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EXTENDED ABSTRACT

Hypervirulent antibiotic-resistant *Clostridium difficile* in Europe

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Abstract

Recently, several *Clostridium difficile* outbreaks due to PCR ribotype 027, associated with increased disease severity and death, have been reported in North America and in several European countries. This strain is toxinA/toxinB-positive, contains the genes for binary toxin and has an 18 bp deletion and a frameshift mutation in the gene *tcdC* hypothesized to result in a deregulated expression of toxins A and B. These strains are high producers of toxins *in vitro* compared with other toxinotypes. Moreover, these strains show a high level of resistance to fluoroquinolones, possibly due to the presence of a transition mutation (C to T) in the *gyr*A, resulting in the amino acid substitution Th82 →Ile. A 2 month prospective study was conducted in 38 hospitals in 14 different European countries to get an overview of the phenotypic and genotypic features of C. *difficile* isolates in 2005. In all, 411 isolates of *C. difficile* were obtained from diarrhoeic patients with suspected *C. difficile*-associated diarrhoea (CDAD); the prevalence of the 027 epidemic strain was 6.2%. All 027 strains were positive for binary toxin genes, had an 18 bp deletion in *tcd*C gene and were resistant to erythromycin and moxifloxacin. Patients infected with an 027 strain were likely to have a more severe disease (OR = 2.52, 95% CI 0.92–6.85, $p = 0.04$) and to have been more specifically treated by metronidazole or vancomycin (OR = 7.23, CI 0.99–149, $p = 0.02$). Ongoing epidemiological surveillance of CDAD cases with periodic characterization of the strains is needed to detect clustering of cases in time and space and to monitor the emergence of a specific hypervirulent clone.

Key words: *Clostridium difficile, toxins A and B, hypervirulence, C. difficile-associated diarrhoea*

Introduction

Infection with toxigenic *Clostridium difficile* results in a spectrum of disease ranging from mild self-limiting diarrhoea to fulminant colitis (1), and asymptomatic toxin-positive carriers have also been identified (2). The prevalence of *C. difficile*-associated diarrhoea (CDAD) may vary depending on the institution and patient population studied (3). The microorganism is the leading cause of nosocomial diarrhoea in adults from industrialized countries (4) and many hospital outbreaks throughout the world have been described (5,6).

Primary virulence factors are two large toxins, toxin A (TcdA) and toxin B (TcdB), which disrupt the actin cytoskeleton of intestinal epithelial cells by the UDP-glucose-dependent glucosylation of proteins of Rho and Ras subfamilies (7). A third toxin, named binary toxin, (actin-specific ADP-ribosyltransferase) was described from *C. difficile* strain CD196 in 1998 (8). It is encoded by two genes (*cdt*A and *cdt*B). Prevalence of binary toxin genes in humans varies from 1.6% to 20.8% and its pathogenic role still remains unclear (9,10).

Standard treatment includes withdrawal of the inducing antibiotic and use of oral metronidazole or vancomycin in seriously ill patients. The major complications of treatment are failure to respond and relapses or re-infections after treatment is discontinued. Since 2003, increasing rates of CDAD with more severe courses have been reported in Quebec (Canada) and the United States (11–13). Investigators from Quebec noted an increase in the frequency and severity of *C. difficile*-associated disease. Furthermore, the disease seemed to be more serious and refractory to therapy with an increased mortality rate.

During the same years, the Centers for Disease Control and Prevention in the USA reported an increased frequency and severity of the CDAD.

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Complications included toxic megacolon, septic shock, requirement for colectomy and death (14). This trend was due to the rapid spread of a specific clone belonging to PCR-ribotype 027/pulsotype NAP1. This strain is toxinA/toxinB-positive, contains the genes for binary toxin and has an 18 bp deletion and a frameshift mutation in the gene *tcdC* hypothesized to result in a deregulated expression of toxins A and B. These strains are high producers of toxins *in vitro* compared with other toxinotypes. Moreover, these strains show a high level of resistance to fluoroquinolones (15).

The European Study

A 2 month prospective study was conducted in 38 hospitals in 14 different European countries, coordinated by the European Study Group on *Clostridium difficile*, to get an overview of the phenotypic and genotypic features of C. *difficile* isolates in 2005. Cultures were performed in each microbiology laboratory according to local standard techniques. Then, strains were sent to three central laboratories for toxinotyping, PCR-ribotyping and antimicrobial susceptibility tests (MIC determination), respectively.

In all, 411 *C. difficile* isolates were obtained from diarrhoeic patients with suspected CDAD and were characterized (16). The great majority (75.7%) were toxinotype O and 24.3% were toxin-variant strains including toxinotypes I, III, IV, V, VI, VIII, IX and XXIV. All strains that were toxin A-negative/toxin B-positive were identified as toxinotype VIII.

All *C. difficile* strains were susceptible to vancomycin and metronidazole. Resistance rates to erythromycin, clindamycin, tetracycline and moxifloxacin varied widely from one country to another; 46% of strains were resistant to fluoroquinolones.

A total of 66 different PCR ribotypes were identified. The six major ribotypes were 001, 002, 014, 017, 020 and 027.

The prevalence of the 027 epidemic strain was 6.2%. All 027 strains were positive for binary toxin genes, had the 18 bp and the 117 deletion in *tcd*C gene and were resistant to erythromycin and moxifloxacin.

Patients infected with a 027 strain were likely to have a more severe disease (OR = 2.52, 95% CI 0.92–6.85, $p = 0.04$) and to have been more specifically treated by metronidazole or vancomycin (OR = 7.23, CI 0.99–149, $p = 0.02$).

Since 2005, *C. difficile* type 027 has caused outbreaks in England and Wales, Ireland, the Netherlands, Belgium, Luxemburg and France and has been also detected in Austria, Scotland, Switzerland, Poland and Denmark (Figure 1) (17).

The impact of CDAD in health-care settings is considerable. Patients require isolation, specific therapy to eliminate *C. difficile*, hygiene in nursing, environmental decontamination and, in serious outbreaks, ward closure.

Figure 1. Detection of *C. difficile* ribotype 027 in different European countries.

Special attention to infection control is required because infection with this strain may be a major nosocomial complication, especially in elderly patients. Outbreaks may require restriction of antibiotic use, especially those implicated in the epidemic, which now include fluoroquinolones, clindamycin and cephalosporins.

Ongoing epidemiological surveillance of CDAD cases with periodic characterization of the strains is needed to detect clustering of cases in time and space and to monitor the emergence of a specific hypervirulent clone.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Fekety R. Antibiotic-associated diarrhoea and colitis. Curr Opin Infect Dis. 1995;8:391–7.
2. Simor AE, Yake SI, Tsimidis K. Infection due to *Clostridium difficile* among elderly residents of a long-term care facility. Clin Infect Dis. 1993;17:672–8.
3. Gerding DN, Johnson S, Peterson I, Mulligan M, Silva J. *Clostridium difficile* associated diarrohea and colitis. Infect Control Hosp Epidemiol. 1995;16:459–76.
4. McFarland LV, Mulligan ME, KwoK RY, Stamm WE. Nosocomial acquisition of *Clostridium difficile* infection. N Engl J Med. 1989;320:204–10.
5. Cartmill TD, Panigrahi H, Worsley MA, McCann DC, Nice CN, Keith E. Management and control of a large outbreak of diarrhoea due to *Clostridium difficile*. J Hosp Infect. 1996;27: 1–15.
6. Muto CA, Pokrywka M, Shutt K, Mendelsohn AB, Nouri K, Posey K, et al. A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of death and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol. 2005; 26:273–80.
7. Voth DE, Ballard JD. *Clostridium difficile* toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005;18: 247–63.
8. Popoff MR, Rubin EJ, Gill DM, Bouquet P. Actin-specific ADP-ribosyltransferase produced by a *Clostridium difficile* strain. Infect Immun. 1998;56:2299–306.
9. Goncalves C, Decre D, Barbut F, Burghoffer B, Petit JC. Prevalence and characterization of a binary toxin from *Clostridium difficile*. J Clin Microbiol. 2004;42:1933–9.
10. Rupnik M, Kato N, Grabnar M, Kato H. New types of toxin A negative, toxin B positive strains among *Clostridium difficile* isolates from Asia. J Clin Microbiol. 2003;41:1118–25.
11. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med. 2005;353:2442–9.
12. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med. 2005;353: 2433–41.
13. Pépin J, Valiquette L, Alary ME, Villemure P, Pelletier A, Forget K, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ. 2004;171:466–72.
14. Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet. 2005;366:1079–84.
15. Pépin J, Saheb N, Coulombe MA, Alary ME, Corriveau MP, Authier S, et al. Emergence of fluoroquinolones as predominant risk factor for *Clostridium difficile*-associated diarrhoea: a cohort study during an epidemic in Quebec. Clin Infect Dis. 2005;41:1254–60.
16. Barbut F, Mastrantonio P, Delmeè M, Brazier J, Ackermann G, Bouza E, et al. for the European Study Group on *Clostridium difficile* (ESGCD). Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterization of the isolates. Clin Microbiol Infect 2007;13:1048–57.
17. Kuijper EJ, Coignard B, Brazier JS, Suetens C, Drudy D, Wiuff C, et al. Update of *Clostridium difficile*-associated disease due to PCR ribotype 027 in Europe. Euro Surveill. 2007;12:E1–2.