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ORIGINAL ARTICLE

Ribotyping of *Clostridioides difficile* in the Liberec Regional Hospital: a tertiary health care facility

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Abstract

The ribotyping of *Clostridioides difficile* is one of the basic methods of molecular epidemiology for monitoring the spread of *C. difficile* infections. In the Czech Republic, this procedure is mainly available in university hospitals. The introduction of ribotyping in a tertiary health care facility such as Liberec Regional Hospital not only increases safety in the facility but also supports regional professional development. In our study, 556 stool samples collected between June 2017 and June 2018 were used for *C. difficile* infection screening, followed by cultivation, toxinotyping, and ribotyping of positive samples. The toxinotyping of 96 samples revealed that 44.8% of typed strains could produce toxins A and B encoded by *tcdA* and *tcdB*, respectively. The ribotyping of the same samples revealed two epidemic peaks, caused by the regionally most prevalent ribotype 176 ($n = 30$, 31.3). *C. difficile* infection incidence ranged between 5.5 and 4.2 cases per 10,000 patient-bed days. Molecular diagnostics and molecular epidemiology are the two most developing parts of clinical laboratories. The correct applications of molecular methods help ensure greater safety in hospitals.

Keywords *Clostridioides difficile* · Toxinotyping · Ribotyping · Health care facility · Clonal spreading

Introduction

Clostridioides difficile (formerly *Clostridium difficile*) is a Gram-positive, spore-forming anaerobic bacterium that is widely distributed in the intestine of humans and animals and in the environment (Nyc et al. 2015; Janezic et al. 2016; Lawson et al. 2016). Its spores are usually found in the human or animal intestine as part of the microbiome (Czepiel et al. 2019), but some strains of *C. difficile* are capable of producing toxins (toxin A, toxin B, and binary toxin) that damage the intestinal mucosa and cause diarrhea. This ability is more important when there is an imbalance in the intestinal microbiota caused by previous antibiotic treatment (Smits et al. 2016; Czepiel et al. 2019). A reduction in microbiome complexity creates space for *C. difficile* to proliferate. Since 2000, hospital strains of *C. difficile* are common hyperproducers of toxins (ribotypes 027, 176, and 001) (Persson et al. 2011). These strains often colonize elderly polymorbid patients, complicating their primary diagnosis and causing life-threatening infections (Beneš et al. 2012).

Laboratory diagnostics of *C. difficile* infections (CDI) is based on examination of the patient´s stool. The two-step algorithm consists of the highly sensitive detection of glutamate dehydrogenase (GDH), which is a *Clostridioides* antigen, followed by toxin detection. The detection of toxigenicity is essential for the diagnosis. Non-toxigenic strains are not considered to be pathogenic (Beneš et al. 2012; Crobach et al. 2016; Krůtová and Nyč 2018). Therefore, closer identification of strains by toxinotyping, ribotyping, or whole-genome sequencing is important. (Huber et al.

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2013; Krutova et al. 2018). Toxinotyping is the basic characterization of a *C. difficile* strain. It describes which toxins the strain produces—toxin A, toxin B, binary toxin, or none. This can be done by enzyme immunoassay or PCR. PCR detects the *cdtA* and *cdtB* genes for toxins A and B, respectively, and the *tcdA* and *tcdB* genes for binary toxin (Delmée et al. 2005; Persson et al. 2008; Rupnik and Janezic 2016). Ribotyping is based on the number and length of intergene sequences between the 16S and 23S rDNA. PCR focused on this region produces several DNA fragments with characteristic lengths. The resulting profile can be assigned to a specific ribotype (Huber et al. 2013). Whole-genome sequencing is the most powerful tool for molecular epidemiology, but it is expensive for tertiary health care facilities. Rep-PCR-based fingerprint DNA testing systems are also commercially available but are not widely used for the diagnosis of *C. difficile* (Corbellini et al. 2014).

Whole-genome sequencing and ribotyping in the Czech Republic is preferably carried out by university hospitals (quaternary medical facility). Tertiary health care facilities, such as Liberec Regional Hospital, had few resources (personnel, financial, and instrumentation) for molecular epidemiology (ribotyping and whole-genome sequencing). This was changed by the SARS-CoV-2 pandemic, which caused a significant development of regional PCR laboratories. The majority of regional laboratories are now able to analyze *C. difficile* strains in more detail. In our study, we use ribotyping to detect outbreaks of CDI in Liberec Regional Hospital.

Material and methods

Hospital

Liberec Regional Hospital is a tertiary health care facility providing specialized care in the Liberec Region, in the north of the Czech Republic, which is inhabited by approximately 500,000 people. The hospital has about 1,200 beds, with 200 beds for follow-up care. As a nonuniversity hospital, it does not have state-funded research activities but has been listed as a research organization since 2019. The average number of patient-bed days in Liberec Regional Hospital in the years 2017–2018 was 224,039.

Sample collection and demographics

Samples for our study were collected over 13 months—from June 2017 till June 2018. In this period, 556 stool samples from hospitalized patients ($n=493$) were sent to the microbiological laboratory of Liberec Regional Hospital. In this group, 46.9% ($n=261$) of samples were from males and 53.1% ($n=295$) from females. The 70–79 age group was the most represented, accounting for 32.7% ($n=182$) of the sample. Internal medicine was the most represented specialty with 35.3% ($n=196$). A more detailed overview is presented in Table 1. A duplicate sample, which was defined as a sample collected within 10 days of the first collection, was not included in the study.

Table 1 Number of samples by sex, age and medical specialty

Medical expertise/age	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	Total
Females	**5**	**8**	**5**	**7**	**8**	**19**	**63**	**94**	**69**	**17**	**295**
Internal medicine			1	2	5	5	25	35	28	6	107
Infectious medicine	1			3		3	12	8	12	3	42
Surgery		2		1	1	2	11	16	6		39
Subsequent inpatient care								6	11	5	22
Rheumatology							1	7	7	1	16
Oncology						4	3	6	1		14
Anesthesiology and intensive care		2	2	1		3	1	2		1	12
Others < 10 samples	4	4	2	0	2	2	10	14	4	1	43
Males	**8**	**5**	**5**	**7**	**15**	**23**	**67**	**88**	**37**	**6**	**261**
Internal medicine			3	3	5	9	23	35	9	2	89
Surgery		1	1	3	3	6	11	17	9	1	52
Infectious medicine		1			1	1	8	8	5		24
Anesthesiology and intensive care			1			4	4	4	2		15
Subsequent inpatient care						1	4	3	5	1	14
Pediatric medicine	7	3									10
Cardiology				1		1	4	4			10
Others < 10 samples	1	0	0	0	6	1	13	17	7	2	47
Total	**13**	**13**	**10**	**14**	**23**	**42**	**130**	**182**	**106**	**23**	**556**

Screening and cultivation

QuikChek Complete (TechLab, USA) was used for CDI screening and basic toxinotyping *C. difficile*. After the CDI screening, GDH-positive samples were selected for stool cultivation. The stool samples were exposed to 96% ethanol (1 mL or a small pea-sized portion of stool in 1:1 mixture with alcohol) for 30 min. Two drops (approximately 50–75 µL) of deposit were inoculated onto Brazier's *Clostridium difficile* Selective agar (Oxoid, Czechia) and spread to obtain single colonies. The inoculated plates were immediately transferred to an anaerobic environment and incubated at 37 °C for 48 h. The identification of *C. difficile* colonies was performed according to the characteristic color and shape of the colonies (irregular with grey to white color) and by Gram-stained microscopy and subsequent toxinotyping by PCR.

PCR toxinotyping

DNA was isolated from the *C. difficile* suspension (1–3 colonies in 500 µLµ nuclease free water) using a PathogenFree DNA Isolation Kit (GeneProof, Czechia) and used for PCR reaction focused on the genes encoding the toxins (cdtA, cdtB, tcdA, and tcdB). Mastermix was 12.5 µL Hotstart Mastermix (Qiagen, Netherlands), 5 µL primer mix (see Table 2 for more details), 5 µL nuclease free water, and 2.5 µL sample DNA. The cycling program was activation for 15 min at 94 °C, followed by 35 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 50 °C, and elongation for 1 min at 72 °C, with the final elongation step for 30 min at 72 °C. The obtained amplicons were detected by capillary electrophoresis in a MultiNa (Shimadzu, Japan) instrument.

PCR ribotyping

This was based on the amplification of an intergene locus between the 16S and 23S rDNA genes. Each ribotype has a specific number of intergene locus with a different length. Amplification thus produced multiple fragments with different lengths. This fragment profile is specific for each ribotype. For ribotyping, the PCR mastermix was 25 µL Hotstar Mastermix (Qiagen, Netherlands), 0.3 µL of each primer (see Table 2 for more details), 22.4 µL nuclease free water, and 2 µL sample DNA. The cycling program was activation for 15 min at 95 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, and elongation for 1 min at 72 °C, with the final elongation step for 30 min at 72 °C. PCR fragments were then analyzed in an ABI310 automatic genetic analyzer (Thermo Fisher Scientific, Waltham, MA, USA) in a 50 cm capillary loaded with POP4 polymer (Thermo Fisher Scientific, Waltham, MA, USA). LIZ600 was used as the size standard (Thermo Fisher Scientific, Waltham, MA, USA). The length of each fragment was determined using the GeneMapper software (Thermo Fisher Scientific, Waltham, MA, USA). The resulting profile was then uploaded to the WEBRIBO database (https://webribo.ages.at/).

Table 2 Ribotyping and toxinotyping primers (Bidet et al. 1999; Persson et al. 2008)

Name	Target	Sequence	Concentration (µM)	Size (bp)
Toxinotyping primer mix				
tcdA-F	tcdA gene	5′-GCATGATAAGGCAACTTCAGTGGTA-3′	3.00	629
tcdA-R		5′-AGTTCCTCCTGCTCCATCAAATG-3′	3.00	
tcdB-F	tcdB gene	5′-CCAAARTGGAGTGTTACAAACAGGTG-3′	2.00	410
tcdB-RA		5′-GCATTTCTCCATTCTCAGCAAAGTA-3′	1.00	
tcdB-RB		5′-GCATTTCTCCGTTTTCAGCAAAGTA-3′	1.00	
cdtA-FA	cdtA gene	5′-GGGAAGCACTATATTAAAGCAGAAGC-3′	0.25	221
cdtA-FB		5′-GGGAAACATTATATTAAAGCAGAAGC-3′	0.25	
cdtA-R		5′-CTGGGTTAGGATTATTTACTGGACCA-3′	0.50	
ctdB-F	cdtB gene	5′-TTGACCCAAAGTTGATGTCTGATTG-3′	0.50	262
cdtB-R		5′-CGGATCTCTTGCTTCAGTCTTTATAG-3′	0.50	
PS-F	16S-rDNA	5′-GGAGGCAGCAGTGGGGAATA-3′	0.25	1062
PS-R		5′-TGACGGGCGGTGTGTACAAG-3′	0.25	
GluD-F	gluD gene	5′-GTCTTGGATGGTTGATGAGTAC-3′	0.50	158
GluD-R		5′-TTCCTAATTTAGCAGCAGCTTC-3′	0.50	
Ribotyping primers				
FAM-16S	16S-23S intergene	5′-GTGCGGCTGGATCACCTCCT-3′	10.00	fragment profile
23S rev		5′-CCCTGCACCCTTAATAACTTGACC-3′	10.00	

Results

Between June 2017 and June 2018, 556 stool samples from hospitalized patients were sent to the laboratory of Liberec Regional Hospital for CDI screening. GDH positivity was detected in 124 (22.3%) samples. Toxin positivity was confirmed in 70/124. Fifty-four GDH-positive samples and all 432 (77.7%) GDH-negative samples were toxin negative. Based on GDH positivity, the incidence of CDI in Liberec Regional Hospital was 5.5 cases per 10 000 patient-bed days. The monthly incidence of CDI ranged from 3 to 13 cases, with a median of 10 and mean of 9.5 cases. It was not possible to estimate any epidemiological trend from the number of CDI occurrences over time.

Stool cultivation was performed on 98 GDH-positive samples. The remaining 26 GDH-positive samples could not be cultured due to lack of material. Cultivation yielded 96 (98.0%) viable cultures. Only 10 GDH-positive samples came from patients without antibiotic treatment. The most commonly used antibiotic in the remaining 88 patients was amoxicillin/clavulanic acid ($n = 45$, 45.9%), followed by cephalosporins ($n = 31$, 31.6%).

PCR toxinotyping revealed that 43 (44.8%) strains carried the genes for toxins A and B (*tcdA* and *tcdB*) only, 33 (34.4%) strains carried the genes for both major toxins and the binary toxin (*tcdA*, *tcdB*, *cdtA*, and *cdtB*), and only 2 (2.1%) strains only carried the genes for toxin B and the binary toxin (*tcdB*, *cdtA*, and *cdtB*). Fourteen (14.6%) strains were non-toxigenic, and in 4 (4.2%) cases, toxinotyping failed or the presence of *C. difficile* was not confirmed. Based on PCR confirmation, the incidence of CDI in Liberec Regional Hospital was 4.2 cases per 10 000 patient-bed days.

PCR ribotyping was performed on 96 strains from cultivation. The most abundant ribotype was 176 ($n = 30$), followed by 012 ($n = 10$) and 014 ($n = 5$). The remaining ribotypes ($n = 24$) were found in less than 5 cases. See Table 3 for a more detailed overview. In 5 cases, ribotyping failed. Ribotype 176 was most commonly seen in the internal medicine department, primarily between August 2017 and November 2018 ($n = 10$). In March 2018, ribotype 176 was detected simultaneously in three departments (2 × internal medicine, 2 × pulmonary medicine, and 2 × surgery). The pulmonary and internal medicine departments share one building in Liberec Regional Hospital.

The strains of ribotype 176 most frequently carried genes for toxins A and B and binary toxin ($n = 24$). In contrast, all strains of ribotypes 012 and 014 only carried genes for the major toxins (A and B). The most frequently detected non-toxigenic ribotype was 010 ($n = 4$) followed by 596 ($n = 3$). Other non-toxigenic ribotypes were only found once.

Table 3 Occurrence of individual *C. difficile* ribotype by month

	176	012	014	010	002	001	078	413	017	596	449	043	440	629	483	051	046	005	667	081	AI-72	AI-60	651	400	020	023	New ribotype	Failed	Total
2017																													
VI	1							1	1																		1		**4**
VII	3								1			1				1										1			**7**
VIII	2	1	1	1		2				1		1																	**9**
IX	**4**		1							1																			**6**
X	**5**							1			1								1						1			1	**10**
XI	**4**	2			1										1														**8**
XII			1	1		1	1	1		1																	1		**7**
2018																													
I	2					1											1											1	**5**
II	1	2		1										1													1	1	**7**
III	**6**												1									1						1	**9**
IV	1		1						1											1							1		**6**
V		2	1		2		1											1						1				**7**
VI	1	**3**		1	1		1				1							1			1		1						**11**
Total	**30**	**10**	**5**	**4**	**4**	**4**	**3**	**3**	**3**	**3**	**2**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**4**	**5**	**96**

Table 4 Occurrence of individual *C. difficile* ribotype by country

Czech Republic				**Poland**	**Slovakia**
Presented data	Beran et al. (2017)	Vaverková et al. (2021)	Krutova et al. (2016)	Aptekorz et al. (2017)	Novakova et al. (2020)
176 (31.3%)	**176 (60.9%)**	001 (20.0%)	**176 (29%)**	027 (82.4%)	001 (59.0%)
012 (10.4%)	014 (9.4%)	014 (13.0%)	001 (24%)	**176 (2.8%)**	**176 (29.5%)**
014 (5.2%)	002 (6.3%)	078 (7.5%)	014 (9%)	014 (2.8%)	017 (3.8%)
010 (4.2%)	078 (3.1%)	**176 (6.5%)**	012 (5%)	010 (1.9%)	020 (1.3%)
002 (4.2%)	043 (3.1%)	020 (6.5%)	020 (4%)		027 (1.3%)
001 (4.2%)	015 (1.6%)		017 (4%)		049 (1.3%)
078 (3.1%)	017 (1.6%)				070 (1.3%)
413 (3.1%)	103 (1.6%)				
017 (3.1%)	174 (1.6%)				
596 (3.1%)					
449 (2.1%)					
043 (2.1%)					

Discussion

Our study found ribotype 176 to be the most frequent in the reporting period. This hypertoxigenous ribotype is common in the Czech Republic and is also specific for Central Europe (Czech Republic, Poland, and Slovakia; see Table 4). It is also called 027-like ribotype because of its quite similar profile to ribotype 027, the hypertoxigenous ribotype widespread in Western Europe (Krutova et al. 2014, 2016; Beran et al. 2017).

Ribotype 176 was the main one that caused local outbreaks in internal medicine and adjacent departments of Liberec Regional Hospital. Table 3 shows two outbreaks in 2017 and 2018. The small outbreak in June 2018 was caused by a different ribotype—012. All other ribotypes represented a maximum of 5% and had no significant peak in the reported period.

The proportion of individual ribotypes in Liberec Regional Hospital is quite different from those detected within the same period in Hradec Kralove University Hospital. There, the most frequent ribotype was 001 (20.0%), followed by 014 (13.0%), 078 (7.5%), 176 (6.5%), and 020 (6.5%) (Vaverková et al. 2021). Both hospitals are located in neighboring regions of the Czech Republic, but patient transfers between them are relatively minimal. The combination of the ribotype spectrums of Liberec and Hradec Kralove is very close to the ribotype spectrum of the Czech Republic as a whole (176–29%, 001–24%, 014–9%, 012–5%, 020–4%, and 017–4%) in 2014 (Krutova et al. 2016). Our observation suggests that the proportions of *C. difficile* ribotypes are probably highly connected to the specific hospital, and the transmission of *C. difficile* strains from one hospital to another is not common. A different view was reported by Beran et al. (2017), when they found two genetically related strains captured in two hospitals. However, they worked with hospitals belonging to the same organization or from the same region of the Czech Republic.

In our cohort, 89.8% of GDH-positive patients received antibiotic treatment. The most commonly used antibiotic was amoxicillin/clavulanic acid (45.9%) followed by cephalosporins (31.6%). High rates of prior treatment with antibiotics have been described in the literature (Beran et al. 2014).

The incidence of *C. difficile* infections in the Liberec Regional Hospital in 2017 and 2018 ranged from 5.5 to 4.2 cases per 10,000 patient-bed days. Both incidence rates correspond to the CDI incidence in the Czech Republic (6.1 to 4.5 cases per 10,000 patient-bed days) (Krutova et al. 2016) and in Europe (4.1 to 3.98 cases per 10,000 patient-bed days) (Bauer et al. 2011).

Molecular diagnostics and molecular epidemiology are one of the most rapidly developing parts of clinical laboratories, helping to make hospitals safer. In our case, it helped to detect local outbreaks of ribotype 176, which could not be detected when evaluating the overall prevalence of CDI due to the high variability of the detected ribotypes. Until recently, tertiary hospitals in the Czech Republic have not had the capacity to use these methods. However, the COVID-19 epidemic changed this. New laboratories have been established, and old ones have been expanded. Spatial and instrument capacities have been increased. The only thing lacking is personnel capacity. If they can be scaled up, molecular methods can change the way hospitals operate.

Author contribution M.K.: data curation, investigation, formal analysis, methodology, writing original draft. I.D.: methodology, writing—review and editing. H.Z.: methodology, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

References

Aptekorz M, Szczegielniak A, Wiechuła B et al (2017) Occurrence of *Clostridium difficil*e ribotype 027 in hospitals of Silesia, Poland. Anaerobe 45:106–113. https://doi.org/10.1016/j.anaerobe.2017.02.002

Bauer MP, Notermans DW, van Benthem BH et al (2011) *Clostridium difficile* infection in Europe: a hospital-based survey. The Lancet 377:63–73. https://doi.org/10.1016/S0140-6736(10)61266-4

Beneš J, Husa P, Nyč O (2012) Recommendations for diagnosis and therapy of colitis caused by *Clostridium difficile*. Klin Mikrobiol Infekcni Lek 18:160–167

Beran V, Chmelar D, Vobejdova J et al (2014) Sensitivity to antibiotics of *Clostridium difficile* toxigenic nosocomial strains. Folia Microbiol (Praha) 59:209–215. https://doi.org/10.1007/s12223-013-0283-1

Beran V, Kuijper EJ, Harmanus C et al (2017) Molecular typing and antimicrobial susceptibility testing to six antimicrobials of *Clostridium difficile* isolates from three Czech hospitals in Eastern Bohemia in 2011–2012. Folia Microbiol (Praha) 62:445–451. https://doi.org/10.1007/s12223-017-0515-x

Bidet P, Barbut F, Lalande V et al (1999) Development of a new PCR-ribotyping method for *Clostridium difficile* based on ribosomal RNA gene sequencing. FEMS Microbiol Lett 175:261–266. https://doi.org/10.1111/j.1574-6968.1999.tb13629.x

Corbellini S, Piccinelli G, De Francesco MA et al (2014) Molecular epidemiology of *Clostridium difficile* strains from nosocomial-acquired infections. Folia Microbiol (Praha) 59:173–179. https://doi.org/10.1007/s12223-013-0281-3

Crobach MJT, Planche T, Eckert C et al (2016) European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect 22:S63–S81. https://doi.org/10.1016/j.cmi.2016.03.010

Czepiel J, Dróżdż M, Pituch H et al (2019) *Clostridium difficile* infection: review. Eur J Clin Microbiol Infect Dis 38:1211–1221. https://doi.org/10.1007/s10096-019-03539-6

Delmée M, Van Broeck J, Simon A et al (2005) Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea: a plea for culture. J Med Microbiol 54:187–191. https://doi.org/10.1099/jmm.0.45844-0

Huber CA, Foster NF, Riley TV, Paterson DL (2013) Challenges for standardization of *Clostridium difficile* typing methods. J Clin Microbiol 51:2810–2814. https://doi.org/10.1128/JCM.00143-13

Janezic S, Potocnik M, Zidaric V, Rupnik M (2016) Highly divergent *Clostridium difficile* strains isolated from the environment. PLoS ONE 11:e0167101. https://doi.org/10.1371/journal.pone.0167101

Krutova M, Kinross P, Barbut F et al (2018) How to: surveillance of *Clostridium difficile* infections. Clin Microbiol Infect 24:469–475. https://doi.org/10.1016/j.cmi.2017.12.008

Krutova M, Matejkova J, Kuijper EJ et al (2016) *Clostridium difficile* PCR ribotypes 001 and 176 - the common denominator of *C. difficile* infection epidemiology in the Czech Republic, 2014. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 21. https://doi.org/10.2807/1560-7917.ES.2016.21.29.30296

Krutova M, Matejkova J, Nyc O (2014) *C. difficile* ribotype 027 or 176? Folia Microbiol (Praha) 59:523–526. https://doi.org/10.1007/s12223-014-0323-5

Krůtová M, Nyč O (2018) Updated Czech guidelines for the laboratory diagnosis of *Clostridium difficile* infections. Epidemiol Mikrobiol Imunol Cas Spolecnosti Epidemiol Mikrobiol Ceske Lek Spolecnosti JE Purkyne 67:92–95

Lawson PA, Citron DM, Tyrrell KL, Finegold SM (2016) Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prévot 1938. Anaerobe 40:95–99. https://doi.org/10.1016/j.anaerobe.2016.06.008

Novakova E, Stefkovicova M, Kopilec MG et al (2020) The emergence of *Clostridium difficile* ribotypes 027 and 176 with a predominance of the *Clostridium difficile* ribotype 001 recognized in Slovakia following the European standardized *Clostridium difficile* infection surveillance of 2016. Int J Infect Dis 90:111–115. https://doi.org/10.1016/j.ijid.2019.10.038

Nyc O, Krutova M, Kriz J et al (2015) *Clostridium difficile* ribotype 078 cultured from post-surgical non-healing wound in a patient carrying ribotype 014 in the intestinal tract. Folia Microbiol (Praha) 60:541–544. https://doi.org/10.1007/s12223-015-0392-0

Persson S, Jensen JN, Olsen KEP (2011) Multiplex PCR method for detection of Clostridium difficile tcdA, tcdB, cdtA, and cdtB and internal in-frame deletion of tcdC. J Clin Microbiol 49:4299–4300. https://doi.org/10.1128/JCM.05161-11

Persson S, Torpdahl and M, Olsen KEP, (2008) New multiplex PCR method for the detection of *Clostridium difficile* toxin A (*tcdA*) and toxin B (*tcdB*) and the binary toxin (*cdtA/cdtB*) genes applied to a Danish strain collection. Clin Microbiol Infect 14:1057–1064. https://doi.org/10.1111/j.1469-0691.2008.02092.x

Rupnik M, Janezic S (2016) An update on *Clostridium difficile* toxinotyping. J Clin Microbiol 54:13–18. https://doi.org/10.1128/JCM.02083-15

Smits WK, Lyras D, Lacy DB et al (2016) *Clostridium difficile* infection. Nat Rev Dis Primer 2:16020. https://doi.org/10.1038/nrdp.2016.20

Vaverková K, Kracík M, Ryšková L et al (2021) Effect of restriction of fluoroquinolone antibiotics on *Clostridioides difficile* infections in the University Hospital Hradec Králové. Antibiotics 10:519. https://doi.org/10.3390/antibiotics10050519