Spread of epidemic *Clostridium difficile* NAP1/027 in Latin America: case reports in Panama

Diana López-Ureña, Carlos Quesada-Gómez, Erick Miranda, Mercedes Fonseca, and Evelyn Rodríguez-Cavallini

1Laboratorio de Investigación en Bacteriología Anaerobia (LIBA), Facultad de Microbiología and Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, 2060, Montes de Oca, San José, Costa Rica
2Director Regional, Caja Seguro Social, David, Chiriquí, Panama
3Hospital Regional Dr. Rafael Hernández, Caja Seguro Social, David, Chiriquí, Panama

The rate and severity of *Clostridium difficile* infection (CDI) have been linked to the emergence and spread of the hypervirulent toxigenic strain NAP1/027. This strain has been responsible for large outbreaks in healthcare facilities in North America and Europe and most recently in Latin America. This is the first report of the NAP1 strain in Panama. It suggests that the spread of *C. difficile* NAP1 throughout Latin America could be a possibility as evidenced in the following case reports. Five isolates typed as NAP1 had tcdA, tcdB, binary toxin gene cdtB and tcdC deletion. All isolates were resistant to clindamycin, fluoroquinolones and rifampicin. Under this scenario, surveillance programmes for CDI should be implemented in public health facilities in Latin America and diagnosis of CDI should be considered, especially in patients with predisposing factors.

**Introduction**

Traditional risk factors associated with *Clostridium difficile* infection (CDI) include age (elderly), antibiotic use, treatment with proton pump inhibitors or histamine H2 receptor antagonists, immunocompromised patients and the presence of gastrointestinal disease as well as other factors (Denève et al., 2009; Morgan et al., 2008; Pépin et al., 2004). However, the rate and severity of CDI have been linked to the emergence and spread of the hypervirulent toxigenic strain NAP1/027 (Denève et al., 2009; O’Connor et al., 2009). The strain has been responsible for large outbreaks in healthcare facilities in North America, Europe and several Asian countries (Freeman et al., 2010; McDonald et al., 2005). Most recently, in Latin America, it has been documented in Costa Rica and Chile (Quesada-Gómez et al., 2010; Hernández-Rocha et al., 2012). Hence, the spread of *C. difficile* NAP1 throughout Latin America could be a possibility, as evidenced in the following case reports from Panama.

**Case Reports**

A total of 57 CDI cases were reported from April 2012 to June 2013 in a Panamanian public hospital (38 cases between October and December). Previously, occasional cases of CDI were reported in this hospital but no epidemiological data were obtained. During this period, toxins were detected in stool samples by the hospital clinical laboratory and *C. difficile* was initially identified as epidemic strain 027 by GeneXpert PCR (Xpert *C. difficile* Epi; Cepheid), according to manufacturer guidelines. Six stool samples were sent to the Research Laboratory for Anaerobic Bacteriology, University of Costa Rica, for further testing. Briefly, *C. difficile* toxin A/B (Xpect test; Remel) was detected in all stool samples. Alcohol shock was performed on the stool sample, followed by culture onto cefoxitin cycloserine fructose agar (CCFA; Oxoid) and fastidious anaerobe broth (LAB M) for 5 and 15 days respectively, incubated under anaerobic conditions (90% N2, 5% H2, 5% CO2) (Miller et al., 2010). The fastidious anaerobe broth was then inoculated onto another CCFA. Characteristic *C. difficile* colonies on the CCFA were inoculated onto Brucella agar (BBL) with 5% lysed horse blood and vitamin K (5 mg ml−1) for identification and molecular tests.

Identification was confirmed by the Rapid ID 32A system (bioMérieux) according to manufacturer guidelines (Agilent Technologies) and tpi gene PCR amplification (Spigaglia & Mastrantonio, 2004; Fig. 1). Molecular strain typing was also confirmed using PFGE with SmaI digestion as described previously (Miller et al., 2010; Quesada-Gómez et al., 2010). Patterns were compared to the National Microbiology Laboratory database using BioNumerics software version 4.6 (Applied Maths). Five strains were typed as NAP1 (Fig. 2). Amplification of the tcdA and tcdB...
PFGE typification of the binary toxin gene \textit{cdtB} gene fragments of \textit{PaLoc} (the pathogenicity locus), the low albumin levels (2.5–3.5 g dl\(^{-1}\)), high serum creatinine levels (100–199 μmol l\(^{-1}\)) and the putative regulatory gene \textit{tcdC} was performed according to conditions described elsewhere (Miller \textit{et al.}, 2010). Five strains had the \textit{tcdA} and \textit{tcdB} genes, the binary toxin gene \textit{cdtB}, and the \textit{tcdC} gene with evidence of a deletion (Fig. 1). Antibiotic susceptibility to clindamycin, moxifloxacin, levofloxacin, ciprofloxacin, rifampicin, metronidazole and vancomycin was determined using the Etest (bioMérieux) according to the Clinical and Laboratory Standards Institute breaking points (Hecht \textit{et al.}, 2007). \textit{C. difficile} ATCC 70057 and \textit{Bacteroides fragilis} ATCC 25285 were used as reference strains. All strains were highly resistant to clindamycin (\(>256 \text{ μg ml}^{-1}\)), moxifloxacin, levofloxacin, ciprofloxacin and rifampicin (\(>32 \text{ μg ml}^{-1}\)) but susceptible to metronidazole (0.25–1 μg ml\(^{-1}\)) and vancomycin (1.5–3 μg ml\(^{-1}\)).

The laboratory data of patients showed that all patients had low albumin levels (2.5–3.5 g dl\(^{-1}\)) (Dubberke \textit{et al.}, 2007), high serum creatinine levels (100–199 μmol l\(^{-1}\)) and a high leukocyte count (10.0–19.9 × 10\(^9\) l\(^{-1}\)) (Pépin \textit{et al.}, 2004). Two patients had a body temperature \(>38.0^\circ\text{C}\) and the average number of diarrhoeal discharges was four per day. The average age of these patients was 66 years and the most frequent causes of hospital admission were malignancies, diabetes, and urinary or renal complications. Two patients were immunocompromised. The average number of days for which four patients were hospitalized at the start of the diarrhoea was 2.5 days; the fifth patient was hospitalized for 26 days. Moreover, patients had been previously prescribed with cephalosporins and fluoroquinolones. One of the patients was undergoing clindamycin treatment. Proton pump inhibitors were used by one patient, and faecal depositions increased in another patient after laxative was taken. Patients were treated with either metronidazole or vancomycin or both. No deaths were associated with CDI, and only one patient had recurrence.

**Discussion**

In this study, among six isolates, five were typed as the hypervirulent strain NAP1. Pulstype NAP1 has been the most frequent isolate in epidemic outbreaks in healthcare facilities in North America and Europe (Loo \textit{et al.}, 2005; McDonald \textit{et al.}, 2005). Even though only six stool samples were analysed, the increase in CDI cases in this Panamanian hospital could be linked to the NAP1 strain, as has been reported in other countries. Moreover, these strains had high antibiotic resistance to clindamycin and fluoroquinolones as previously reported for NAP1 strains in Costa Rica and other countries (Labbé \textit{et al.}, 2008; Quesada-Gómez \textit{et al.}, 2010; Razavi \textit{et al.}, 2007). Furthermore, epidemiological data for NAP1 patients were grouped for analysis based on risk factors previously associated with CDI (Morgan \textit{et al.}, 2008). Patients had risk factors that have been linked to CDI (Denève \textit{et al.}, 2009; Morgan \textit{et al.}, 2008; Pépin \textit{et al.}, 2004). Among them were age, malignancies and antibiotic use.

Infections are considered to be nosocomial cases if the diagnosis of CDI is made more than 48 h after admission (Dubberke \textit{et al.}, 2007). Nonetheless, in these reports, the strains could also be considered community acquired as NAP1 strains have been isolated from community cases (Limbago \textit{et al.}, 2009), or they could be recurrences as one patient had previously been hospitalized with a history of diarrhoea; however, CDI had not been diagnosed before.

Hence, attention must be drawn to these reports by clinical professionals and health authorities in Latin America as the NAP1 strain has spread in North America and Europe causing hospital outbreaks. Moreover, three Latin American countries have had reports of the pulstype NAP1, which suggest that the spread of this epidemic strain throughout Latin America is a possibility. Under this scenario, surveillance programmes for CDI should be implemented in public health facilities in Latin America and a diagnosis of CDI should be considered, especially in patients with...
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References


