et al.*, 2007; Kuijper *et al.*, 2007; Bacci *et al.*, 2009; O'Connor *et al.*, 2009; Riley *et al.*, 2009; Rupnik *et al.*, 2009; Tae *et al.*, 2009; Baldan *et al.*, 2010; Freeman *et al.*, 2010). In Latin America, however, there has only been one publication describing the isolation of this strain in a Costa Rican hospital (Quesada-Gómez *et al.*, 2010).

Since there is little information concerning *C. difficile* infections in Latin American countries, and awareness of this emerging problem remains low, this review aims to summarize the reports published over the years, which have identified the presence of this pathogen in these few countries.

Clostridium difficile in Latin America

Although incidences of *C. difficile* infection in North America and Europe are well documented, little is known about the spread of this disease in Latin America. The most notable publication in this area, Quesada-Gómez *et al.* (2010), described, for the first time, the isolation of strain 027/BI/NAP-1 from patients with CDAD in a Costa Rican hospital. The detection of strain 027/BI/NAP-1 in a Latin American country highlights the problem of *C. difficile* dissemination around the world. It must only be a matter of time before this hypervirulent strain is detected in other countries in Latin America. In this review, we attempt to summarize the main publications of the last 10 years concerning the epidemiology of *C. difficile*, as well as studies related to CDAD in Latin American countries, that are available in PubMed and Lilacs databases. It is

important to describe the relevant studies that have been conducted in these countries.

Argentina

Fernandez Canigia *et al.* (2001) described a 15-month study using 245 stool samples obtained from 217 inpatients and 28 ambulatory patients with diarrhoea. It was found that only 6.5% (16/245) of the cases, representing 13 inpatients and three ambulatory patients without previous hospitalization, were associated with *C. difficile*. These findings are in agreement with the results of other studies concerning risk groups for the development of CDAD, which include advanced age, hospitalization, underlying diseases and antimicrobial and/or immunosuppressive therapy. Excluding one paediatric patient, the mean age of the positive cases was 72.9 years (ages ranging from 47 to 88 years). Most of the inpatients presented severe underlying disease, as opposed to outpatients, who had sinusitis, pharyngitis and polymyositis. All of the patients had a history of chemotherapy or antibiotic therapy, β -lactams being the main drug type administered (87% of the cases) followed by quinolones (60%), aminoglycosides and fluconazole (33%) and macrolides (20%).

In a 1-year study conducted with symptomatic patients from a general hospital, Legaria *et al.* (2003) observed a higher frequency of CDAD, which was detected in 36.8% (32/87) of the patients analysed. Again, the expected risk groups for CDAD development were observed. The main underlying conditions were AIDS (28%), pulmonary infections (28%) and cancer (21%). The ages of the patients ranged from 26 to 87 years, 52% of them being older than 64 years of age. The main drugs used preceding the development of symptoms were clindamycin (24% of cases), ampicillin, amoxicillin or third-generation cephalosporins (52%) and steroids or chemotherapeutic agents (20%). Interestingly, 15% (5/32) of the cases of CDAD were detected in outpatients presenting diarrhoea, highlighting the problem of community acquisition of *C. difficile*. This situation was revised by Freeman *et al.* (2010), who suggested that increased pressure on hospitals to discharge patients could cause dissemination of the bacterium and increase the circulation of strains in the community.

Goorhuis *et al.* (2009) published a retrospective study, conducted in a general hospital in Buenos Aires, analysing data concerning the frequency of CDAD from May 2000 to December 2005. The incidence rates of CDAD per 10 000 admissions were 37, in the year 2000; 84, in 2001; 67, in 2002; 43, in 2003; 48, in 2004; and 42, in 2005. Of the 131 isolates analysed in this study, 119 were identified as PCR-ribotype 017 ($tcdA^-/tcdB^+$), seven were identified as ribotype 001, four were identified as ribotype 014 and one was identified as ribotype 039. The high prevalence of ribotype 017 led the authors to conclude that not only has this *C. difficile* type gradually replaced other circulating PCR-ribotypes in this hospital, but it could also be

associated with a similar spectrum of clinical disease as that caused by $tcdA^+/tcdB^+$ strains.

Brazil

Ferreira *et al.* (2003) analysed 181 stool samples that were collected over 1 year from children (0–5 years of age) with acute diarrhoea who were admitted to three different hospitals. Samples were also collected from healthy children (without diarrhoea). The samples were screened for the presence of clostridia, including *C. difficile*. A total of 10% (18/181) of the hospitalized children were positive for species of *Clostridium*, and virtually the same result was obtained with healthy children. On the other hand, none of the children in the healthy group were positive for *C. difficile*, while 5.5% (10/181) of the hospitalized children harboured this species. The cytotoxicity of the *C. difficile* isolates was assayed on Vero cells and only one strain was considered non-toxicogenic. Molecular analyses using multiplex-PCR confirmed the absence of *tcdA* and *tcdB* genes in this strain and revealed that among the toxigenic strains, six possessed the $tcdA^+/tcdB^+$ genotype, while the other three were $tcdA^-/tcdB^+$. It is important to highlight that this was the first and only description of the presence of $tcdA^-/tcdB^+$ strains in Brazil. As previously mentioned, this group of strains has already been described in other countries, where it is considered clinically relevant, having been associated with outbreaks (Alfa *et al.*, 2000; McDonald *et al.*, 2005; Drudy *et al.*, 2007).

Another study with paediatric patients was conducted by Pinto *et al.* (2003), in which 210 faecal samples were analysed from children aged 3 months to 7 years, divided into two groups: 114 outpatients (51 with diarrhoea and occasionally under antibiotic therapy, 40 without diarrhoea and not using antibiotics and 23 without diarrhoea from day care centres) and 96 inpatients (30 with diarrhoea and taking antibiotics, 49 under chemotherapy and 17 without diarrhoea but under antibiotic therapy). *C. difficile* could be recovered from 6.7% (14/210) of the samples examined and toxigenic strains of *C. difficile* were isolated from 4.2% of the inpatients and from 3.5% of the outpatients. Interestingly, both toxigenic and non-toxicogenic strains were observed in five children presenting diarrhoea, confirming that *C. difficile* can also be a component of the intestinal microflora. All the toxigenic strains were defined as $tcdA^+/tcdB^+$ by PCR. In the strains tested, no resistance was found to metronidazole or vancomycin but resistance to clindamycin was observed and the MIC values were higher among community-acquired strains, showing that the selective pressure provided by this drug is important not only in the hospital environment.

Marcon *et al.* (2006) conducted an epidemiological case-control study to identify the main risk factors associated with the development of nosocomial diarrhoea in the intensive care unit (ICU) of a public hospital. Samples collected during January–October 2002 from 49 patients with diarrhoea and 49 patients without diarrhoea, who

were matched for age and sex, were included in the study. Immunocompromised patients, such as those who were HIV-positive, were excluded from the study because they generally suffer from infectious diarrhoea of non-bacterial and non-hospital origin. The mean age of the patients in both groups was 54 years (ages ranging from 13 to 94 years). It was found that nosocomial diarrhoea could be attributed to several factors, such as the use of antibiotics in general (ceftriaxone in particular), the length of stay in the hospital ICU, the use of enteral diets and the presence of a previous infection (respiratory, urinary tract or bloodstream). Diarrhoea was found to be the result of infection with *Pseudomonas aeruginosa* in 14 cases (28.6%) and *C. difficile* in 22 cases (44.9%). Six patients presented co-infection with both micro-organisms. Of the patients with diarrhoea, 86% had one or more roommates with diarrhoea in the ICU. The study, therefore, highlighted the importance of hand washing before and after procedures, as well as a need to adopt precautionary measures for contact with patients suffering from diarrhoea.

Alcides *et al.* (2007) conducted a study with the aim of characterizing *C. difficile* strains isolated from children (3 months–7 years of age) with or without diarrhoea, as well as strains isolated from hospital environments. A total of 39 strains were obtained: 15 from hospitalized children with diarrhoea, 17 from outpatients with diarrhoea, four from healthy children, and four from the general hospital environment. All isolates were susceptible to vancomycin and metronidazole; however, high levels of clindamycin resistance were also observed. Twenty-one strains were non-toxigenic, reinforcing the theory that infants are colonized by *C. difficile*, which forms part of the intestinal microflora. All of the 18 toxigenic strains possessed the TcdA⁺/TcdB⁺ phenotype, as determined by ToxA immunoassay and cytotoxicity testing on Vero cells. The strains could be divided into eight serogroups based on the results of SDS-PAGE of whole-cell proteins or into 13 groups based on PCR ribotyping (001, 015, 031, 043, 046, 131, 132, 133, 134, 135, 136, 142 and 143). It is interesting that the last seven PCR ribotypes were considered to be novel, as they had not been previously recognized and entered into the PCR ribotype database (Stubbs *et al.*, 1999). These types, which accounted for 78.5% of all the isolates tested, were considered henceforth as Brazilian ribotypes. PCR ribotype 133 was the most common, being detected in 10 of the 39 strains.

Another study (Balassiano *et al.*, 2009) also attempted to characterize *C. difficile* strains isolated from hospitalized patients but focused on the adult population. All of the 21 patients enrolled in the study had undergone broad-spectrum antibiotic therapy; three of the patients were HIV-positive and one had a central nervous system neoplasm. Active CDAD was observed in 28.5% (6/21) of the inpatients and *C. difficile* was isolated from 66.7% (4/6) of faecal samples from these subjects. The four strains were toxigenic, presenting a TcdA⁺/TcdB⁺ profile, as confirmed by PCR and by immunoassay to detect both toxins.

Genes that encode binary toxin (CDT) production were not detected in any of the strains. According to the PCR-ribotyping method used, two strains, which were isolated in the same month (October 2006), were shown to belong to PCR-ribotype 014. The other two strains, isolated within 6 months of each other (January and July 2007), belonged to PCR-ribotype 106. Interestingly, PFGE analysis confirmed that the ribotype 014 strains represented the same clonal type, while the ribotype 106 strains were closely related, showing about 90% DNA sequence similarity. It is of note that ribotype 106 is one of the most common types in the UK, being previously considered a British strain. This was reportedly the first time the presence of this ribotype had been detected outside the UK, since other studies conducted in several European countries, the USA and Canada had failed to detect it (Brazier *et al.*, 2007). The spread of *C. difficile* internationally was therefore demonstrated and was identified as a potential contributor to the occurrence of worldwide outbreaks, as was seen with strain 027/BI/NAP-1.

Dias *et al.* (2010) published an interesting study, in which they reported a suspected outbreak of CDAD in a private hospital. It was observed that in February 2002, three cases of CDAD were detected, and in March of the same year, seven cases occurred. An outbreak was implied, but the increase in the number of cases coincided with a change in the diagnosis test, from an immunoassay to detect TcdA to another that allowed the detection of TcdA and TcdB. Based on this, the authors concluded that, in fact, a pseudo-outbreak had occurred in this hospital. After this suspicion, the Infection Control Department started active surveillance for CDAD. From March 2002 to December 2003, the surveillance programme identified 138 cases of CDAD, 30% of which were present on admission, highlighting the problem of community-acquired *C. difficile*. The mean age of the patients was 64 years and almost all of them had been under antibiotic therapy prior to CDAD diagnosis. The incidence of CDAD among hospitalized patients was determined to be 3.3 patients for every 1000 admitted. *C. difficile* was recovered from 16/138 stool samples. The isolates were genotyped by arbitrary primed-PCR (AP-PCR), which identified 13 different clonal profiles, reinforcing the fact that there was no outbreak in the hospital, as this would have resulted in the same clonal profile being detected in all strains.

The first CDAD outbreak in Brazil was described by Balassiano *et al.* (2010). The study was carried out on a medical surgical intensive care unit (ICU) of a tertiary hospital. Medical records of all individuals who were patients of the ICU between January 2006 and July 2009 were reviewed and of the 218 patients presenting nosocomial diarrhoea, 43 (19.7%) of the cases were linked to CDAD. The mean rate of CDAD was 1.8/1000 patient days, with the highest incidence between December 2007 and August 2008 (mean 5.5/1000 patient day), during which the outbreak occurred. During this period, 101 stool samples were collected from patients and tested and TcdA

and TcdB were detected in 32 (31.5%) of them. No severe cases of CDAD were identified and no mortality due to CDAD was observed during the outbreak. In general, the patients were elderly, with 80% of them being over 65 years of age (mean age 77.8 years). All patients affected in the outbreak were immunocompromised and under antibiotic therapy, piperacillin/tazobactam being the main drug (81% of cases). Some stool samples were subjected to culture, resulting in three strains being isolated. Tests of the hospital environment were conducted, resulting in one strain being isolated. These four strains were found to be toxigenic (TcdA⁺/TcdB⁺), none presented binary toxin (*cdt*) genes. All the strains were sensitive to metronidazole and vancomycin, as expected, and were also sensitive to moxifloxacin. On the other hand, the isolates were resistant to clindamycin, ciprofloxacin and levofloxacin. No mutations in quinolone resistance-determining regions (QRDR) of *gyr* genes were found. Molecular fingerprinting analysis revealed that the strains could be divided into two PCR ribotypes: 038 and 135, with two strains of each ribotype, the latter being an exclusively Brazilian type, mentioned previously. PFGE analysis confirmed the distribution of the strains among the clonal types. The strains of ribotype 038 were isolated from a stool sample and from an environmental swab, which could reflect the contamination of the environment with *C. difficile* spores. Cross-infection may also have occurred, since both strains belonging to ribotype 135 were obtained from different stool samples. These two situations could be a consequence of inadequate protocols for the disinfection of the hospital environment and the hygiene of health care professionals at the time of the outbreak, which was brought under control only when the infection control program was adopted. It is important to reinforce the notion that cross-infection and environmental contamination are two crucial causal factors that can be associated with CDAD outbreaks (Hookman & Barkin, 2009).

The most recent publication on this subject described a study conducted in a university hospital from March 2008 to September 2009 (Balassiano *et al.*, 2011). The patients enrolled in this study were using broad-spectrum antimicrobials prior to getting diarrhoea and were immunosuppressed due to co-morbidities or as a result of their therapy. The mean age of the patients was 48 years (ages ranging from 27 to 79 years). Of the 70 patients suffering from nosocomial diarrhoea during the study period, CDAD was confirmed by immunoassay in 19 (27.1%) of the cases. *C. difficile* was recovered from eight stool samples, seven being isolates that were confirmed as toxigenic (TcdA⁺/TcdB⁺) and only one being confirmed as non-toxigenic. None of the isolates presented binary toxin (*cdt*)-related genes. All of these strains were sensitive to metronidazole, vancomycin and moxifloxacin, and resistant to clindamycin, ciprofloxacin and levofloxacin. No mutations in QRDR of *gyr* genes, that could be associated with quinolone resistance, were found. The eight isolates could be divided into four clonal groups, according

to PFGE and PCR-ribotyping analyses. PCR-ribotypes 010, 020, 133 and 233 were detected. Of the strains, 50% belonged to ribotype 133, an exclusively Brazilian type, as mentioned previously, and 25% belonged to ribotype 233. These results show that, once again, cross-infection among hospitalized patients was observed; a problem that could reflect failures in surveillance protocols and may occasionally contribute to the emergence of CDAD outbreaks, as observed in another Brazilian hospital (Balassiano *et al.*, 2010).

Chile

Gardilic *et al.* (2000) published a descriptive study, conducted in a tertiary-care teaching hospital. Medical records of patients presenting diarrhoea between June and September 1999, whose faecal samples were submitted for testing for TcdA using a commercial immunoassay, were analysed. During this period, 27 cases of CDAD were confirmed. The mean age of the patients was 56.5 years (ages ranging from 26 to 87 years) and 77.7% (21/27) of them were older than 65 years of age. A total of 59.2% (16/27) of the patients had been subjected to gastrointestinal procedures, including the use of nasogastric and nasojejunal probes and upper digestive endoscopes. All of these CDAD patients had previously also been under antimicrobial therapy, ciprofloxacin in particular, reinforcing the importance of quinolones as inducers of clinical problems associated with *C. difficile*. The mortality rate attributable to this infection was 4%. It is of note that one of these patients developed toxic megacolon, a classic and important complication of CDAD that increases the risk of mortality in certain cases.

Alvarez *et al.* (2001) published a study searching for clinical parameters that were unrelated to the use of antimicrobials and the presence of diarrhoea. The authors tried to determine a clinical prediction model for CDAD diagnosis. The researchers conducted a prospective 5-month study in a university tertiary hospital on all patients who were tested for *C. difficile* cytotoxicity at that time. A total of 92 stool samples were assayed and CDAD was confirmed in 28.2% (26/92) of cases. Parameters such as age, underlying diseases, class of antimicrobials administered before diarrhoea, temperature, characteristics of the stools and presence or absence of abdominal pain were compared among CDAD-positive and -negative groups. A logistic regression model was applied and revealed that an age of over 60 years, temperatures higher than 37.8 °C in the previous 24 h and presence of mucus in the stools were significant predictors of infection. The combination of these variables proved to be useful as a clinical prediction model for CDAD diagnosis and was accurate in 85.5% of the cases when compared with proportions of CDAD cases confirmed via culture and toxin detection methods.

Herrera *et al.* (2003) conducted a retrospective study of all cases of CDAD that had occurred in a university hospital (Universidad de Chile) between June 2000 and May 2001.

The study compared incidence rates, clinical presentations, complications and mortality rates associated with CDAD among inpatients in the nephrology unit and those in other hospital units. The authors reported that the global incidence of CDAD in the hospital was 0.53 cases per 100 discharges per year, while the incidence of CDAD in the nephrology unit was about 13 times higher at seven cases per 100 discharges per year. The main co-morbidities associated with these patients were chronic renal failure in haemodialysis (48%), uraemic syndrome (36%) and renal transplant (6%). The disease in one of these patients (3%) progressed to fatal toxic megacolon and 12% of patients presented lower digestive bleeding. Among the patients with renal diseases, 69% were elderly (>60 years old), and 79% had a previous history of antimicrobial use, quinolones and cephalosporins being the most frequently administered. The authors reported that immunodeficiency and abnormalities in the intestinal motility characteristics of renal patients could not only increase the risk of *C. difficile* acquisition and the development of CDAD, but could also explain CDAD cases in patients with no history of antimicrobial therapy, as observed in this study.

Recently, Jensen *et al.* (2010) reviewed medical records of inpatients of a health unit from June 2003 to March 2008, which correlated with data in other literature. Of the 706 patients in which TcdA was detected, CDAD could be confirmed in 112 cases (15.9%). Most of the patients were in the critical patients unit; 45% (51/112) of them presented type 2 diabetes and 41% (47/112) were hypertensive. It is interesting that 44.6% (50/112) of the CDAD-positive patients had been previously subjected to a gastrointestinal procedure (colonoscopy, endoscopy or parenteral nutrition) and 98% were undergoing antimicrobial therapy involving ceftriaxone, clindamycin or ciprofloxacin.

Costa Rica

The first study reporting the presence of *C. difficile* among adults in Costa Rica was published in 2008 by Zumbado-Salas *et al.* (2008). In a 13-month study, stool samples collected from 104 patients presenting diarrhoea and receiving antimicrobial drugs were subjected to CDAD testing via culture and toxin detection methods. The frequency of CDAD in these patients was 30% (31/104), which is similar to previously reported detection rates in Ireland (27%) and Sweden (20%), suggesting that *C. difficile* infections are not governed by the socioeconomic factors that distinguish high- and low-income countries.

After this pioneering description of the presence of *C. difficile* infection in Costa Rica, Quesada-Gómez *et al.* (2010) published one of the most important articles about this subject in Latin America. They described, for the first time, the isolation of 027/BI/NAP-1 strains from patients with CDAD in a Costa Rican hospital. Among the 37 isolates recovered in this study, 54% were characterized as 027/BI/NAP-1 strains by PFGE. These strains presented the

same key characteristics as 027/BI/NAP-1 strains previously isolated in other countries, characterized by a deletion in the *tcdC* gene, the production of binary toxin and high levels of resistance against fluoroquinolones. The authors emphasized that the presence of an epidemic and hyper-virulent strain of *C. difficile* in a Latin American country deserves attention, particularly to prevent further dissemination. So far, there is no information about detection of this *C. difficile* strain in other countries in this geographical region.

Jamaica

There are no publications available concerning the *C. difficile* situation in Jamaica. However, Camacho-Ortiz *et al.* (2009b) published a review in which the work of Heslop and colleagues (unpublished results), Jamaican investigators, is cited. In this study, 113 stool samples from three different groups were analysed, which included 21 samples from patients who had previously been treated with immunosuppressive drugs, 39 from patients under radiotherapy and 53 from patients where no immunosuppressive treatments were employed. It was found that 14.1% (16/113) of the samples were positive for CDAD; five were from the patients under immunosuppressive therapy and 11 were from the patients receiving no medication or treatment.

Mexico

García-Osogobio *et al.* (2000) presented a case report of a 52-year-old male patient with ulcerative colitis that progressed to toxic PMC. This patient had been treated for amoebiasis with metronidazole but, after 2 weeks of recovery, the symptoms recurred. Ulcerative colitis was confirmed by sigmoidoscopy and biopsy and the patient was properly treated. After 2 months with no symptoms, he presented rectal bleeding and an inflamed and distended colon with free inflammatory liquid in the peritoneum, which was diagnosed as toxic megacolon. A total abdominal colectomy and a temporary ileostomy were performed and the histopathology and microbiology analyses confirmed PMC associated with *C. difficile*. The authors concluded that, although toxic megacolon due to this micro-organism is a rare complication in patients with ulcerative colitis, it should be suspected in those under antibiotic therapy that present relapses.

Another case report was published by Sánchez-Pérez *et al.* (2010), describing a 63-year-old woman who was suffering from abdominal pain, bloating and diarrhoea for over 3 days. The patient had been administered amoxicillin/clavulanate 1 week earlier, which had been used to treat an upper respiratory tract infection. Presumptive diagnosis of CDAD was initially excluded, since abdominal tomography did not show the usual signs and the toxin test was negative. The patient was hospitalized for rehydration and treated with levofloxacin and metronidazole. During the first 24 h the symptoms had worsened and the patient developed septic shock. Additional examinations

confirmed the presence of PMC and a diagnosis of toxic megacolon was established. The authors commented that in such cases, surgical intervention immediately following diagnosis is necessary since toxic megacolon is a life-threatening complication of CDAD.

In 2009, Camacho-Ortiz *et al.* (2009a) published the results of a four-year study conducted in a tertiary-care hospital in Mexico City, which aimed to determine the incidence and risk factors associated with CDAD. Two groups were compared: CDAD cases, defined as diarrhoea or toxic megacolon coupled with TcdA toxin detection or pseudo-membrane identification, and control cases, defined as inpatients without diarrhoea that were hospitalized at the same time as the CDAD group. Of the 3130 faecal samples submitted for TcdA detection, 170 (5.43%) were positive. An outbreak was detected during the study in August 2005 when the incidence of CDAD increased from 5.04 to 29.5 cases per 1000 hospital discharges. Based on multivariate analysis, the authors found that significant risk factors relating to the development of CDAD followed the same criteria as those already described in the literature, namely the use of H₂ blockers, age of over 65 years, prior hospitalization within 12 weeks of diagnosis, prior use of cephalosporins and fluoroquinolones, hospitalization at an ICU and extended hospital stays.

Peru

García *et al.* (2007a) studied the prevalence, incidence and epidemiology of CDAD in a tertiary-care hospital in Peru from September 2005 to May 2006. Among the 4264 patients admitted to the hospital over the study period, 156 (3.7%) developed nosocomial diarrhoea and 55 (35.5%) of these cases were associated with *C. difficile*. The overall incidence of CDAD was 12.9 per 1000 admissions. Compared with a control group, CDAD patients had been hospitalized for a significantly prolonged time before the onset of diarrhoea, had made use of diapers, had generally received clindamycin and were often resident in the same room as another CDAD patient. All these factors were considered as risk factors for the development of CDAD but faecal incontinence associated with the use of diapers was considered the most important. The authors highlighted that this characteristic has not been described before in an adult setting and suggested that the presence of multiple diapered patients in the same room is unfavourable when trying to prevent cross-infection.

García *et al.* (2007b) reported a unique case of a 30-year-old HIV-positive patient who died due to complications associated with PMC and co-infections of *C. difficile*, cytomegalovirus (CMV) and possibly *Mycobacterium tuberculosis*. The patient presented chronic diarrhoea over 1 year and was admitted to hospital with a cough, difficulty breathing and sensory disorder. Preliminary examinations did not detect either alcohol-acid resistant bacilli in the sputum nor bacteria or parasites in the faeces. Even though the presumptive diagnosis was negative, based on the high

incidence of HIV-positive patients with tuberculosis in Peru, anti-tuberculosis therapy was started and administered with ceftriaxone, co-trimoxazole and clindamycin. Five days later, the patient developed upper gastrointestinal bleeding, which was diagnosed as a peptic ulcer. The necrotic tissue tested positive for multiple acid-fast Gram-positive bacteria, as determined by biopsy. After the third week of hospitalization, the diarrhoea became worse and the colonoscopy revealed the presence of PMC. TcdA and TcdB were detected in the faeces. Metronidazole was administered, but the patient succumbed to shock and died. Colon and ileum necropsies revealed cytopathic effects characteristic of CMV and the presence of numerous acid-fast Gram-positive bacteria. The authors highlighted that in AIDS patients who develop PMC and do not respond to treatment for *C. difficile* infection, CMV should be considered as a possible contributing factor in differential diagnoses.

Puerto Rico

The only study that mentions the presence of *C. difficile* infection in Puerto Rico was conducted by Carrer *et al.* (2005). A pilot study was undertaken to identify the main pathogens associated with nosocomial diarrhoea that could be linked to community-acquired enteric pathogens. Seventy-six stool samples were subjected to culture, toxin assay or ELISA to detect classical enteropathogens belonging to bacterial, parasite and virus groups. The authors observed that the main community-acquired enteric pathogens are a very rare cause of diarrhoea in hospitalized patients in Puerto Rico. They based this conclusion on the fact that species of the genera *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and Rotavirus could not be detected, and *Giardia* (1.3%), Adenovirus (2.6%) and Shiga toxin-producing *E. coli* (1.3%) were detected only at low levels. On the other hand, 10.3% of the stool samples were positive for *C. difficile* toxins, and this micro-organism was considered by the authors to be the only clinically significant factor among patients presenting nosocomial diarrhoea.

Conclusions

In the new millennium, infections around the world involving *C. difficile* and the number of reports concerning this pathogen have increased, especially because of its epidemiology and changes in clinical presentation. When the worldwide scenario of *C. difficile* infection was examined by studying the epidemiology of the micro-organism, it was observed that, after 2002, the United States and Canada started to have problems with CDAD, especially considering the increase in cases of severe infections that progressed to toxic megacolon, resulting in high rates of mortality among elderly people. However, when populations that once were not considered to be of high risk, such as pregnant women and community healthy individuals, started to develop infections associated with *C. difficile*, a new search for the possible cause of the increase

in the number of cases started. A hypervirulent strain was identified, 027/BI/NAP-1, which was associated with the more severe cases, changing again the epidemiology of this pathogen in many countries. Looking at all the developed countries that are facing problems in their health care units and experiencing high ratios of mortality, one question comes to mind: what about cases in Latin American countries? There are only a few reports concerning the detection and involvement of this pathogen in cases of diarrhoeal disease. Quesada-Gómez et al. (2010) made by far the most important contribution when the authors described, for the first time, the isolation of 027/BI/NAP-1 strains from patients with CDAD in a Costa Rican hospital. But again, if this strain circulates in North America and Europe and has now been found in Costa Rica, why are there no publications reporting the isolation of this strain in Latin America? One of the explanations for this is that diagnostic testing for anaerobic bacteria is not a routine procedure in laboratories in Latin America. Most of the clinical laboratories that are responsible for the isolation and identification of bacteria that are the main causes of infections do not identify anaerobic bacteria. The common practice is to release the result as a 'possible anaerobe'. If the hospital is interested in the identification of the species, the strain is sent to a research laboratory, commonly located at a university, which are few because most of the microbiologists are unable to identify anaerobic bacteria. It is also quite expensive and many laboratories cannot grow anaerobic bacteria routinely. Since the research laboratories do not have the routine tests to identify these bacteria, it takes a while for a result to be given. In the meantime, the patient is already being treated with broad-spectrum antibiotics. As mentioned previously, there are only a few anaerobic research groups in Latin America that work specifically with *C. difficile*, making it difficult to detect the presence of epidemic strains, such as 027/BI/NAP-1. There are, perhaps, several possible scenarios in the countries of Latin America: (a), 027/BI/NAP-1 strains are circulating in the countries cited in this review but have not yet been found; (b), 027/BI/NAP-1 strains are not present; (c), Latin America has specific ribotypes that are not usually found in North America or Europe; or (d), some of the strains mentioned in studies are of the 027/BI/NAP-1 type, which, in the absence of ribotyping, have been difficult to identify.

It seems likely that, if infections caused by *C. difficile* become a significant problem in Latin America, it will be important to be aware of both the methods of detection and current strategies for diagnosis and treatment in order to avoid serious public health problems. In this review, we have attempted to survey the well-documented situation in Europe and North America, as well as analyse the more fragmented data derived from the relatively few reports that have been made in Latin America. We hope that this review will raise awareness of *C. difficile* infections, especially where the hypervirulent strain is involved, particularly among health professionals in Latin America

and other developing regions, and will also promote collaboration.

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