Clostridioides difficile ribotype distribution in a large teaching hospital in Serbia

Miloš Korać
Hospital for Infectious and Tropical Diseases, Medical Faculty University of Belgrade
Maja Rupnik
National laboratory for health, environment and food, Centre for Medical Microbiology, University of Maribor, Faculty of Medicine
Nataša Nikolić
Hospital for Infectious and Tropical Diseases, Medical Faculty, University of Belgrade
Milica Jovanović
Microbiology Department, Clinical Centre of Serbia
Tanja Tošić
Microbiology Department, Clinical Centre of Serbia
Jovan Malinić
Hospital for Infectious and Tropical Diseases, Medical Faculty, University of Belgrade
Nikola Mitrović
Hospital for Infectious and Tropical Diseases, Medical Faculty, University of Belgrade
Marko Marković
Hospital for Infectious and Tropical Diseases, Belgrade
Ankica Vujović
Hospital for Infectious and Tropical Diseases, Belgrade
Sanja Peruničić
Hospital for Infectious and Tropical Diseases, Belgrade
Ksenija Bojović
Hospital for Infectious and Tropical Diseases, Medical Faculty, University of Belgrade
Vladimir Djordjević
Clinic for Digestive Surgery, Belgrade
Aleksandra Barać
Hospital for Infectious and Tropical Diseases, Medical Faculty, University of Belgrade
Ivana Milošević (ivana.milosevic00@gmail.com)
Hospital for Infectious and Tropical Diseases, Belgrade https://orcid.org/0000-0002-4331-3328

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Abstract

Background: The global epidemic of nosocomial diarrhea caused by *Clostridioides (Clostridium) difficile* started in 2003, with high mortality rates and emergence of a new hypervirulent strain NAP1/BI/027. The aim of this study was to assess the presence of ribotype 027 and other *C. difficile* ribotypes in a Serbian University Hospital, to compare the temporal variability of ribotypes three years apart, as well as to compare clinical, demographic and laboratory characteristics and disease outcome among patients infected with 027 and non-027 ribotype. This was a prospective observational study addressing four months time intervals during 2014/2015 and 2017/2018. Results: Ribotyping was performed in 64 non-duplicate *C.difficile* strains. Ribotype 027 was the most prevalent, and was detected in 53 (82.8%) patients (43/45 and 10/19 patients in 2014-2015 and 2017/2018, respectively). Other detected ribotypes were 001/072 in 4 (6,3%), 002 in 4 (6,3%), 014/020 in 2 (3,1%) and 176 in 1 (1,5%) patient. The percentage of patients infected with ribotype 027 significantly decreased during the three-year period, from 95.6% to 52.6% (p <0.001). Ribotype 027 infection was associated with fluoroquinolone treatment more frequently than infection with other ribotypes [33 (62.3%) vs. 2 (18.2%), p= 0.010)]. Severe *C.difficile* infection was diagnosed more often in patients with detected ribotype 027 compared to those infected with non-027 ribotypes (p=0.006). No significant difference in the mortality and recurrence rates was found between patients infected with ribotype 027 and those infected with other ribotypes [10/53 (18.8%) vs. 2/11 (18.2%), p=0.708, and 10/35 (28.6%) vs. 0/2 (0%), p=1.000, respectively]. Conclusion: *C. difficile* ribotype 027 was the most prevalent ribotype among patients in a large Serbian hospital, but there is a clear decreasing trend.

1. Background

The global epidemic of nosocomial diarrhea caused by Clostridioides (Clostridium) difficile started in 2000 in the USA and Canada (1). Increased prevalence and higher mortality rates were registered in 2001, especially among elderly. NAP1/BI/027 (North American pulsed-field 1/PCR-ribotype 027), a new hypervirulent strain associated with severe C. difficile infection (CDI) has been detected in 2002 (1). The majority of NAP1/BI/027 isolates are resistant to fluoroquinolones. They also possess virulence factors such as increased sporulation and modified surface layer protein adherence (2). The NAP1/BI/027 strain was detected in almost all European countries, with a different prevalence rate.

The reports on C. difficile in Serbia are scarce (3–11). Since 2008, University Hospital for Infectious and Tropical Diseases, Clinical Center of Serbia in Belgrade has been central Serbian institution for the treatment of patients with CDI (3).

The aim of this study was to assess the presence of ribotype 027 and other C. difficile ribotypes in large teaching hospital in Serbia, to compare the temporal variability of ribotypes three years apart, as well as to compare clinical, demographic and laboratory characteristics and disease outcome among patients infected with ribotype 027 and non-027.

2. Methods

2.1. Patient population

This study enrolled 64 randomly selected patients with confirmed CDI who were treated as inpatients in Hospital for Infectious and Tropical Diseases in Belgrade. This hospital is a tertiary-care facility where patients with infectious diseases from the entire country are referred. Study was designed as a prospective cohort study which was performed during two periods, three years apart. The first period was from November the 1st 2014 to February 28th 2015 and the second from November the 1st 2017 to February 28th 2018. Severity of disease was defined by IDSA/SHEA (Infectious Disease Society of America/Society of Hospital Epidemiologists of America) criteria (1). The consent for participation was obtained from all subjects, and the study was approved by the Clinical Centre of Serbia Ethics Committee.

2.2. C. difficile diagnosis and strains characterization

In patients with diarrhea diagnosis of CDI was established by microbiological tests. Microbiological testing was based on detection of glutamat dehydrogenase (GDH) (RIDA®QUICK Clostridium difficile GDH, R-Biopharm AG, Darmstadt, Germany) and in the case of positive GDH test a rapid test for detection of C. difficile toxin A and B in stool (RIDA®QUICK Clostridium difficile Toxin A/B (R-Biopharm AG, Darmstadt, Germany). Stool culture for C. difficile (medium Chrom ID C. difficile, bioMérieux, 69280, Marcy l'Etoile, France) was performed using GDH and toxin positive stools, depending on the medium availability.

Ribotyping of C. difficile strains was performed in a National laboratory for health, environment and food, Centre for Medical Microbiology, Department for microbiological research in Maribor, Slovenia. C. difficile strains were typed with agarose based ribotyping with Bidet primers as described by Janezic S. and Rupnik M. (12).

2.3. Statistical analyses

Demographic characteristics (age, gender), clinical presentation and severity of the disease (fever, abdominal crapms, vomiting, abdominal distension, hypotension, initial number of stools

presence of loose (mucoid) stool and ascites), baseline laboratory analyses (complete blood count, C-reactive protein, albumin concentration) and disease outcome (resolution of diarrhea, death or relaps) were compared in patients with ribotype 027 and other non-027 ribotypes of C. difficile. All statistical analyses were performed using an electronic database organized in SPSS version 21.0. The independent-samples t test was used to compare means. The non-parametric variables were analyzed using the Chi-square or Fisher’s exact test, as appropriate. The level of significance was 0.05.

Ethical approval for the study was obtained from the Ethical Committee Faculty of Medicine, University of Belgrade.

All procedures performed involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants (or their caregivers) included in the study.

3. Results

Fifty-seven C. difficile strains were cultivated from CDI patients admitted to hospital from November 2014 to February 2015 and 19 C. difficile strains from samples taken during second study period (November 2017 - February 2018). Only 64 strains out of 76 original strains were typed since there was no growth in 12 samples.

Ribotype 027 was the most prevalent among 64 detected strains, found in 53 (82.8%) cases. Other isolated C.difficile ribotypes were 001/072 in 4 (6,3%), 002 in 4 (6,3%), 014/020 in 2 (3,1%) and 176 in 1 (1,5%) patient. The percentage of patients infected with ribotype 027 significantly decreased during the three-year period, from 95.6% (43/45) in 2014–2015 to 52.6% (10/19) in 2017–2018 ($p < 0.001$) (Table 1).

All study patients received antibiotics for various reasons, mostly due to urinary tract infection or pneumonia, during the two months period preceding CDI. The majority of patients (35 (54.7%)), were previously treated with fluoroquinolones, while 23 (35.9%) and 6 (9.4%) patients were treated with cephalosporins and carbapenems, respectively. Patients infected with ribotype 027 received fluoroquinolones prior to CDI more often than patients infected with other ribotypes [33 (62.3%) vs. 2 (18.2%), $p = 0.010$)]. The rate of patients receiving fluoroquinolones decreased over the time [29 (64.4%) during first vs. 6 (31.6%) during second study period, $p = 0.027$].

Clinical and laboratory characteristics of 53 patients with 027 ribotype infection and 11 patients with non-027 ribotype infection were compared. (Table 1). The mean leukocyte count in patients with isolated ribotype 027 was significantly higher in comparison to patients with non-027 ribotype infection ($16.31 \pm 8.4 \times 10^9$/l vs.$10.39 \pm 4.14 \times 10^9$/l, $p = 0.027$). Patients infected with ribotype 027 presented more often with severe form of disease ($p = 0.006$), but it didn't affect clinical course and outcome, since mortality and recurrence rates were similar in patients infected with 027 and those infected with non-027 ribotypes [10/53 (18.8%) vs. 2/11 (18.2%), $p = 0.708$, and 10/35 (28.6%) vs. 0/2 (0%), $p = 1.000$, respectively] (Table 1). Two (16%) patients with ribotype 027 died of severe, complicated disease, and remaining ten (84%), regardless of the ribotype, due to their underlying comorbidities. Predictive factors associated with mortality in patients with ribotype 027 infections were high WBC count ($22.74 \pm 9.75 \times 10^9$/l in those who died vs. $14.7 \pm 7.07 \times 10^9$/l, in those who survived, $p = 0.003$), high CRP (168.86 ± 61.63 mg/l vs. 97.07 ± 73.96 mg/l, $p = 0.006$) and creatinine level (209.25 ± 170.03 mg/l vs. 123.06 ± 71.13 mg/l, $p = 0.017$).

Table 1
Clinical and laboratory characteristics of patients with CDI infected with ribotype 027 and other ribotypes

	Ribotype 027 (N = 53) n (%)	Other ribotypes (N = 11) n (%)	p
2014/2015 (N = 45)	43 (95.6%)	2 (4.4%)	< 0.001
2017/2018 (N = 19)	10 (52.6%)	9 (47.4%)	
Age > 65 years	45 (84.9%)	8 (72.7%)	0.330
Fever	23 (43.3%)	3 (27.3%)	0.076
Abdominal cramps	44 (83%)	5 (45.5%)	0.007
Vomiting	9 (16.9%)	0	0.140
Abdominal distension	7 (13.2%)	1 (9%)	0.805
Hypotension	23 (43.4%)	6 (54.5%)	0.499
Initial number of stools (n)	1.67 ± 0.8	1.50 ± 0.7	0.766
Loose (mucoid) stools	44 (83%)	7 (63.4%)	0.325
CRP > 100 mg/l	21 (39.6%)	5 (45.5%)	0.353
WBC > 15×10^9/l	27 (50.9%)	1 (9%)	0.274
Albumin < 30 g/l	34 (64.1%)	7 (63.6%)	0.618
Ascites	7 (13.2%)	1 (9%)	0.707
Time to resolution of diarrhea (days)	5.5 ± 2.8	6.8 ± 4.8	0.275
Severe CDI	41 (77.3%)	4 (36.4%)	0.006
Severe, complicated CDI	5 (9.4%)	1 (0.9%)	1.000
First episode	29 (54.7%)	4 (36.4%)	0.637
Death	10 (18.8%)	2(18.2%)	0.708
WBC-white blood cell count; CRP-C-reactive protein			

4. Discussion

Hospital for Infectious and Tropical Diseases, Clinical Center of Serbia in Belgrade is the biggest infectious diseases facility in Serbia. Over 2700 patients with CDI were treated in this institution from 2008–2018. The overall number of CDI patients treated annually decreased notably from 494 patients treated in 2015, to 391 patients in 2016, 322 patients in 2017, and 275 patients in 2018.

In spite of huge number of treated patients, number of isolated and characterized strains presented in this study is much lower. There are several reasons for this discrepancy: samples were cultured and strains were collected only in four months' period and not during the whole year. Diagnosis was based on typical endoscopic presentation in some patients, so no microbiological testing was required. Furthermore, stool cultures were negative for C.difficile in some patients, although they had positive GDH and toxin A/B tests. In addition, some of stored C. difficile strains could not be recultivated for typing.

The vast majority of patients in this study had been infected with the hypervirulent NAP1/BI/027 strain. During the period 2014/2015 the rate of patients infected with this ribotype was as high as 95.6%, suggesting that NAP1/BI/027 was causative strain in the outbreak of CDI in Serbia. The high rate of ribotype 027 infection was also observed in Romania (82.6%) and Poland (82.4%) during 2013/14 (13,14). Study data reflecting the predominance of NAP1/BI/027 infection are in concordance with previously reported prevalence of this ribotype in Serbia and neighboring Bosnia and Herzegovina (4). The other most prevalent strains in the region were 176, 001/072 and 014/020 which is consistent with presented study (4). Davies et al. demonstrated the same distribution of dominant C. difficile strains (027, 001/072 and 014/020) in European countries, although with lower rates compared to those recorded in Serbia (15).

An important observation of the study is that, although ribotype 027 remained the most prevalent strain, a significant decrease in the rate of patients infected with this ribotype was noted during the three-year period. Compared to 95.6% in 2014/2015, this strain was isolated in 52.6% of cases in 2017/2018, accompanied with decrease of overall number of patients treated for CDI. A similar trend in the prevalence of ribotype 027 was first observed by authors from Netherlands in 2009 due to responsible use of antibiotics and other preventive measures (16). Although international comparisons are difficult, decline in number of CDI caused by 027 ribotype was reported by other countries, such as Belgium and Great Britain. (17,18,19). As opposed to these results, Italy and Germany reported the increase in the rate of patients with ribotype 027 infection reaching 38% and 30%, respectively (20,21).

The decline in the prevalence of ribotype 027 infections, and CDI in general in Great Britain was the result of antibiotic stewardship with fluoroquinolone prescribing (19). Namely, one of the explanations for the spread of this ribotype in the epidemic era is the overuse of this class of antibiotics, considering that NAP1/BI/027 strain is resistant to fluoroquinolones. Presented patients infected with ribotype 027 were more often pretreated with fluoroquinolones comparing to other patients. Furthermore, study demonstrated a significant decrease in the rate of patients treated with fluoroquinolones (mainly ciprofloxacin) between the two periods. It might be one of the explanations for the decrease in the rate of ribotype 027 infection and overall number of CDI patients, along with other preventive measures.

Ribotype 027 infection caused severe form of CDI more often than other ribotypes, but it did not affect mortality and recurrence rates. There are controversial reports concerning the influence of ribotype 027 on the severity, recurrence and mortality rates in CDI. It was assumed that patients infected with NAP1/BI/027 strain develop more severe CDI forms and have greater risk of experiencing relapse, complications,

and death (22–25). The Canada-wide CDI study analyzed the role of infecting strain type and patient age on the severity of CDI: a severe outcome, defined as CDI requiring intensive care unit care, colectomy, or causing death within 30 days after diagnosis, was detected in 12.5% of patients with ribotype 027 and 5.9% of patients with other ribotypes (25). Thirteen (24.5%) patients with ribotype 027 infection and two (18.8%) patients with non-027 ribotype infection experienced severe outcome in presented study. Portuguese authors demonstrated that lethal outcome was related to older age, leukocytosis and renal failure, and fatal comorbidities according to McCabe score (26). This study data also indicate higher mortality rate in patients with leukocytosis, renal failure and higher CRP. According to Michel and Gardner, all cause mortality at 30 days varied from 9–38%, and inhospital mortality ranged from 8–37.2% in patients with CDI infection (27). All cause mortality at 30 days was 18.75% in analyzed patients, regardless of the ribotype. However, the epidemiology of C. difficile is changing rapidly, and a number of studies suggest that strain type, including NAP1/BI/ 027, is not associated with more severe form of disease (28–29).

The results presented in the study have to be seen in light of some limitations. The small sample size is a main limitation in given results, so findings addressing the association of ribotype 027 with disease severity, mortality and recurrence rates needs further investigation. On the other hand, authors consider that although the sample is small, the declining trend of ribotype 027 infection rate and its association with lower fluoroquinolone utilization is undoubted.

5. Conclusions

Ribotype 027 is the most prevalent ribotype among CDI patients in Serbia, but shows declining trend in its rate. Ribotype 027 infection was associated with prior fluoroquinolone treatment and severe form of the disease, but it did not affect death and recurrence rates compared with non 027 ribotype infection. Further studies involving more patients need to be conducted to confirm the results and establish trend in C. difficile ribotypes distribution in Serbia.

Declarations

Ethics approval and consent to participate

The survey protocol was conducted according to Declaration of Helsinki. All survey participants signed informed consent forms for all personally identifiable data including demographic, clinical, and biomedical data.

The study was approved by the Clinical Centre of Serbia Ethics Committee (No. 29/V-16).

Consent for publication

Written informed consent for publication of their clinical details was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author [I.M.]. The data are not publicly available since containing information compromise research participant privacy/consent.

Competing interests

The authors declare that they have no financial and non-financial competing interests.

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Authors' contributions

Each author made substantial contributions of the work.

MK and IM (corr. author) designed the study and drafted the work, MR performed ribotyping of *C. difficile* strains, NN performed statistical analysis and assisted with data interpretations, MJ and TT performed microbiological testing and *C.difficile* stool culture, JM, NM, MM, AV, SP collected data and clinically monitored the patients, VDj performed endoscopy procedures when necessary, AB was a contributor in writing the manuscript. All authors read and approved the final manuscript.

All authors guarantee that the order of the authors are in accordance with their scientific contribution, and that all made a meaningful contribution to the work.

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