10.1111/j.1469-0691.2009.02807.x

First isolation of *Clostridium difficile* PCR ribotype 027 from a patient with severe persistent diarrhoea in Hungary

G. Terhes[1], E. Urbán[1], M. Konkoly-Thege[2], É. Székely[2], J. S. Brazier[3], E. J. Kuijper[4] and E. Nagy[1]

1) *Institute of Clinical Microbiology, University of Szeged, Szeged and* 2) *St László Hospital, Budapest, Hungary,* 3) *Anaerobe Reference Laboratory, NPHS Microbiology Cardiff, University Hospital of Wales, Cardiff, UK and* 4) *National Reference Laboratory for Clostridium difficile, Leiden University Medical Centre, Leiden, the Netherlands*

A recent Supplement to *Clinical Microbiology and Infection* entitled 'Infection control measures to limit the spread of *C. difficile*' pointed out that the incidence of *C. difficile*-associated diarrhoea (CDAD) has been increasing worldwide, and stressed the importance of research in the fields of epidemiology and infection control [1]. Since 2003, one of the main causes of the increasing prevalence of CDAD has been claimed to be the emergence of PCR ribotype 027/NAP1, which has caused epidemics in North America, the UK, the Netherlands, Belgium and France. The presence of PCR ribotype 027 in Austria, Japan, Ireland, Germany and Switzerland has also been reported recently [2,3]. The majority of publications have emphasized that the presence of this strain is usually associated with more severe symptoms and signs than those associated with the other more common toxin-positive strains [4,5]. Whereas PCR ribotype 027 was present in the population earlier, the majority of the historic strains were fluoroquinolone sensitive [6]. The overuse of antibiotics such as fluoroquinolones may lead to the selection and emergence of resistant strains, and may contribute to the spread of PCR ribotype 027, which is usually resistant to erythromycin. Here, the Eastern European spread of *C. difficile* PCR ribotype 027 is reported.

Keywords: *Clostridium difficile*, nosocomial diarrhoea, PCR ribotype 027

Original Submission: 4 September 2008; **Revised Submission:** 1 November 2008; **Accepted:** 14 November 2008
Editor: D. Raoult

Clin Microbiol Infect 2009; **15:** 885–886

Corresponding author and reprint requests: E. Nagy, Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, H-6725 Szeged, Semmelweis u. 6., Hungary
E-mail: nagye@mlab.szote.u-szeged.hu

On 14 June 2007, a 53-year-old male patient with severe diarrhoea and nausea was admitted to hospital in Budapest, Hungary. Medical history included hospitalization 2 weeks before his recent illness because of systemic lupus erythematosus, and previous antibiotic therapy (ceftriaxone, clarithromycin and imipenem). Stool specimens were negative for *Salmonella* spp., *Shigella* spp., *Yersinia eneterocolitica*, *Campylobacter* spp. and yeasts. In spite of the supportive therapy, the patient's condition did not improve, and diarrhoea persisted. Colonoscopy revealed that the ileal mucosa was intact, but ulcers were visible in the colon. The patient was therefore treated with a higher dose of steroid and empiric metronidazole. *Clostridium difficile* toxin detection (using VIDAS® *Clostridium difficile* Toxin A II assay; bioMérieux, Craponne, France) yielded positive scores. Because of the severe and persistent diarrhoea and the therapeutic failures, antibiotic therapy was changed to oral vancomycin. On 25 July, high fever, right-sided pneumonia and respiratory distress developed; the patient was admitted to the ICU, where he died shortly thereafter. *Escherichia coli* sepsis was confirmed by a positive blood culture. At that time, the patient's death was not attributed to *C. difficile* infection.

Six months later, as part of a national *C. difficile* survey in the National Reference Laboratory for Anaerobes in Hungary, *C. difficile* was isolated from the faeces of this patient, which had been kept frozen in the local laboratory. The isolated strain was positive for toxin A and B genes, and the presence of binary toxin genes was confirmed.

At the 3′ end of the *tcd*A gene, no deletion was detected using NK9 and NK11 primers as described by Kato *et al.* [7]. Sequence analysis of the partial *tcd*C gene of this strain revealed an 18-bp and single nucleotide deletions, which are characteristic of the ribotype 027.

Comparison of the results of PCR ribotyping with the pattern of ribotype 027 in the ARL library (Cardiff, UK) and with the patterns of ribotype 027 control strains (Lume 1 and 10) allowed characterization of this strain as ribotype 027. Using E test, the strain was resistant to erythromycin (≥256 mg/L) and moxifloxacin (≥32 mg/L), whereas it was sensitive to metronidazole (1 mg/L) and rifampicin (≤0.002 mg/L), and intermediate resistant to clindamycin (4 mg/L); the vancomycin MIC was 1 mg/L.

This case demonstrates the importance of risk factors such as previous hospitalization, antibiotic therapy, and immunosuppression in the development of *C. difficile*-associated diarrhoea (CDAD), all of which were obvious in this patient's medical history.

Although there was no evidence of subsequent severe cases, or of any outbreak situation in the ward or in the ICU, this case emphasizes the importance of timely microbial diagnosis of *C. difficile* as a causative agent of diseases in hospitalized patients and the importance of the need for increasing awareness of the severity of CDAD and its incidence.

Transparency Declaration

This work was supported by grants K69044 from the Hungarian National Research Fund and KMA 0304 the Hungarian National Office for Research and Technology. All authors declare no conflicts of interest.

References

1. Riley TV, Huovinen P, (eds). Infection control measures to limit the spread of *Clostridium difficile*. *Clin Microbiol Infect* 2008; 14 (suppl 5): 1–20.
2. Kuijper EJ, Coignard B, Brazier J *et al.* Update of *Clostridium difficile*-associated disease due to PCR ribotype 027 in Europe. *Euro Surveill* 2007; 12: pii=714.
3. Fenner L, Widmer AF, Stranden A *et al.* First cluster of clindamycin-resistant *Clostridium difficile* PCR ribotype 027 in Switzerland. *Clin Microbiol Infect* 2008; 14: 514–515.
4. McDonald LC, Killgore GE, Thompson A *et al.* An endemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med* 2005; 353: 2433–2441.
5. Warny M, Pepin J, Fang A *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: 1079–1084.
6. Drudy D, Kyne L, Mahony R, Fanning S. *gyr*A mutations in fluoroquinolone-resistant *Clostridium difficile* PCR-027. *Emerg Infect Dis* 2007; 13: 504–505.
7. Kato H, Kato N, Watanabe K *et al.* Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. *J Clin Microbiol* 1998; 36: 2178–2182.