Rapid communications

FIRST CASES OF *CLOSTRIDIUM DIFFICILE* PCR RIBOTYPE 027 ACQUIRED IN AUSTRIA

A Indra (alexander.indra@ages.at)¹, S Huhulescu¹, S Kernbichler¹, HW Kuo^{1,2}, G Feierl³, A Holler³, F Skrabal³, G Tucek⁴, F Allerberger¹

1. Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Vienna, Austria

2. European Programme of Intervention Epidemiology Training (EPIET)

3. Medizinische Universität Graz, Graz, Austria

4. Sozialmedizinisches Zentrum Süd, Vienna, Austria

In Austria, *Clostridium difficile* is the leading cause of community-acquired bacterial diarrheal illness and the most frequently identified cause of hospital-acquired diarrhea [1]. It is the causative agent of pseudomembranous colitis. A new emerging hypervirulent strain of *C. difficile* – PCR ribotype 027 – causes more severe disease and is associated with a higher case-fatality-ratio than other types [2]. This increased virulence is associated with two deletions in a toxin regulator gene resulting in hyperproduction of toxins A and B. The incidence of *C. difficile*-associated disease (CDAD) due to type 027 is increasing in the United States, Canada, Asia and Europe [2,3,4,5,6]. In Austria, *C. difficile* type 027 has so far only been discovered once, in a British tourist with pseudomembranous colitis in 2006 [7]. In April 2008, *C. difficile* PCR ribotype 027 was found in two cases of *C. difficile*-associated disease affecting Austrian citizens treated in hospitals in Vienna and Graz, Austria.

Case reports

The first case, a female hospital employee from Vienna in her forties was treated for chronic paranasal sinusitis with co-amoxiclav (oral, 625mg every eight hours) for 14 days, when she developed severe abdominal pain, diarrhea, cramps and fever. A stool specimen gained in March 2008, one day before admission to Hospital A, yielded *C. difficile* 027. In the year before this admission, she had neither been hospitalised nor visited a healthcare facility as an outpatient. Computer tomography scanning revealed pseudomembranous colitis, a further stool specimen gained during hospitalisation tested positive for *C. difficile* toxin (no culture performed). The patient refused colonoscopy. Since adolescence, the patient shows leucopenia with values around 2,000 cells/microL (range 1,000 to 3,100), which previously was diagnosed to be the consequence of an Epstein-Barr-virus (EBV) infection in childhood. Patient's charts revealed a peak temperature of 40°C, peak leucocytosis of 3,700 cells/microL, peak serum creatinine 1.4 mg/dL (norm: 0.66-1.09 mg/gL), and peak lactat 2.4 mmol/L (norm: 1-2 mmol/L). Serum albumin was normal. The patient was treated with metronidazole (i.v., 500 mg every eight hours) for 17 days. Cefotaxime (i.v., 2 g every 8 hours) was administered additionally from day 3 till 10 of hospitalisation and piperacillin-tazobactam (i.v., 4.5 g every 8 hours) from day 11 till 17 because of fear of "secondary peritonitis". After 20 days, the previously seemingly healthy adult was discharged from hospital.

Apart from attending a conference in Switzerland, no travel abroad was documented since October 2005.

The second case, a male patient in his eighties with indwelling Foley catheter (a flexible latex tube that is passed through the urethra during urinary catheterization and into the bladder to drain urine), living non-institutionalised in Graz and without hospitalisation during the previous three months, suffered diarrheal illness for four weeks, after which his general condition deteriorated. No antibiotics were taken during the last three months. He was admitted to a hospital with the suspicion of pneumonia in February 2008. Under stationary treatment with ceftriaxone (i.v., 2 g/day), the patient, who no longer had diarrhea, developed signs and symptoms of septicemia. The patient further deteriorated and was transferred to an intensive care unit on day five of hospitalisation. There he was treated with imipenem (i.v., 500 mg every six hours). Computer tomography scanning did not reveal signs of pseudomembranous colitis. Patient's charts revealed a peak temperature of 38.5°C, peak leucocytosis of 33,000 cells/microL, normal creatinine, and through serum albumin 4.0 g/L (norm: 37-53 g/L). Lactat was not tested for. A stool culture gained on day 6 of hospitalisation yielded *C. difficile*. Bloodcultures and cultures of respiratory fluids were not performed. His clinical condition worsened and he died on day 7 of hospitalisation, supposedly due to pneumonia. Colonoscopy and post mortem autopsy were not performed. No travel abroad was documented for at least the last 10 years.

Laboratory characterisation

Stool specimens of both patients were positive for *C. difficile*. The isolates were further characterised by the Austrian Agency for Health and Food Safety (AGES) in April 2008 as *C. difficile* PCR ribotype 027 by PCR ribotyping [7]. Both isolates contained the genes for toxin A, toxin B, and the binary toxin. In addition, an 18 base pair deletion in *tcdC* was present. The two isolates were further characterised by multilocus variable-number tandem-repeats analysis (MLVA). The MLVA profiles of the two isolates differed from each other by 18 repeats at locus A, by 9 repeats at locus B, by 4 repeats at locus C, and by 2 repeats at locus E; no data are available for locus G [8]. The differences indicate that the two isolates must not be considered as clonal.

Susceptibility testing by E-tests revealed patterns of clindamycin-susceptible and fluoroquinolone-susceptible type 027; moxifloxacin

resistance was determined using a breakpoint of < 2 µg/ml for susceptible. Disc diffusion tests revealed erythromycin resistance for both isolates. This pattern is different from that of the major European O27 strains [9,10].

Conclusion

Although we hypothesize that both Austrian patients had community-acquired infections of *C. difficile* PCR ribotype O27 and suffered severe illness, important questions remain unanswered for both cases. We cannot exclude that the female hospital employee had a healthcare-acquired infection with community onset. We can not exclude for sure, that the patient in his eighties was a case of merely asymptomatic colonization with a strain acquired in the hospital. There is no consensus definition for severe CDAD, nor is there agreement as to the most important clinical indicators that should be used to differentiate severity [11,12]. Clinicians in the setting of the Quebec outbreak identified a white blood cell count >20,000 cells/microL and an elevated serum creatinine as potential indicators of complicated disease [10]. A group in the United States devised a scoring system to identify patients with severe infection by giving one point each for age >60 years, temperature >38.3°C, serum albumin <2.5 mg/dL (25 g/L), or peripheral white blood cell count >15,000 cells/microL within 48 hours of enrollment; two points were given for endoscopic evidence of pseudomembranous colitis or treatment in the intensive care unit, and patients with two or more points were considered to have severe disease [12].

The number of cases of enterocolitis due to *C. difficile* (ICD10: A04.7) reported by Austrian hospitals has drastically increased during the last few years: from 813 in 2002 (including 55 lethal cases) to 2,192 in 2006 (including 150 lethal cases). This constitutes a 3.7-fold increase of the number of reported cases with CDAD between 2002 and 2006. The potential problem of reporting bias for gastroenteric diseases has been addressed recently [13]. However, Burckhardt et al. even reported a six-fold increase of the yearly average of *C. difficile* incidence rates for a province in neighboring Germany for the same period of time [14].

The occurrence of autochthonous cases of *C. difficile* PCR ribotype O27 is just another argument underlining the urgent need for the implementation of a *C. difficile* surveillance system throughout Austria. Given the epidemic potential and the severity of the disease, especially among the elderly, surveillance of *C. difficile* must be introduced along with enhanced prevention and treatment strategies [15]. We strongly advise microbiological laboratories to perform microbiological cultures on toxin-positive stool samples and to apply typing methods on all isolates of severe clinical cases of *C. difficile* associated disease. For laboratories using capillary-sequencer-based PCR ribotyping, the web based WEBRIBO@-system (<http://webribo.ages.at>) allows fast and reliable identification of PCR-ribotype patterns.

Acknowledgments

We are grateful to Jon Brazier from the University Hospital of Wales (Anaerobe Reference Laboratory, NPHS Microbiology Cardiff) for confirming the microbiological results.

References

- Huhulescu S, Kiss R, Brettlecker M, Cerny RJ, Hess C, Wewalka G, Allerberger F. (2008) Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection*. Submitted.
- Kuijper EJ, Coignard B, Brazier J, Suetens C, Drudy D, Wiuff C, et al. Update of *Clostridium difficile*-associated disease due to PCR ribotype O27 in Europe. *Euro Surveill*. 2007;12(6):pii=714. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=714>
- Vonberg R, Schwab F, Gastmeier P (2007) *Clostridium difficile* in discharged inpatients, Germany. *Emerg Infect Dis* 13: 179-180.
- Indra A, Schmid D, Huhulescu S, Hell M, Gattringer R, Hasenberger P et al. Characterization of clinical *Clostridium difficile* isolates by PCR-ribotyping and detection of toxin genes – Austria, 2006/2007. *J Med Microbiol* 57: 1-7
- McDonald LC, Owings M, Jernigan DB (2006) *Clostridium difficile* infection in patients discharged from US short-stay hospitals, 1996-2003. *Emerg Infect Dis* 12: 409-415.
- Kato H, Ito Y, van den Berg R, Kuijper E, Arakawa Y. First isolation of *Clostridium difficile* O27 in Japan. *Euro Surveill*. 2007;12(2):pii=3110. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3110>
- Bidet P, Barbut F, Lalande V, Burghoffer B, Petit J-C (1999) Development of a new PCR-ribotyping method for *Clostridium difficile* based on ribosomal RNA gene sequencing. *FEMS Microbiol Lett* 175: 261-266.
- Van den Berg RJ, Schaap I, Templeton KE, Klaassen CH, Kuijper EJ (2007) Typing and subtyping of *Clostridium difficile* isolates by using multiple-locus variable-number tandem-repeat analysis. *J Clin Microbiol* 45: 1024-1028.
- Barbut F, Mastrantonio P, Delmée M, Brazier J, Kuijper EJ, Poxton I (2007) Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterization of the isolates. *Clin Microbiol Infect* 13: 1048-1057.
- Fenner L, Widmer AF, Stranden A, Conzelmann M, Goorhuis A, Harmanus C, Kuijper EJ, Frei R (2008) First cluster of clindamycin-resistant *Clostridium difficile* PCR ribotype O27 in Switzerland. *Clin Microbiol Infect* 14: 514-517.
- Pépin J, Valiquette L, Alary ME, Villemure P, Pelletier A, Forget K, Pépin K, Chouinard D. (2004) *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 171: 466-472.
- Zar FA, Bakkanagari SR, Moorthi KM, Davis MB (2007) A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. *Clin Infect Dis* 45: 302-307.
- Wilcox M, Fawley W (2007) Viral gastroenteritis increases the reports of *Clostridium difficile* infection. *J Hosp Infect* 6: 395-396.
- Burckhardt F, Friedrich A, Beier D, Eckmanns T (2008) *Clostridium difficile* surveillance trends, Saxony, Germany. *Emerg Infect Dis* 14: 691-692.
- Vonberg RP, Kuijper EJ, Wilcox MH, Barbut F, Tüll P, Gastmeier P, on behalf of the European *C. difficile*-Infection Control Group and the European Centre for Disease Prevention and Control (ECDC), van den Broek PJ, Colville A, Coignard B, Dahan T, Debast S, Duerden BI, van den Hof S, van der Kooij T, Maarleveld HJH, Nagy E, Notermans DW, O'Driscoll J, Patel B, Stone S, Wiuff C (2008) Infection control measures to limit the spread of *Clostridium difficile*. *Clin Microbiol Infect* 14 (Suppl. 5): 2-20.

This article was published on 15 May 2008.

Citation style for this article: Indra A, Huhulescu S, Kernbichler S, Kuo H, Feierl G, Holler A, Skrabal F, Tucek G, Allerberger F. First cases of *Clostridium difficile* PCR ribotype O27 acquired in Austria. *Euro Surveill*. 2008;13(20):pii=18875. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18875>