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PRACA ORYGINALNA
ORIGINAL PAPER

Outbreak of *Clostridioides difficile* infection in Silesian district hospital

Ognisko epidemiczne *Clostridioides difficile* w śląskim szpitalu powiatowym

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ABSTRACT

INTRODUCTION: In order to evaluate a *Clostridioides difficile* infection (CDI) outbreak between December 2018 and February 2019 in the internal medicine ward of a district hospital in Silesia, 6 stools from 5 patients were examined.
MATERIAL AND METHODS: *C. difficile* was identified, genes encoding glutamate dehydrogenase (GDH) – *gluD*, A/B – *tcdA/tcdB* and binary – *cdtA/cdtB* toxins, *ermB* were determined by mPCR and antibiotic resistance by means of E-Tests.
RESULTS: Women predominated among the patients (4/5). All the 6 *C. difficile* isolates belonged to hyperepidemic ribotype 027, were positive for all genes and were resistant to moxifloxacin, erythromycin, clindamycin, rifampicin, imipenem, and chloramphenicol.
CONCLUSIONS: The obtained results indicate that the hyperepidemic *C. difficile* clone is spreading in the ward.

KEY WORDS

Clostridioides difficile infection, outbreak, *Clostridioides difficile* toxins

STRESZCZENIE

WSTĘP: W celu oceny ogniska zakażenia *Clostridioides difficile* (*Clostridioides difficile* infection – CDI) w okresie od grudnia 2018 r. do lutego 2019 r. na oddziale chorób wewnętrznych szpitala powiatowego na Śląsku objęto badaniem materiały kliniczne od 5 pacjentów (6 stolców).

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MATERIAŁ I METODY: Zidentyfikowano izolaty *C. difficile*, geny kodujące dehydrogenazę glutaminianową (GDH) – *gluD*, toksyny A/B – *tcdA/tcdB* oraz geny *cdtA/cdtB* kodujące toksynę binarną, *ermB* wykryto za pomocą mPCR, a antybiotykooporność za pomocą E-testów.
WYNIKI: Wśród pacjentów dominowały kobiety (4/5). Wszystkie szczepy *C. difficile* (6) należały do hiperepidemicznego rybotypu 027, we wszystkich szczepach wykazano obecność badanych genów oraz wykryto oporność na: moksyfloksacynę, erytromycynę, klindamycynę, ryfampicynę, imipenem i chloramfenikol.
WNIOSKI: Uzyskane wyniki świadczą o szerzeniu się na oddziale hiperepidemicznego klonu *C. difficile* o rybotypie 027.

SŁOWA KLUCZOWE

zakażenie *Clostridioides difficile*, ognisko epidemiczne, toksyny *Clostridioides difficile*

INTRODUCTION

According to the reports of the Polish Chief Sanitary Inspectorate in 2020, 41.1% (200/535 except SARS-CoV-2) of the reported outbreaks were caused by *Clostridioides difficile* (*C. difficile*); in 2019 *C. difficile* infection (CDI) accounted for 30% [1].
Clostridioides difficile is an anaerobic, Gram-positive spore-forming bacillus. The bacterium produces spores under favorable conditions, e.g. access to oxygen, nutrients, etc. The spores demonstrate resistance to many factors, including alcohol-based disinfectants commonly used in healthcare units [2]. The main risk factor predisposing patients to the development of CDI are: older age, antibiotic usage, especially fluoroquinolones, cephalosporins, clindamycin etc., causing disorders in the intestinal microbiota, promoting the multiplication of *C. difficile* [3].
The clinical symptoms of CDI depend on the toxin production by *C. difficile*: toxin A (*tcdA*) – enterotoxin, toxin B (*tcdB*) – cytotoxin and binary toxin (*cdtA/cdtB*), which is ADP-ribosyltransferase and is produced by about 20% of strains. *Clostridioides difficile* infections are manifested as mild antibiotic-associated diarrhea (defined as 3 or more bowel movements a day) and more severe forms such as pseudomembranous colitis, toxic megacolon, sepsis and death [4]. The hypervirulent epidemic *C. difficile* strain belonging to PCR RT 027 (BI/NAP1/027) is the main cause of severe CDIs due to the increased production of toxins A and B and the production of binary toxin [5].
An increase in severe CDI caused by the *C. difficile* ribotype (RT) 027 strain was reported in Canada and the USA in 2000–2003. During the next 10 years, the dominance of the hyperepidemic strain was noted in European countries, including Poland and on other continents [6,7]. The frequent occurrence of this strain has been observed especially in southern Poland and also in Western Australia, South Korea, Hong Kong and Costa Rica [8].
A proper antibiotic policy plays a leading role in reducing the CDI risk [9,10]. Pharmacotherapy for CDI has changed in recent years. Recommendations for the treatment of CDI have been updated (Europe vs US), e.g. in both Europe and the US metronidazole has been withdrawn based on recent reports of vancomycin superiority to metronidazole. Society for Healthcare Epidemiology of America/Infectious Diseases Society of America (SHEA/IDSA) recommends starting therapy with fidaxomicin, in special circumstances together with bezlotoxumab [10,11,12].
The aim of the study was to evaluate an epidemic outbreak of CDI, between December 2018 and February 2019 at the internal medicine ward.

MATERIAL AND METHODS

The outbreak of CDI was noted in the 33-bed internal medicine ward in the Silesian district hospital between December 2018 and February 2019. The outbreak included 5 patients with antibiotic-associated diarrhea. Fecal samples from those patients were collected for testing for CDI (from one patient 2 samples were collected).
The fecal samples were examined in accordance with a two-stage algorithm: first, detecting the *C. difficile* somatic antigen – glutamate dehydrogenase (GDH) and then toxins A/B – (TechLab, Blacksburg, USA) [13]. Next, (in the case of one or both positive results) the stool samples were cultured on chromID *C. difficile* and CLO plates (bioMérieux, Marcy L'Etoile, France), incubated at 37°C under anaerobic conditions (Whitley A35 Workstation, UK) for 48 h. Colonies with characteristic morphology (CDIFF – black colonies, CLO – gray, jagged with yellow-green fluorescence under UV light, and horse odor) were isolated for further biochemical identification (VITEK 2 Compact System, bioMérieux, Marcy L'Etoile, France), antibiotic susceptibility testing and ribotyping [6].
The genes encoding *C. difficile* toxins and GDH were detected by performing the multiplex polymerase chain reaction (mPCR) according to Stubbs, and using the HotStarTaq Plus PCR Master Mix Kit (Qiagen, Germany) [14]. For this purpose, brain-heart infusion culture DNA was isolated from *C. difficile* (QIAamp DNA Mini Kit, Qiagen, USA), mPCRs were performer (*gluD, tcdA, tcdB, 16S rDNA*), and additional PCR for the *ermB* gene encoding the MLS_B resistance mechanism (to macrolides, lincosamides and streptogramin B) was done (Table I). The obtained amplicons were subjected to electrophoretic separation. The results were interpreted on the basis of gel visualization in a BOX Chemi XR5 apparatus

(Syngene, UK). Ribotyping of the isolates was performed as described previously [15].

The antibiotic (minimum inhibitory concentration – MIC) susceptibility of the isolated strains was determined by the E-test (bioMérieux, Marcy L'Etoile, France) for 10 antibiotics: metronidazole (range 0.016––256 µg/mL), vancomycin (0.016–256 µg/mL), chloramphenicol (0.016–256 µg/mL), moxifloxacin (0.002–32 µg/mL), piperacillin with tazobactam (0.016–256 µg/mL), erythromycin (0.016–256 µg/mL), clindamycin (0.016–256 µg/mL), benzylpenicillin (0.016–256 µg/mL), imipenem (0.002–32 µg/mL), rifampicin (0.002–32 µg/mL). The plates were incubated at 37°C for 48 h anaerobically according to the manufacturer's instructions. The antibiotic susceptibility results were interpreted in accordance with the recommendations of EUCAST (European Committee on Antimicrobial Susceptibility Testing, Version 10.0, valid 2020.01.01). Interpretations for Gram-positive anaerobes and *C. difficile* were used, and MIC values > 256 µg/ml were considered as resistant to erythromycin [16].

Table I. Primers used in mPCR of *C. difficile* strains isolated during course of outbreak
Tabela I. Startery *C. difficile* wykorzystane w reakcji mPCR przy opracowaniu ogniska epidemicznego

Gene target	Name	Sequence	Amplicon size [bp]
mPCR			
gluD	908CLD_gluDs	5' – GTCTTGGATGGTTGATGAGTAC – 3'	158
	909CLD_gluDas	5' – TTCCTAATTTAGCAGCAGCTTC – 3'	
tcdA	CD_tcdA-F3345	5' – GCATGATAAGGCAACTTCAGTGGTA – 3'	629
	CD_tcdA-R3969	5' – AGTTCCTCCTGCTCCATCAAATG – 3'	
tcdB	CD_tcdB-F5670	5' – CCAAARTGGAGTGTTACAAACAGGTG – 3'	410
	CD_tcdB-R6079A	5' – GCATTTCTCCATTCTCAGCAAAGTA – 3'	
	CD_tcdB-R6079B	5' – GCATTTCTCCGTTTTCAGCAAAGTA – 3'	
16S-rDNA	CD_PS13	5' – GGAGGCAGCAGTGGGGAATA – 3'	1062
	CD_PS14	5' – TGACGGGCGGTGTGTACAAG – 3'	
Binary toxin			
cdtA	cdtA-F739A	5' – GGGAAGCACTATATTAAAGCAGAAGC – 3'	221
	cdtA-F739B	5' – GGGAAACATTATATTAAAGCAGAAGC – 3'	
	cdtA-R958	5' – CTGGGTTAGGATTATTTACTGGACCA – 3'	
cdtB	ctdB-F617	5' – TTGACCCAAAGTTGATGTCTGATTG – 3'	262
	cdtB-R878	5' – CGGATCTCTTGCTTCAGTCTTTATAG – 3'	
Mechanism MLS_B			
ermB	2980	5' – AATAAGTAAACAGGTAACGTT – 3'	688
	2981	5' – GCTCCTTGGAAGCTGTCAGTAG – 3'	

RESULTS

In the period between December 29, 2018 and February 3, 2019, diarrhea was noted in 5 patients of the internal medicine ward. The infection control team stated a CDI outbreak based on the clinical symptoms of the patients (> 3 bowel movements per day, abdominal pain, malaise, and in some of them fever), and laboratory results. The characteristics of individual patients from this outbreak, as well as the results of microbiological tests are shown in Table II.

Sample numbers 4 and 4a belonged to one patient with the recurrence of diarrhea in the period of 4 weeks after the previous episode. A 68-year-old woman had several accompanying diseases: type 2 diabetes mellitus, hypertension and heart complaints; she had been previously treated surgically in the same hospital. The interview showed that the patient's previous hospitalization was completed on December 18, 2018. Since the patient was previously treated with an antibiotic (co-amoxiclav), it was decided to test her for antibiotic-associated diarrhea. When CDI was confirmed by the laboratory results, patient treatment

with oral metronidazole (500 mg 3 times daily for 7 days) was started. On the third day of treatment the number of bowel movements was reduced. When metronidazole treatment was finished and the patient's condition improved, the abdominal pain was gone and diarrhea subsided, she was discharged. Her subsequent hospitalization was noted on January 28, 2019 due to resumed diarrhea (7 bowel movements per day), abdominal pain, malaise and weakness. Stool samples were tested for CDI as described above. After confirming positive results for GDH and *C. difficile* A/B toxins, CDI treatment with oral vancomycin (250 mg 4 times daily for 10 days) was started. The patient also received rehydration therapy. After stabilization of the patient's condition and the resolution of diarrhea, she was discharged home on February 9, 2019. During the next 3 months this patient was not hospitalized for treatment of diarrhea.

From the 5 patients' stools, 6 *C. difficile* strains were isolated. All the *C. difficile* isolates belonged to PCR RT 027. The mPCR showed in all the strains the presence of the following genes: *gluD* (encoding GDH antigen), *tcdA* (toxin A), *tcdB* (toxin B) and *cdtA*/*cdtB* (responsible for encoding binary toxin), as well as *ermB* – (MLS_B resistance; Figure 1).

Women (4/5) dominated among the outbreak patients. All the patients lived in the Silesian region of Poland. The age of the patients was in the range of 39–86 years. Between admission to the hospital and receiving positive results for CDI an average of 5 days passed. A high level of C-reactive protein (CRP) was noted in all the 5 patients. Patient no. 5 with diarrhea was initially hospitalized in the internal medicine ward; however, due to the suspicion of appendicitis, she was transferred to the department of surgery.

All the tested *C. difficile* strains demonstrated sensitivity to metronidazole, vancomycin, and piperacillin with tazobactam; 5/6 strains (83.3%) were resistant to penicillin. All the 6 isolates showed resistance to moxifloxacin, chloramphenicol, imipenem, rifampicin, as well as erythromycin and clindamycin (confirmed by the presence of the *ermB* gene). The results of the antibiotic susceptibility testing are presented in Table III.

Table II. Characterization of patients and *C. difficile* isolates during CDI outbreak
Tabela II. Charakterystyka pacjentów oraz izolatów *C. difficile* podczas ogniska CDI

Sample numbers	Gender	Age	Date of stool collection and testing for *C. difficile*	CRP mg/dl	*C. difficile* genes			*C. difficile* ribotype
					GDH	toxins A/B	binary toxin	
1	M	61	2019-01-08	62.6	+	+	+	RT 027
2	W	78	2018-12-29	313.1	+	+	+	RT 027
3	W	86	2018-12-28	41.2	+	+	+	RT 027
4*	W	68	2018-12-29	102.6	+	+	+	RT 027
4a			2019-01-28	52.3	+	+	+	RT 027
5b	W	39	2019-02-03	346	+	+	+	RT 027

* Isolates no. 4 and 4a are from same patient; b – patient was transferred to surgical ward due to suspicion of appendicitis; CRP – C-reactive protein; GDH – glutamate dehydrogenase; M – man; W – woman; RT 027 – ribotype 027.

Table III. MIC50, MIC90, geometric mean of tested *C. difficile* strains derived from 5 patients during studied CDI outbreak
Tabela III. MIC50, MIC90 oraz średnia geometryczna badanych szczepów *C. difficile* pochodzących z próbek pacjentów włączonych do ogniska CDI

Antibiotic	*Clostridioides difficile* isolates (n = 6)					EUCAST µg/mL[a]
	MIC_{50} µg/mL	MIC_{90} µg/mL	GM	Range µg/mL	% strains resistant [EUCAST]	
1	2	3	4	5	6	7
Metronidazole	1	1.5	1.16	0.75–1.5	0	> 2
Vancomycin	0.19	0.25	0.18	0.125–0.25	0	> 2
Moxifloxacin[b]	32	32	32	32	100	4
Erythromycin	256	256	256	256	100	IE
Clindamycin[c]	256	256	256	256	100	> 4
Piperacillin/Tazobactam[c]	4	4	3.64	3–4	0	> 16
Imipenem[c]	24	32	25.9	12–32	100	> 4

cd. table III

1	2	3	4	5	6	7
Benzylpenicillin[c]	0.75	1	0.66	0.25–1	83.3	> 0.5
Chloramphenicol[c]	24	64	26.42	12–64	100	> 8
Rifampicin[b]	32	32	32	32	100	0.004

[a]resistance according EUCAST; [b]ECOFF for *C. difficile* was used because lack of them according EUCAST; [c]MICs for Gram-positive anaerobes were used because lack of them according EUCAST; GM – geometric mean; IE – lack of limit value; Range [µg/mL] = range of antibiotic susceptibility test results from minimum to maximum.

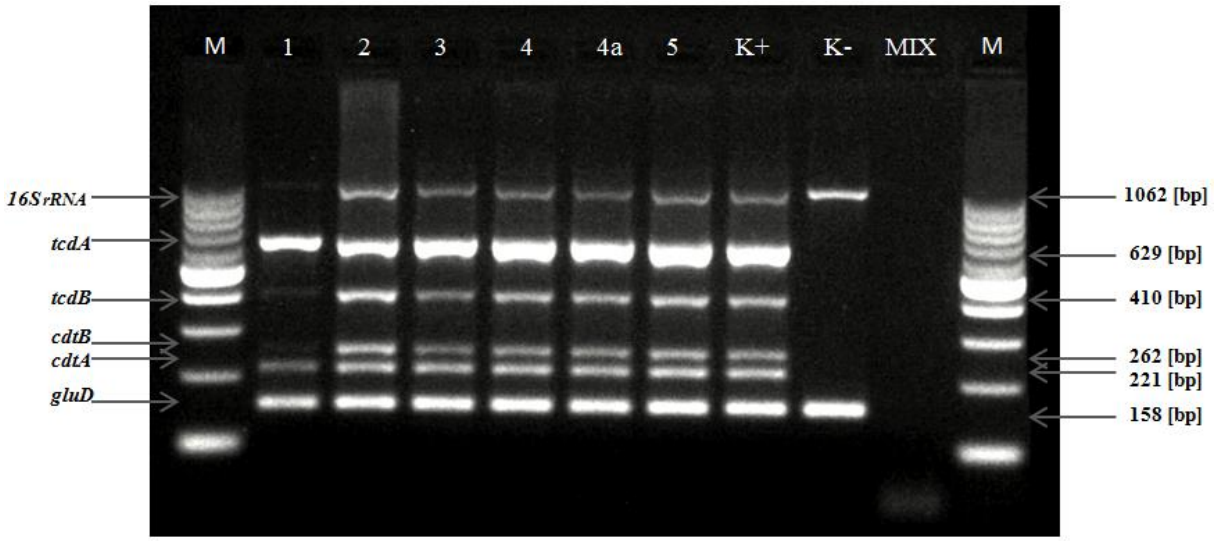

Fig. 1. mPCR results of 6 *C. difficile* isolates from CDI outbreak in internal medicine ward of district hospital in Silesia, PL (separation of PCR products in a 1% agarose gel). Lanes: **M** – DNA marker; **1–5** – *C. difficile strains tested;* **K (+)** – positive control *C. difficile* [16S-rDNA – 1062 pz; *tcdA* – 629 pz; *tcdB* – 410 pz; *cdtB* – 262 pz; *cdtA* – 221 bp; *gluD* – 158 pz]; **K (-)** – negative control; **MIX** – *reaction mix.*
Ryc. 1. Wyniki mPCR 6 izolatów *C. difficile* pozyskanych z próbek pacjentów podczas ogniska CDI na oddziale chorób wewnętrznych szpitala powiatowego na Śląsku (produkty PCR w 1-proc. żelu agarozowym). Ścieżki: **M** – marker DNA; **1–5** – badane szczepy *C. difficile*; **K (+)** – kontrola pozytywna *C. difficile* [16S-rDNA – 1062 pz; *tcdA* – 629 pz; *tcdB* – 410 pz; *cdtB* – 262 pz; *cdtA* – 221 pz; *gluD* – 158 pz]; **K (-)** – kontrola negatywna; **MIX** – mieszanina reakcyjna.

DISCUSSION

A major contributor to outbreaks and healthcare--associated infections is the hypervirulent *C. difficile* strain RT 027, characterized by multidrug-resistant (MDR-resistant) and an increased ability to produce toxins and spores. The result of a two-year study conducted in 2016 allowed the dominance of *C. difficile* RT 027 (19%) to be determined in the 125 *C. difficile* isolates, derived from various European countries [7]. Aptekorz et al. [5] indicated a significant dominance of *C. difficile* RT 027 among isolates from 15 different hospitals in Silesia. During the presently investigated outbreak, all of the *C. difficile* isolates belonged to PCR RT 027. Infection with a hypervirulent strain of *C. difficile* results in recurrences, estimated at 25–30%. Recurrences of CDI (rCDI) are found in 20––30% of patients within 8 weeks after the first episode. Recurrences of CDI are a major clinical and economic challenge; the estimated costs of treating CDI in the European Union are over €3 billion and in the United States about $796 million annually, with an upward trend [17]. The risk factors of rCDI are: age > 65 (20 times more often compared to patients under 20 y/o), a severe course of the first episode of CDI, prolonged hospitalization and long-term antibiotic therapy [18].

During the studied CDI outbreak, one patient demonstrated a recurrence of diarrhea 4 weeks after treatment. In a study by Dharbhamulla et al. [18], a recurrence of CDI within 14 days of the first episode was observed in 22.1% of the respondents. At the same time, from the first episode of CDI (14 days) Cioni et al. [19] observed a recurrence of CDI in 14.6% of patients. Recurrences of CDI are common and as described by Aptekorz et al. [5], in 7 out of the 9 studied patients rCDI was caused by the same strain (one patient from the study group died).

In the treatment of CDI, discontinuation of antibiotic therapy is essential, if possible; also, rehydration and electrolyte supplementation are required. Disturbing information is described by Lee et al. [13], pointing to a decreased sensitivity to metronidazole among 40%

of the tested *C. difficile* strains (also from Poland). By reviewing the literature, Clancy et al. [12] noticed a significant decrease in the effectiveness of metronidazole against CDI over the years (before 2000 – 3% of failures in pharmacotherapy with metronidazole, after 2000 – as much as 18%). Although at this moment according to European and also USA recommendations, metronidazole is not recommended for the treatment of even moderate cases of CDI, at the time (2018/2019) of the studied outbreak, metronidazole was still in use in Poland [10,11].

Among our *C. difficile* isolates, no metronidazole or vancomycin resistant strains were found. In the recommendations of IDSA and SHEA for the treatment of recurrent CDI, the antibiotic next to vancomycin should be rifaximin for multiple recurrences [10]. In the CDI outbreak we investigated, all of the 6 *C. difficile* isolates were resistant to rifampicin; we also demonstrated in a previous study that there is a high percentage of resistance to rifampicin [7].

All the 6 CDI outbreak isolates were resistant to moxifloxacin, chloramphenicol and imipenem. Vernon et al. [20] reported 7/75 *C. difficile* isolates from the hospital environment, resistant to moxifloxacin; in addition there were 3 confirmed cases of patients with toxic megacolon (a severe course of clinical CDI).

The presence of the *ermB* gene determines resistance to erythromycin, clindamycin and streptogramin B [6]. All 6 of our *C. difficile* isolates belonging to RT 027 showed the presence of the *ermB* gene (MLS_B-type resistance).

All the 6 strains of *C. difficile* from the studied outbreak were sensitive to piperacillin with tazobactam, but only one exhibited sensitivity to penicillin, which is possibly caused by the production of beta-lactamase. Lachowicz et al. [9] obtained antibiotic susceptibility results similar to ours; 253 isolates were sensitive to metronidazole, vancomycin and 209/253 were sensitive to clindamycin. Only 1 strain possessed reduced sensitivity to metronidazole.

The ribotyping of *C. difficile* isolates showed that all isolates from the epidemic outbreak we investigated belonged to PCR RT 027. Multiplex PCR performed with the above-mentioned strains in order to detect genes encoding toxins A, B and binary, confirmed the toxin profile typical for *C. difficile* RT 027 strains.

The distribution of the *C. difficile* PCR RT 027 strain in Poland is determined at the level of 48% [21]. Such a high spread is related to bacterial virulence factors, but it also results from the negligence of medical staff and the patients themselves. Proper hand hygiene, disinfection by using sporicides and optimization of the treatment can be effective to reduce CDI hospital outbreaks.

CONCLUSIONS

All the 6 *C. difficile* isolates belonged to the same hyperepidemic PCR RT 027, were MDR-resistant to moxifloxacin, erythromycin, clindamycin, rifampicin, imipenem, and chloramphenicol.

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Author's contribution

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