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RISK FACTORS FOR *CLOSTRIDIUM DIFFICILE* INFECTIONS IN BARANYA COUNTY, SOUTHERN HUNGARY

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In the past decade, *Clostridium difficile* infections (CDIs) have become a major public health challenge. Their epidemiology has radically changed with a significant rise in the number of cases and an increase in severe episodes. Recurrence and failure of conventional treatments are very common. Furthermore, a spread of CDI has emerged in general population without the usual risk factors (unexposed to antibiotic treatment, young people, etc.). The conventional treatments (metronidazole and vancomycin) are still effective and are the first-line antibiotics with new recommendations. New therapeutic strategies are now available. Recent studies show a better efficacy of vancomycin compared with metronidazole for severe episodes. Fidaxomicin is a novel antibiotic drug with an efficacy similar to vancomycin and a lower risk of recurrence. Finally, for relapsing forms, fecal microbiota transplantation (FMT) seems to be the best option. We determined risk factors for CDI among patients treated at the infectious diseases ward of our hospital in Pécs. The study included 886 patients with CDI from 2009 to 2014. The average number of recurrent episodes was 2.16 and the proportion of severe cases was 66%. Among our patients, 726 (82%) had taken antibiotics and 769 (86.8%) had been hospitalized in the prior 3 months before developing CDI. We have found that prior statin use could be a significant risk factor of CDI (OR: 1.7765, 95% CI: 1.3966–2.2597, $p < 0.0001$). Finally, we present the comparative efficacy of different types of treatment (metronidazole, vancomycin, fidaxomicin, and FMT).

Keywords: severe *Clostridium difficile* infection, risk factors, proton pump inhibitors, statin intake, fecal microbiota transplantation

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Introduction

Clostridium difficile, a Gram-positive, obligate anaerobic, spore-forming bacterium, was first isolated from the feces of healthy neonates in 1935 [1]. Since the association between this agent and antibiotic-associated diarrhea has been recognized in 1978 [2], the interest in this pathogen is increasing. The incidence of *C. difficile* infections (CDIs) has increased to epidemic proportion over the past decades worldwide. Between 1996 and 2003, the prevalence of CDI in the USA doubled, reaching 61 per 100,000 [3], and the rate of CDI listed as any diagnosis of hospitalized patients rose from 3.82 per 1,000 discharges in 2000 to 8.75 per 1,000 discharges in 2008 [4]. Clinicians noticed a larger proportion of severe and recurrent cases occurring in these countries than previously reported. The increasing incidence of such infection can be partly explained by the spread of fluoroquinolone-resistant strains belonging to the PCR ribotype 027, the production of binary toxin [5–7]. The European Study Group of *C. difficile* reported the mean incidence of healthcare-associated CDI as 4.1 per 10,000 hospital patient days [8, 9]. Subsequently, epidemics caused by PCR ribotype 027 strains have been recognized in the hospitals in many European countries [10]. The changing epidemiology of CDI is well illustrated with the recent reports of community-associated infection in patients without predisposing conditions [11]. In a hospital-based survey, Bauer et al. [12] identified 65 different ribotypes from isolates, of which 014/020 (16%), 001 (9%), and 078 (8%) were the most prevalent, whereas 027 was detected in 5% of isolates. PCR ribotypes 018 and 056 were significantly associated with poor disease outcome [12]. In Hungary, we have experienced that the CDI outbreak has been started in 2009. These facts inspired us to evaluate the changing epidemiology of CDIs in our ward.

Patients and Methods

We conducted a case–control study at the infectious diseases ward of the Clinics of University of Pécs. A CDI patient was defined as an adult with at least three non-formed stool per day and positive test results for *C. difficile* toxin. Stool samples were collected at the department and were sent to the stool laboratory of the National Public Health and Medical Officer Service of Baranya County. At the first period, only ELISA tests for detecting toxins and culture were available. Both examinations were performed by the laboratory in each of the cases. Later, C. Diff Quik Chek Complete[®] (TechLab Inc., 2001 Kraft Drive Blacksburg, VA, USA) was introduced. This is a rapid membrane enzyme immunoassay for the simultaneous detection of *C. difficile* glutamate

dehydrogenase antigen and toxins A and B in a single reaction well. Cultivation was done only in those cases where the toxin assay had been found negative.

According to international guidelines, severe disease was defined as CDI being in an intensive care unit, the presence of pseudomembranes upon endoscopic examination, or the existence of two or more of the following factors: fever of more than 38.3 °C, albumin of less than 2.5 mg/dl, white blood cell count of more than 15,000 μ^{-1} , or a serum creatinine level greater than or equal to 1.5 times the premorbid level within 48 h of admission.

Controls were randomly selected from those patients of the same ward who were hospitalized with diagnosis other than CDI. Exclusion criteria were diarrhea in the past 3 months or previous CDI in the past medical history. Data were collected on arrival of the patients between January 1, 2009 and December 31, 2014. Medical and laboratory records were retrospectively reviewed both from case and control patients. Univariate analysis using *t*-tests for continuous variables and the chi-square or Fisher's exact test for categorical variables identified risk factors in patients with CDI. A two-tailed $p \leq 0.05$ was considered statistically significant. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for categorical variables.

Results

In this 6-year period, 6,218 hospitalizations were made, of whom 886 (14.2%) cases were CDI-associated. Rates of CDI have been gradually increasing in our department. It was rare as a given diagnosis in 2009, it occurred in 271 of our 1,047 patients (25.9%) in 2013 (Figure 1). From the total of 886 cases, 493 (55.6%) were females and 393 (44.4%) were males. The mean age of the patients was found to be 70.1 years with the range of 26–92 years. The average number of recurrent episodes was 2.16 and the proportion of severe cases was 66%. Exposure to antibiotics (OR: 12.9807, 95% CI: 10.1172–16.6547, $p < 0.0001$) and admission to a healthcare facility have been found to be the two biggest risk factors. Other factors were proton pump inhibitor (PPI) intake (OR: 6.8598, 95% CI: 5.4055–8.7055, $p < 0.0001$) and statin intake (OR: 1.7765, 95% CI: 1.3966–2.2597, $p < 0.0001$).

Discussion

Patients with CDI typically have extended lengths-of-stay in hospitals, and CDI is a frequent cause of large hospital outbreaks of disease [13]. The data

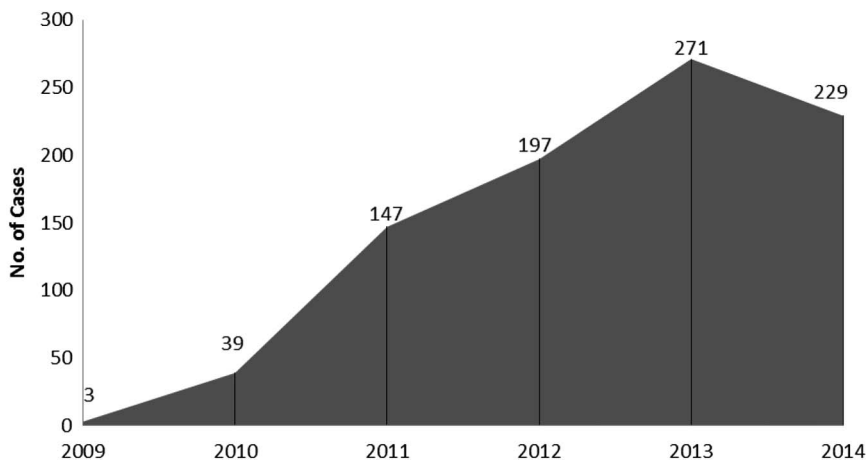


Figure 1. Number of diagnosed CDI cases in our department between 2009 and 2014

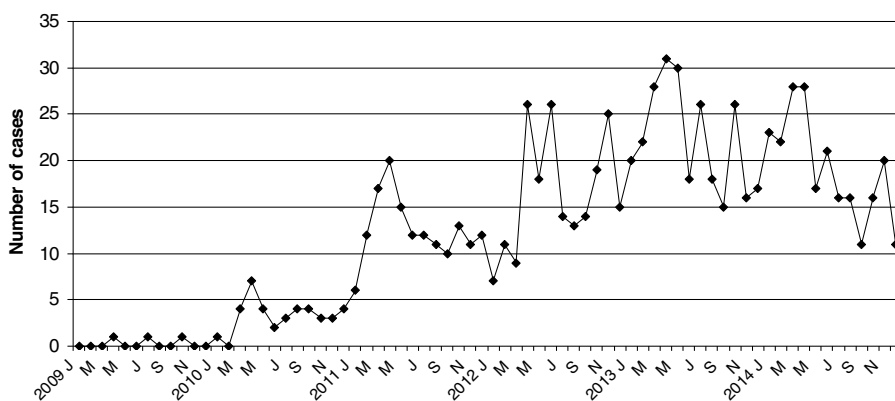


Figure 2. Reported CDI cases by month

collected from the database of our ward have shown that CDI outbreak has reached Southern Hungary in 2010.

The monthly distribution of CDI cases is shown in Figure 2. We used a geometrical model based on the harmonic technique of Edwards to examine seasonal variation in CDI occurrence. Analysis was performed by using *Episheet.xls*[®]. The peak-to-low ratio was 1.44 (95% CI: 1.188–1.764) and the peak was on 27th April. A small burden of cases can be recognized during spring–early summer in each year. It can be explained by the higher antibiotics intake of the population during the previous months to treat upper respiratory tract infections.

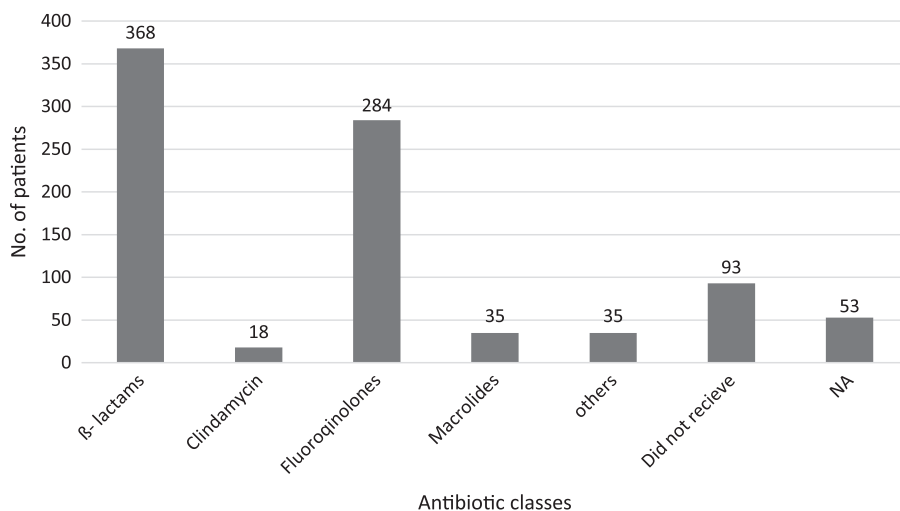


Figure 3. Occurrence of *Clostridium difficile*-associated diarrhea, by type of preceding antibiotic

Of the total 886 CDI cases, 726 (83.50%) had taken antibiotics in the preceding 3 months (OR: 12.9807, 95% CI: 10.1172–16.6547, $p < 0.0001$) (Figure 3). We found that 226 (25.51%) cases were community-associated CDI (Figure 4). Figure 5 shows the proportion of patients by different age groups.

About 64.8% of the patients have taken PPI (OR: 6.8598, 95% CI: 5.4055–8.7055, $p < 0.0001$) and 32.1% of them have taken statin (OR: 1.7765, 95% CI: 1.3966–2.2597, $p < 0.0001$). These findings correlate with international epidemiological studies, of which many have found an association with PPI and CDI [14, 15], and this association is confirmed and the evidence that the PPI use increases the risk of CDI is strengthened by the recent meta-analyses [16–18]. The effect of statins on the evolution of CDI is not clear. Several studies suggest that prior statin use can be a protective factor or can be associated with successful treatment response [19–21]. Saliba et al. [22] have found that the 30-day mortality rate was lower among current statin users 89/669 (13.3%) compared with 251/1219 (20.6%) in non-users ($p < 0.001$). Some other papers have not found such an association [23] or suggest that statin intake can be a risk factor and that statins interact with *C. difficile* toxins A and B causing an increase in the rate and severity of *C. difficile*-associated disease (CDAD) [24]. However, it is important to note that further examinations are needed to prove this hypothesis.

It is important to note that one of the major limitations of this study is the lack of information regarding the distribution of the different strains. Collecting any information regarding the distribution of different ribotypes was impossible, because ribotyping is not a routine test conducted by the laboratory, and our

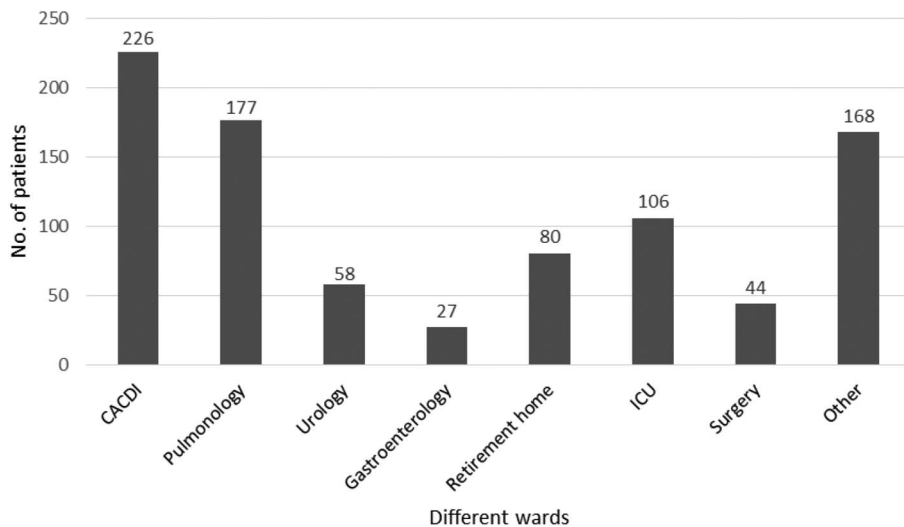


Figure 4. Occurrence of *Clostridium difficile*-associated diarrhea, by type of hospital ward

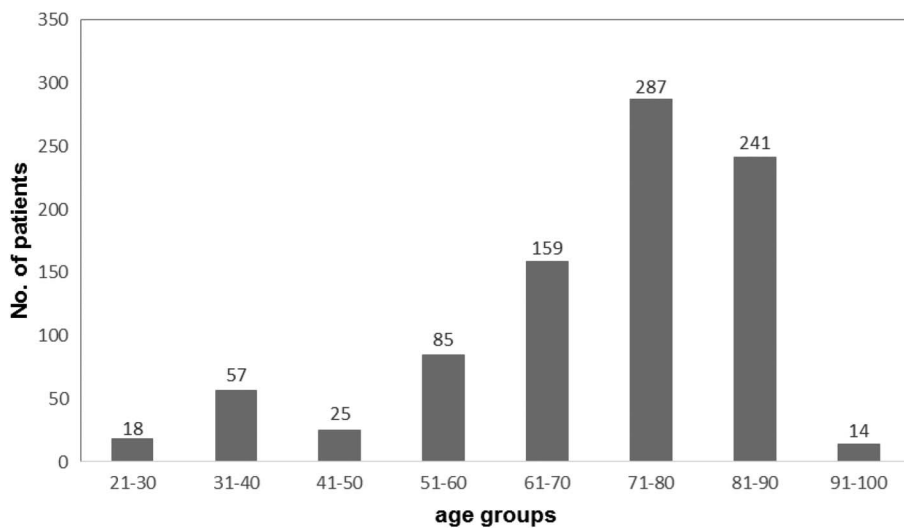


Figure 5. Number of cases by different age groups

investigation was retrospective. However, data from Hungary are available in the international literature [25, 26]. Terhes et al. [26] have examined 150 fecal samples collected from patients suffering from in the southeastern part of Hungary. About 80% of the isolates were found to be positive to both *tcdA* and

ctdB. Eight isolates (5.3%) harbored the cdtB gene responsible for the production of binding component of binary toxin. Only one strain showed the pattern, which was identical to PCR ribotype 027, four strains belonged to PCR ribotype 078, and the remainder was PCR ribotype 131 [26].

Treatment of CDI is very complicated, mainly in those cases where conventional approaches fail to resolve the problem. Recently presented papers showed that overall metronidazole was inferior to vancomycin, and there is also an evidence of inferior microbiological efficacy of the previous drug [27]. In addition, Musher et al. [28] have found that metronidazole therapy alone has a relatively poor outcome. Later, a systematic literature revealed that fidaxomicin provides improved sustained cure rates in patients with CDI compared with vancomycin and metronidazole [29, 30]. Another analysis has shown that metronidazole, as the first-line treatment for CDIs, is less costly. Fecal microbiota transplantation (FMT) and vancomycin are more effective [31]. We have experienced that in 652 cases (73.6%), metronidazole alone was effective and in 90 cases (10.2%), the metronidazole therapy was switched to vancomycin, because of its poor effectiveness and according to the guidelines of CDI [13, 29, 32], we started *vancomycin* therapy immediately on the day of the hospitalization in 142 cases (16%). Fidaxomicin was used just in two cases (0.2%). In our practice, the symptomatic cure was achieved in 88.5% of patients who received metronidazole and in 95% of patients who received vancomycin. The recurrence rate has been found higher when metronidazole was used (19.6% vs. 10.1%).

It is important to note that according to Hungarian and international guidelines [29, 33], in those cases where conventional treatment had failed, we have done 30 FMTs with an excellent outcome. In our practice, FMT through the upper gastrointestinal tract was found to have an overall primary cure rate of 89.7% and a secondary cure rate of 96.5% [34].

A meta-analysis has found all-cause mortality at 30 days varied from 9% to 38%. Three studies reported attributable mortality at 30 days, varying from 5.7% to 6.9%. In hospital, mortality ranged from 8% to 37.2% [35]. In our department during that time, CDI resulted death in 11.9% (106 cases).

During the past decade, *C. difficile* has become one of the most important infections and nosocomial problems. Previous antibiotic treatment, elderly, and immunosuppression are the risk factors. In addition to the earlier observations, we observed the possible role of prior statin use in higher and more severe CDI prevalence. However, further examinations are needed to prove this hypothesis. Similar to the worldwide tendencies in our ward, we could detect important changes in the course of this disease (i.e., mortality rates, proportion of severe cases, etc.). In conclusion, we can note that the *C. difficile* pandemic has reached Hungary and shows the same features elsewhere in the developed world.

In treatment strategies, oral metronidazole therapy is recommended as treatment in initial CDI in mild/moderate disease, because it has been found to be effective, has the advantage of low cost, and is less likely inducing the selection of vancomycin-resistant enterococci. Based on studies, guidelines, and our experiences, vancomycin is considered superior to metronidazole in severe cases. FMT seems to be highly effective in treating multiple recurrent CDI.

Conflict of Interest

The authors work in a University Hospital, where fecal microbiota transplantation is a treatment option for patients suffering from recurrent or severe CDI. All FMT procedures were carried out with the written ethical approval (permission number: 16014) of the appropriate Scientific and Ethics committee (Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság, ETT – TUKEB) and the written informed consent of the patients.

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