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Introduction

Clostridium difficile is a Gram-positive spore-forming anaerobe that can be found in the stomach and the intestines of healthy people. There are two forms of *C. difficile* bacteria – an active form that cannot survive in the environment for long periods of time and the dominant form, a spore, which can survive for a long period of time. Spores are very difficult to remove from surfaces and therefore can contaminate the environment by living on them for weeks to months. Spores cause infection after they have been ingested and have germinated into the active form of *C. difficile*. When the normal flora of the intestinal tract is disrupted (e.g. with antibiotics), *C. difficile* can multiply and produce toxins that cause mild to very severe diarrhea known as *Clostridium difficile* infection or CDI [1,2].

Clostridium difficile infection is prevalent in health-care facilities and presents the leading cause of the antibiotic-associated nosocomial diarrhea and colitis in the industrialized world. Less often, it is acquired in the community from an unknown source. The main risk factors for developing CDI are advanced age, (over 65 years), with comorbidity and the previous use of antibiotics. Administration of the broad-spectrum antibiotics leads to the elimination of the healthy microflora of the gut, followed by the loss of colonization resistance, which may allow microbes such as *C. difficile* to colonize, adhere and replicate to levels that cause disease [2,3]. According to the Centre for Disease Control (CDC), 1 in 20 hospitalized patients will acquire a health care-associated infection, and while most

Case Report

The First Evidence of Epidemic Strain *Clostridium Difficile* (027/NAP1/BI) in Eastern Croatia

Abstract

A case of the first evidence of epidemic strain *Clostridium difficile* (027/NAP1 (BI) in a patient in Slavonia region (Eastern Croatia) is presented. *Clostridium difficile* infection presents the leading cause of the antibiotic-associated nosocomial diarrhea and colitis in the industrialized world. PCR-ribotype 027 is a hypervirulent strain with great epidemic potential and since 2005 spread to European countries. A study published in 2011 years did not prove the presence of ribotype 027 in Croatia.

health care-associated infections such as methicillin-resistant *Staphylococcus aureus* are decreasing, *C. difficile* rates continue to rise rapidly [4].

At the beginning of the 21st century increasing rates of *C. difficile* infection have been reported in Northern Hemisphere, first in the United States of America (USA) and Canada. The prevalence of hospital-acquired *Clostridium difficile* infection (HA-CDI) has recently increased with the spread of the hypervirulent, fluoroquinolone-resistant strains belonging to the Polymerase Chain Reaction (PCR) – ribotype 027 [5,6]. Ribotype 027 was the causative agent of the largest *C. difficile* epidemic recorded to date, in which over 2 000 fatalities occurred in Quebec, Canada during 2005. Reports of this outbreak described much higher rates of morbidity and mortality associated with the ribotype 027 than with the other, typical endemic strains (such as ribotype 078). Because of its epidemic potential ribotype 027 is called “hypervirulent” strain. Clear disambiguation between the hypervirulent and typical strains is currently precluded by the incomplete understanding of what causes some strains to generate outbreaks with substantial morbidity [7]. In addition to the usual toxins A and B, these fluoroquinolone-resistant strains produce a binary toxin.

In Europe, PCR-ribotype 027 was first reported in 2005 in England and shortly after in the Netherlands [8,9]. In 2005, when performing a Europe-wide survey of 38 hospitals in 14 countries, the European Study Group of *C. difficile* found a novel ribotype 027/NAP1/BI in Ireland, the Netherlands and Belgium. Within 3 years this PCR ribotype had spread to at least 16 European countries. Epidemics of CDI caused by the PCR-ribotype 027 have been reported in hospitals in many European countries [10-12]. In November, 2008, European *Clostridium*

difficile infection study group (ECDIS) set up a network of 106 laboratories in 34 European countries, in one to six hospitals per country, and tested the stool samples of the patients with suspected *C. difficile* infection. That study showed that PCR ribotypes other than 027 are prevalent in the European hospitals and not one stool from Croatia was positive for 027 ribotype [13].

We report a case of hospital-acquired colitis with diarrhea caused by *Clostridium difficile* PCR- ribotype 027 in a female patient in general hospital in Slavonia, middle-east region of Croatia.

Clinical case management

In March 2016, 75-year-old woman was brought to the emergency room of Slavonski Brod, General Hospital due to long-term pain in the lower abdomen which had amplified in recent weeks, with the more than three watery stools a day and a frequent urge to vomit. Her medical history showed that in December 2015 she was admitted to the Osijek, Clinical Hospital for aortic valve surgery. After surgery, she was released to home care, but was readmitted to the Clinical Hospital in Osijek due to abdominal pain accompanied by watery stool. Laboratory tests indicated the elevated C-reactive protein (CRP) – 363 mg/L, Leukocytes – $20.00 \times 10^9/L$, and were negative for *Clostridium difficile* with Enzyme Immunoassay Method (EIA). She was treated with amoxicillin-clavulanic acid, ciprofloxacin, metronidazole and oseltamivir, and there was a short-term improvement of the patient's condition. After she was discharged from hospital, the patient's condition got worse again and after 16 days at home was brought to the emergency room of the General Hospital in Slavonski Brod with the aforementioned symptoms. Laboratory tests indicated elevated CRP – 158.5 mg/L, Leukocytes – $18.36 \times 10^9/L$ and the stool examination was positive for *Clostridium difficile* toxins. After confirming the presence of *Clostridium difficile*, oral vancomycin therapy was introduced. The patient's condition improved and she was discharged to home care.

Microbiological investigation and hospital policy

General Hospital „Dr Josip Bencevic“ is the biggest Croatian general hospital with 575 beds, settled in middle-east Slavonia region, on the Sava river, which is the natural border with the Republic of Bosnia and Herzegovina. Hospital policy is such that surveillance samples are taken from all newly admitted patients, primarily those coming from large teaching hospitals and/or hospitals in other cities. The samples are taken from defined anatomical areas such as swab of vestibulum nasi, axillary swab, and oropharyngeal swab, urine in patients with catheter, wound swab after surgery, stool and/or rectal swab in patients with diarrhea or other intestinal symptoms.

Microbiological investigation of *C. difficile* in our laboratory is a two-step testing process recommended by The Society for Healthcare Epidemiology of America (SHEA), Infectious Diseases Society of America (IDSA) and accepted by CDC and European Centre for Disease Prevention and Control (ECDC) [14].

First step in *C. difficile* diagnosis is the detection of *C. difficile* antigen, glutamate dehydrogenase (GDH), by the automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) principles (mini VIDAS®, BioMérieux, France). This test detects an antigen that is produced in high amounts by *C. difficile*, both the toxin and non-toxin producing strains. Our patient had positive GDH antigen (TV neg 0.10 – TV poz 0.10) with value 7.11.

Positive results are followed by PCR assay Xpert® *C. difficile* (Cepheid, USA). Xpert *C. difficile* /Epi is the first commercially available test in the world for detecting and differentiating the epidemic strain of *C. difficile* (027/NAP1/BI – the strain, which is restriction endonuclease analysis group BI, pulse-field gel electrophoresis type NAP1, and polymerase chain reaction ribotype 027, is designated BI/NAP1/027). This additional testing showed the presence of the toxigenic *C. difficile* and the presumptive positivity of the 027 ribotype.

After the presence of the toxigenic *C. difficile* in the stool had been confirmed, the patient was moved to a single room at the Department of Infectious Diseases, with the instructions for healthcare workers about the contact isolation and enhanced disinfection of room. Hospital room in which the patient previously resided was disinfected with glutaraldehyde, as well as the room to which she was moved. In the therapy was given oral vancomycin, and patient was required to remain in the single room until she had passed normal stool for 72 hours.

Discussion

C. difficile spores are highly desiccation resistant and can persist on hard surfaces for more than 5 months. The accumulation of spores over the bacterial growth cycle demonstrated that hypervirulent strains sporulated earlier and accumulated significantly more spores per total volume of culture than non-hypervirulent strains. Increased rate of sporulation may explain the observation of unusually high relapse rates associated with hypervirulent strains because patients are more likely to contaminate their local environment and subsequently re-infect themselves. Conducted studies have shown three significant differences between the hypervirulent 027 ribotype and other non-hypervirulent strains; hypervirulent strains are more infectious than the epidemic strains; they result in higher rate of symptomatic disease, and outcompete endemic strains in the host's gut [7,15,16]. Due to the dramatic change in the epidemiology of CDI in the last two decades, with the rise of incidence and outbreaks in large and small hospitals, microbiological laboratories must be prepared for these diagnostic challenges, and aware of the limitations of certain diagnostic methods used in previous years. For these reasons, molecular tests in the diagnosis of CDI become necessary.

GDH is considered to be very sensitive, but it is not very specific for toxin-producing *C. difficile*. This test indicates if *C. difficile* is present but like bacterial culture do not distinguish toxin-producing from non-toxigenic *C. difficile* isolates. The reason for this approach is that GDH is produced in significantly higher quantities than the *C. difficile* toxin and should yield a more sensitive assay than the solid-phase toxin A/B EIA's. The commercial GDH tests offer a turnaround time

of 15–45 min, use it as a screening test to distinguish between the negative and the positive sample, and then go for further processing. Numerous studies have proved that approximately 20 % of the patients who are positive for the GDH antigen of *C. difficile* carry a non-toxigenic strain of *C. difficile* [17]. In our patient, confirmation test was carried out on Xpert® platform, a very practical and very simple Real-time Polymerase Chain Reaction (RT-PCR) device suitable for small laboratories with a lot of samples and a small number of employees. This is a rapid and very sensitive method for confirming the presence of the *C. difficile* toxin, but because of the very high cost of the test, it is reasonable to perform the GHD screening test first. Before PCR assay we used ELFA method (EIA test), but it is not sensitive enough and misses up to 30 % of cases. However, this PCR assay is not recommended for use by some professional organizations [17,18].

C. difficile spores are transmitted via fecal/oral pathway; they are ubiquitously present on inanimate objects, resistant to commonly used decontaminants and can persist for a long period of time in the spore form without the loss of viability. The resistance of the hypervirulent *C. difficile* strain BI/NAP1/027 spores to regular disinfectants has been researched in different studies. The effectiveness of chlorine-releasing agents, peroxygen-releasing agents and quaternary ammonium compounds in spore killing has been compared and chlorine-releasing agents are proved to be more efficient than any other disinfectant for killing spores. But, some previously conducted studies point to the need to apply different types of disinfection depending on the frequency and incidence rates of *C. difficile* in the hospital, so we choose glutaraldehyde for the room disinfection due to low rates of CDI in our hospital, <3.0 cases per 1000 patient-days [19,20]. Hand hygiene which includes washing with soap and water as opposed to alcohol-based hand rubs was recommended during the Intrahospital Infection Control Team (IHI-Team) visit of the department with the CD 027 positive patient. Infection control barriers, antimicrobial stewardship, education of health-care workers and patients can lead to reduction in CDI incidence and decrease in severity, but first step in CDI control is the availability of specific and sensitive diagnostic platforms that must be part of every microbiological laboratory today.

References

- Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J Jr, (1995) *Clostridium difficile* - associated diarrhea and colitis. *Infect Hosp Epidemiol* 16: 459–477. [Link: https://goo.gl/OVCi0l](https://goo.gl/OVCi0l)
- Kuijper EJ, Coignard B., Töll P (2006) *Clostridium difficile* – associated disease in North America and Europe. *Clin Microbiol Infect* 12: 12–18. [Link: https://goo.gl/CLWoRI](https://goo.gl/CLWoRI)
- Lawley TD, Walker AW (2013) Intestinal colonization resistance. *Immunology* 138: 1–11. [Link: https://goo.gl/081ghU](https://goo.gl/081ghU)
- Miller BA, Chen LF, Sexton DJ, Anderson DJ (2011) Comparison of the burdens of hospital – onset, healthcare facility-associated *Clostridium difficile* Infection and of healthcare-associated infection due to methicillin-

resistant *Staphylococcus aureus* in community hospitals. *Infect Control Hosp Epidemiol* 32: 387–390. [Link: https://goo.gl/p7kD6s](https://goo.gl/p7kD6s)

- Warny M, Pepin J, Fang A, Killgore G, Thompson A, et al. (2005) Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 366: 1079–1084. [Link: https://goo.gl/p3FBVl](https://goo.gl/p3FBVl)
- McDonald LC, Killgore GE, Thompson A, Robert C. Owens Jr, et al. (2005) an epidemic, toxin gene-variant strain of *Clostridium difficile* –associated diarrhea with high morbidity and mortality. *N Engl J Med* 353: 2442–2449. [Link: https://goo.gl/IT6pPO](https://goo.gl/IT6pPO)
- Laith Y, Riley TV, Paterson DL, Marquess J, Soares Magalhaes RJ, et al. (2015) Mechanisms of hypervirulent *Clostridium difficile* ribotype 027 displacement of endemic strains: an epidemiological model. *Scientific Reports*. [Link: https://goo.gl/TJqzTs](https://goo.gl/TJqzTs)
- Smith A (2005) Outbreak of *Clostridium difficile* infection in an English hospital linked to hypertoxin-producing strains in Canada and the US. *Euro Surveill*. [Link: https://goo.gl/Ae5aHY](https://goo.gl/Ae5aHY)
- Kuijper EJ, van den Berg RJ, Debast S, Caroline EV, Dick V, DV, et al. (2006) *Clostridium difficile* ribotype 027, toxinotype III, the Netherlands. *Emerg Infect Dis* 12: 827–830. [Link: https://goo.gl/Kh2fyf](https://goo.gl/Kh2fyf)
- Barbut F, Mastrantonio P, Delmée M, Brazier J, Kuijper E, et al. (2007) Prospective study of *Clostridium difficile*-associated disease in Europe with phenotypic and genotypic characterisation of the isolates. *Clin Microbiol Infect* 13: 1048 – 1057. [Link: https://goo.gl/lKnV7S](https://goo.gl/lKnV7S)
- Kuijper EJ, Barbut F, Brazier JS, Kleinkauf N, Eckmanns T, et al. (2008) Update of *Clostridium difficile* infection due to PCR ribotype 027 in Europe, 2008. *Euro Surveill*. [Link: https://goo.gl/coL96c](https://goo.gl/coL96c)
- Søes L, Mølbak K, Strøbaek S, Truberg JK, Torpdahl M, et al. (2009) The emergence of *Clostridium difficile* PCR ribotype 027 in Denmark – a possible link with the increased consumption of fluoroquinolones and cephalosporins? *Euro Surveill*. [Link: https://goo.gl/SPnaKn](https://goo.gl/SPnaKn)
- Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, et al. (2011) *Clostridium difficile* infection in Europe: a hospital-based survey. *Lancet* 377: 63–73. [Link: https://goo.gl/mtF0zP](https://goo.gl/mtF0zP)
- Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, et al. (2010) Clinical Practice Guidelines for *Clostridium difficile* Infection in adults: Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect Control Hosp Epidemiol* 31: 431–455. [Link: https://goo.gl/bZ04ls](https://goo.gl/bZ04ls)
- Marsh JW, Arora R, Schlackman JL, Shutt KA, Curry SR, et al. (2012) Association of Relapse of *Clostridium difficile* Disease with BI/NAP1/027. *J Clin Microbiol* 50: 4078–4082. [Link: https://goo.gl/XzPLEI](https://goo.gl/XzPLEI)
- Robinson CD, Auchtung JM, Collins J, Britton R (2014) Epidemic *Clostridium difficile* strains demonstrate increased competitive fitness over non-epidemic isolates. *Infect Immun* 82: 2815–2825. [Link: https://goo.gl/11FM0T](https://goo.gl/11FM0T)
- Goldenberg SD, Cliff PR, French GL (2010) Glutamate Dehydrogenase for Laboratory Diagnosis of *Clostridium difficile* Infection. *J Clin Microbiol* 48: 3050–3051. [Link: https://goo.gl/wrJzsV](https://goo.gl/wrJzsV)
- Bassetti M, Villa G, Pecori D, Arzese A, Wilcox M (2012) Epidemiology, Diagnosis and Treatment of *Clostridium difficile* Infection. *Expert rev Anti infect Ther* 10: 1405–1423. [Link: https://goo.gl/GAFJcN](https://goo.gl/GAFJcN)
- Mayfield JL, Leet T, Miller J, Mundy LM (1998) Environmental Control to Reduce Transmission of *Clostridium difficile*. *Clin Infect Dis* 31: 995–1000. [Link: https://goo.gl/P3KtQK](https://goo.gl/P3KtQK)
- Ghose C (2013) *Clostridium difficile* infection in the twenty-first century. *Emerging microbes & Infections*. 2: e62. [Link: https://goo.gl/chSuUr](https://goo.gl/chSuUr)

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