Changing CDI epidemiology in a Croatian University Hospital (2011-2016)

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University of Split School of Medicine
Universitas Studiorum Spalatensis Facultas Medica

Anita Novak*[1,2] Žana Rubić[1,2] Ivana Goic Barisic[1,2] Ivana Mareković[3,4] Ana Budimir[3,4] Ante Tonkic[1,2] Marija Tonkic[1,2]

[1] University of Split, School of Medicine Split, Croatia • [2] University Hospital of Split, Croatia • [3] University Hospital Centre Zagreb, [4] University of Zagreb, School of Medicine

* Presenting author: anitanovak1@net.hr

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Background: The epidemiology of *Clostridium difficile* infection (CDI) has been dramatically changed over the last two decades, mostly because the emergence of hypervirulent strains (PCR-ribotypes 027, 078, and more recently 176). Those ribotypes are associated with higher rate of severe CDI, recurrences and mortality (1, 2, 3). During the first Croatian CDI study, performed in 2010-2011 in University Hospital of Split, 16 different PCR-ribotypes were identified. The most common PCR-ribotypes were 001 and 014/020, while hypervirulent strains were not detected (4). However, in study performed in 2016, in another Croatian healthcare setting, outbreak caused by PCR-ribotype 176 was described (5). The aim of this study was to determine changes in CDI epidemiology in a University Hospital of Split (UHS), Croatia, in the light of improved diagnostic approach and increased clinical awareness.

Materials/methods: During the study period (January-December 2016), all faecal specimens received in Department of Clinical Microbiology, UHS, from patients with a clinical picture compatible with CDI, were tested for the presence of glutamate dehydrogenase (GDH) and free *C. difficile* toxins A/B, using combined rapid chromatographic immunoassay card (CoproStrip *C. difficile* GDH + Toxin A/B, Savyon Diagnostics, Israel), according to the manufacturer's instructions. According to ESCMID guidelines, GDH positive, ToxinA/B negative strains were further tested with molecular tests, Loop-Mediated Isothermal Amplification, LAMP, (*Ilumigene C. difficile*, Meridian Bioscience, Italy) for the presence of toxigenic strain (6). All toxigenic samples were cultured on commercial selective *C. difficile* agar plates (CLO, bioMérieux, Marcy l´Etoile, France), with alcohol pretreatment. The plates were incubated in an anaerobic jar at 37°C for 48 h. Strains were identified as *C. difficile* according to colony morphology, Gram staining and commercial identification system (Vitek 2 Compact ANC Card bioMerieux). Identification was confirmed with MALDI-TOF (Bruker) in University Hospital Centre Zagreb. All isolated strains were stored at -80°C. A CDI case was defined as a patient with diarrhoea and microbiological evidence of active *C. difficile* toxin A or/and B production in stool. Ten randomly selected strains were sent for ribotyping to collaborative abroad laboratory (Leiden University Medical Center, NL) and seven of them were suitable for capillary gel-based electrophoresis, CE-ribotyping, (Life Technologies, Paisley, UK). The results were compared with those obtained in previous studies, performed during 2010-2012 and 2015.

Results: A total of 1912 diarrhoeal stool samples were tested by a rapid test and 499 CDI cases were laboratory confirmed. Eight stool samples harbored untoxigenic *C. difficile* strain, while toxigenic strain without active toxin production was detected in 19 samples (Fig1). Testing rate was significantly higher than in previous studies; 8.1, 7.5, 6.2 and 1.4 times than in 2010, 2011, 2012 and 2015, (Fig 2).

Three different PCR-ribotypes were identified for the first time: 176, 027 and 220, while in 2010-2011, the most prevalent PCR-ribotypes were 001 and 014/020.

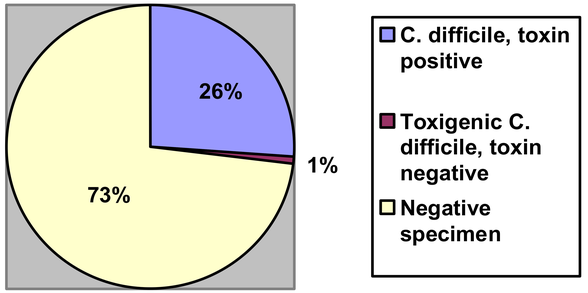

Figure 1. *Clostridium difficile* testing results.

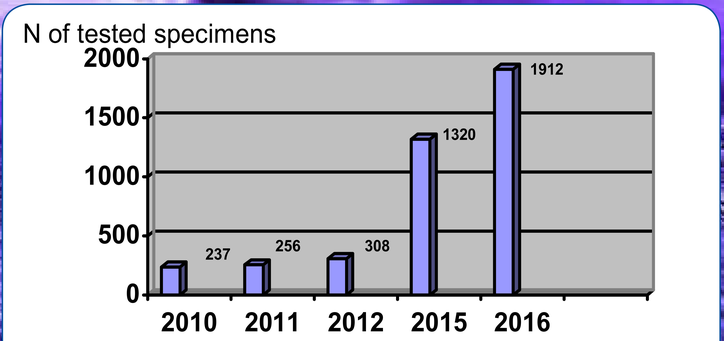

Figure 2. *Clostridium difficile* testing rate.

Conclusions: The emergence of hypervirulent PCR-ribotypes 176 and 027, which are associated with severe CDI, higher rate of mortality and recurrences, highlights the need for continuous CDI surveillance on a local and national level. In order to determinate the true evolution of *C. difficile* strains over the space and time, establishing of a national reference center for *C. difficile* ribotyping is strongly recommended. Continuous raising testing rate indicates better awareness among clinicians which implicate better healthcare practice and final outcome. Even though the rate of toxigenic strain without active toxin production was small, it's still worth mentioning. CDI-related complications may occasionally occur among toxin-negative patients diagnosed by PCR. Therefore, careful clinical evaluation among them is strongly recommended (7).

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