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Emergence of *Clostridium difficile* NAP1 in Latin America[▽]

The NAP1 and NAP2 strains of *Clostridium difficile* have been linked to nosocomial outbreaks of antibiotic-associated diarrhea (AAD) and pseudomembranous colitis in North American and European countries (4, 5). We found these strains, together with seven additional pulsed-field gel electrophoresis (PFGE) patterns, among 37 isolates recently recovered from patients with AAD in a Costa Rican hospital. Herein we present the macrorestriction patterns of the isolates as well as data regarding their toxin genotypes and susceptibility to selected antibiotics.

The isolates were recovered by inoculating a loopful of diarrheic stool samples onto cefoxitin-cycloserine fructose agar plates (CCFA; Oxoid). They were identified with the rapid ID32A system (bioMérieux) and a PCR targeting the triose phosphate isomerase gene (9). We typed the isolates by PFGE (1) and amplified fragments of the *tcdA*, *tcdB*, *tcdC*, and *cdtB* genes by PCR with oligonucleotides and conditions reported elsewhere (3, 9, 10). These genes code for toxin A, toxin B, the negative regulator of the pathogenicity locus, and the binding domain of the binary toxin, respectively. MICs of clindamycin, metronidazole, vancomycin, moxifloxacin, ciprofloxacin, and amoxicillin-clavulanate were determined using Etest strips (AB bioMérieux). *C. difficile* ATCC 700057 and *Bacteroides fragilis* ATCC 25285 were used as reference strains. The breakpoints recommended by the CLSI (2) and Peláez et al. (6) were used for antimicrobial susceptibility interpretation.

Nine different PFGE types were identified in the collection (Fig. 1). All isolates were positive for both *tcdA* and *tcdB* and susceptible to vancomycin and metronidazole, which are the first antibiotics to be prescribed for this type of infection. They were also susceptible to amoxicillin-clavulanic acid.

More than half the isolates exhibited the macrorestriction pattern of the NAP1 strain ($n = 20$; 54%). All these bacteria had the gene for the binding domain of the binary toxin and a deletion in *tcdC* (Table 1). Only one of the NAP1 strains was susceptible to clindamycin. In fact, 10 (50%) were categorized as intermediate and another 9 (45%) as resistant to this antibiotic. One clindamycin-resistant NAP1 strain had a MIC of ≥256 μg ml^{-1}. In

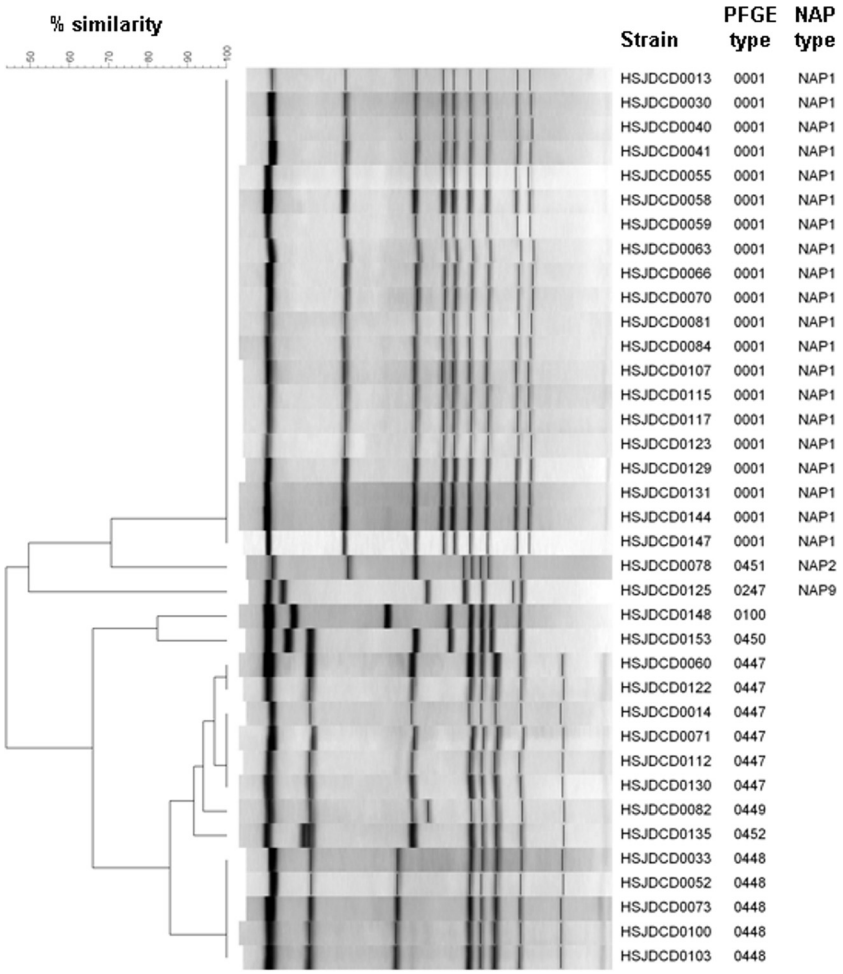

FIG. 1. Pulsed-field gel electrophoresis. SmaI dendrogram of 37 isolates of *Clostridium difficile* recovered from patients with antibiotic-associated diarrhea in a Costa Rican hospital.

TABLE 1. SmaI macrorestriction patterns, toxin genotype, and MICs of clindamycin, metronidazole, vancomycin, moxifloxacin, ciprofloxacin, and amoxicillin-clavulanic acid of 37 isolates of *C. difficile* recovered from patients with antibiotic-associated diarrhea in a Costa Rican hospital

Toxin genotype	SmaI pattern	Range of MICs (μg ml^{-1})					
		Clindamycin	Metronidazole	Vancomycin	Moxifloxacin	Ciprofloxacin	Amoxicillin/ clavulanic acid
tcdA$^+$ *tcdB*$^+$ *cdtB*$^+$ *tcdC* with deletion	001 (NAP1, n = 20)	2–>256	0.5–4	0.5–1.5	>32	>32	0.38–1.5
tcdA$^+$ *tcdB*$^+$ *tcdC* with deletion	447 (n = 6)	>256	0.09–0.38	0.5–4	>32	>32	0.75–1
	448 (n = 5)	>256	0.09–0.75	1–2	>32	>32	0.75–1
	449 (n = 1)	>256	0.38	4	>32	>32	1
	452 (n = 1)	>256	0.38	4	>32	>32	0.75
tcdA$^+$ *tcdB*$^+$ *tcdC* without deletion	100 (n = 1)	12	0.19	0.75	ND[a]	>32	0.38
	247 (NAP9, n = 1)	>256	0.75	1.5	>32	>32	1.5
	450 (n = 1)	>256	0.5	0.75	ND	>32	0.19
	451 (NAP2, n = 1)	16	0.25	1	2	>32	0.38

[a] ND, not determined.

agreement with recent data (7), all NAP1 isolates were highly resistant to the two fluoroquinolones tested (Table 1).

The 13 isolates with the SmaI patterns 447, 448, 449, and 452 clustered together (Fig. 1). These 13 isolates lacked the binary toxin and had a deletion in the *tcdC* gene (Table 1). In addition, they were without exception categorized as highly resistant to clindamycin, moxifloxacin, and ciprofloxacin (Table 1). One strain with the PFGE pattern 447 and the strains with the PFGE patterns 449 and 452 exhibited the highest MICs to vancomycin overall.

The isolates with the PFGE patterns 100, 450, and 451 (NAP2) and 247 (NAP9) were negative for the binary toxin and did not have deletions in the *tcdC* gene (Table 1). The strains with the PFGE patterns 100, 450, and 247 (NAP9) were resistant to clindamycin, moxifloxacin, and ciprofloxacin (Table 1). In contrast, the isolate with the PFGE pattern 451 (NAP2) was moderately resistant to clindamycin (MIC = 16 μg ml^{-1}) and highly resistant to ciprofloxacin (MIC > 32 μg ml^{-1}) but susceptible to moxifloxacin (MIC = 2 μg ml^{-1}). NAP9 strains have been recently isolated from retail meat (8).

The finding of the NAP1 strain in Latin American countries is novel and deserves attention from infectious disease specialists and epidemiologists to prevent its dissemination.

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Carlos Quesada-Gómez
César Rodríguez*
María del Mar Gamboa-Coronado
Evelyn Rodríguez-Cavallini
Laboratorio de Investigación en Bacteriología Anaerobia
Facultad de Microbiología
Universidad de Costa Rica
Ciudad Universitaria Rodrigo Facio
2060, San Pedro de Montes de Oca
San José, Costa Rica
Centro de Investigación en Enfermedades Tropicales
Universidad de Costa Rica

Tim Du
Michael R. Mulvey
National Microbiology Laboratory
Winnipeg, Manitoba, Canada

Manuel Villalobos-Zúñiga
Ricardo Boza-Cordero
Hospital San Juan de Dios
San José, Costa Rica

*Phone and fax: (506) 2511-4364
E-mail: cesar.rodriguezsanchez@ucr.ac.cr

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