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Original article

ANTIBIOTICS: CURE AND RISK FACTOR FOR *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Abstract

Introduction: The major risk factor for acquiring an infection with *Clostridioides difficile* (CDI) is a long-term antibiotic treatment. Contrarily, the treatment of severe CDI cases involves application of antibiotics like vancomycin or metronidazole. Our aim was to investigate the percentage of resistance to eight antibiotics (vancomycin, metronidazole, tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin) among *Clostridioides difficile* isolates, indirectly evaluating the risks of acquiring CDI and the risks of therapeutic failure.

Materials and methods: Eighty isolates of *Clostridioides difficile*, collected from fecal samples from as many patients during a four-year period, were subject to PCR ribotyping and to antibiotic susceptibility testing by using the E test.

Results: Ribotyping of the 80 isolates of *C. difficile* showed that they belonged to 20 different ribotypes. The most common one was 001/072, with 40% of the isolates. All 80 of *C. difficile* isolates in this study showed a good sensitivity towards vancomycin and metronidazole. Resistance percentages towards tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin were 1.25%, 49%, 55%, 57%, 100% and 45%, respectively. The highest antimicrobial resistance percentages were detected in isolates taken from patients hospitalized in surgical clinics and in isolates belonging to the dominant ribotype 001/072 and hypervirulent ribotypes 017 and 027.

Conclusions: Vancomycin and metronidazole should remain the first option therapy for CDI. Therapy with clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin could be a risk factor for CDI. Excessive use of a particular antibiotic plays a major role in selecting and multiplying resistant clones of *Clostridioides difficile* strains.

Keywords: *Clostridioides difficile* infection, *C. difficile*, antimicrobial susceptibility

Introduction

Clostridioides difficile is one of the most important intrahospital pathogens. This sporogenic anaerobic bacterium has commonly been isolated from feces, mostly from elderly hospitalized patients on antibiotics and has been associated with several clinical manifestations ranging from diarrhea to pseudomembranous colitis[1].

The major risk factor for acquiring an infection with *Clostridioides difficile* (CDI) is a long- term antibiotic treatment. Although all antibiotics can lead to CDI, studies have shown that mostly involved are the wide spectrum antibiotics, like: fluoroquinolones, penicillins, third generation cephalosporins and clindamycin[2]. Non-severe cases of CDI can be solved by terminating the use of the given antibiotic and by using probiotics[3]. On the other hand, the treatment of severe CDI cases involves application of antibiotics, such as vancomycin or metronidazole[4]. In many hospitals, including the University Clinical Complex "Mother Teresa" in Skopje, this therapy is given empirically, although there are few reports of emerging resistance worldwide[5].

Our aim with this study was to investigate the percentage of resistance to eight antibiotics (vancomycin, metronidazole, tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin) among *Clostridioides difficile* isolates, indirectly evaluating the risk of acquiring CDI (by using the last six of them) or the risk of therapeutic failure in treating CDI (by using the first two).

Materials and methods

All fecal samples received in the 2016-2020 period at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, in order to diagnose *Clostridioides difficile* infection (CDI), were subject to immunochromatographic detection of glutamate dehydrogenase (GDH) antigen and toxins A and B of *Clostridioides difficile*. In order to cultivate them, the samples were planted on two plates: directly on Cycloserine-Cefoxitin-Fructose agar (CCFA) and on Columbia blood agar after performing the alcohol shock test. Such planted plates were incubated anaerobically for 48 hours at 37^0C in order to isolate *Clostridioides difficile*. The grown colonies were identified by characteristic macroscopic appearance and also microscopically by Gram staining. The definitive identification was made by using the automated system VITEK 2.

Eighty isolates of *Clostridioides difficile* from as many patients were collected from the cultures and were later typed using the PCR ribotyping method as the most commonly used typing method for this bacterium in Europe[6].

The antimicrobic susceptibility towards the eight antibiotics: vancomycin, metronidazole (according to EUCAST breakpoints) and tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin (according to CLSI break points), was also determined by using the E test on all eighty isolates. Interpretation criteria (breakpoints) for the susceptibility testing of the isolates are shown in Table 1.

Results

In the period of 2016-2020, we received 1380 fecal samples from symptomatic patients for CDI, and in 182 of them presence of *Clostridioides difficile* was confirmed. After keeping only the first isolate from patients that had multiple samples tested and eliminating the isolates that had not survived the laboratory manipulation and subcultivation, we finally collected 80 isolates of *Clostridioides difficile* for further examination.

The origin of the isolates (clinics where the symptomatic patients were hospitalized) is shown in Table 2.

The isolates originated from 41 male and 39 female patients. The average age of patients was 54. Fifty-six percent of the patients were over 60 years old. The percentage of toxigenic strains among the isolates of *Clostridioides difficile* was 92.

Table 1. Interpretation criteria for the antimicrobial susceptibility testing of *Clostridioides difficile* isolates according to their minimal inhibitory concentrations (MIC)

	Vancomycin**	**Metronidazole****	**Tetracycline***	**Erythromycin***	**Clindamycin***	**Ciprofloxacin***	**Moxifloxacin***	**Imipenem***
Susceptible (μg/ml)	≤2	≤2	≤4	≤2	≤2	≤2	≤2	≤4
Intermediate (μg/ml)	-	-	8	4	4	4	4	8
Resistant (μg/ml)	>2	>2	≥16	≥8	≥8	≥8	≥8	≥16

** Interpretation is based on the European Committee on Antimicrobial Susceptibility Testing, *Interpretation is based on CLSI M100-S25

Table 2. Origin of *Clostridioides difficile* isolates

Origin of fecal samples (isolates) sent		**Collected isolates (percentage of total isolates collected)**	**Total samples received (percentage of positive samples received)**
Surgery Clinics	Number	22	70
	%	27.5%	31.4%+
Internal Diseases Clinics	Number	31	256
	%	38.8%	12.1%+
Pediatric Clinic	Number	7	413
	%	8.7%	1.7%+
Infectious Diseases Clinic	Number	10	442
	%	12.5%	2.3% +
Private ambulances, outpatients and the rest	Number	10	199
	%	12.5%	5%+

Table 3. Ribotypes confirmed among isolates of *Clostridioides difficile*

	***C. difficile* ribotypes**	**Number (N)**	**%**
1	001/072	32	40.00%
2	002	5	6.25%
3	003	1	1.25%
4	005	3	3.75%
5	012	1	1.25%
6	014/020	10	12.50%
7	015	1	1.25%
8	017	5	6.25%
9	023	1	1.25%
10	027	5	6.25%
11	046	1	1.25%
12	070	1	1.25%
13	255/258	3	3.75%
14	SLO 046	3	3.75%
15	SLO 047	3	3.75%
16	SLO 069	1	1.25%
17	SLO 110	1	1.25%
18	SLO 120	1	1.25%
19	SLO 160	1	1.25%
20	SLO 187	1	1.25%
Total		80	100%

In order to classify the isolates in groups, for better correlation with their antimicrobial susceptibility, we performed the PCR ribotyping as the most widely used *Clostridioides difficile* typing method in Europe. PCR ribotyping results are shown in Table 3.

Antimicrobial resistance of the 80 *Clostridioides difficile* isolates toward the 8 examined antibiotics is shown in Table 4. Four of the examined antibiotics, which the isolates showed variable susceptibility to, were also analyzed in terms of associating the resistance of the strain with its origin. This association is shown in Table 5.

Table 4. Resistance of *Clostridioides difficile* strains towards the eight examined antibiotics

Antibiotic	Result Interpretation	Ribotype 001/072 n (%)	014/020 n (%)	002 n (%)	017 n (%)	027 n (%)	Others* n (%)	Total n (%)
Vancomycin	Susceptible	32(100)	10(100)	5(100)	5(100)	5(100)	23(100)	80(100)
	Resistant	-	-	-	-	-	-	-
	Intermediate	-	-	-	-	-	-	-
Metronidazole	Susceptible	32(100)	10(100)	5(100)	5(100)	5(100)	23(100)	80(100)
	Resistant	-	-	-	-	-	-	-
	Intermediate	-	-	-	-	-	-	-
Tetracycline	Susceptible	32(100)	10(100)	5(100)	2(40)	5(100)	22(95.65)	76(95)
	Resistant	-	-	-	-	-	1(4.35%)	1(1.25)
	Intermediate	-	-	-	3(60%)	-	-	3 (3.75)
Clindamycin	Susceptible	2(6.25)	1(10)	3(60)	-	1(20)	13(56.52)	20(25)
	Resistant	28(87.50)	1(10)	-	5(100)	-	5(21.74)	39(48.75)
	Intermediate	2(6.25)	8(80)	2(40)	-	4(80)	5(21.74)	21(26.25)
Erythromycin	Susceptible	4(12.50)	9(90)	5(100)	-	-	18(78.26)	36(45)
	Resistant	28(87.50)	1(10)	-	5(100)	5(100)	5(21.74)	44(55)
	Intermediate	-	-	-	-	-	-	-
Imipenem	Susceptible	6(18.75)	4(40)	-	-	-	11(47.83)	21(26.25)
	Resistant	23(71.88)	3(30)	3(60)	5(100)	4(80)	8(34.78)	46(57.5)
	Intermediate	3(9.38)	3(30)	2(40)	-	1(20)	4(17.39)	13(16.25)
Ciprofloxacin	Susceptible	-	-	-	-	-	-	-
	Resistant	32(100)	10(100)	5(100)	5(100)	5(100)	23(100)	80(100)
	Intermediate	-	-	-	-	-	-	-
Moxifloxacin	Susceptible	6(18.75)	9(90)	4(80)	1(20)	-	23(100)	43(53.75)
	Resistant	26(81.25)	1(10)	-	4(80)	5(100)	-	36(45)
	Intermediate	-	-	1(20)	-	-	-	1(1.25)

*others= 003, 005, 012, 015, 023, 046, 070, 255/258, SLO 046, SLO 047, SLO 069, SLO 110, SLO 120, SLO160, SLO 187

Table 5. Antibiotic resistance of *Clostridioides difficile* isolates to clindamycin, erythromycin, imipenem and moxifloxacin, according to the origin of the isolates

Percentage of Resistant Isolates	Antibiotic	Origin of the isolate: Surgery Clinics	Internal Diseases Clinics	Pediatric Clinic	Infectious Diseases Clinic	Private Ambulances, Outpatients and the Rest	Total (%)
	Clindamycin	77	51	14	30	20	49
	Erythromycin	77	65	16	30	30	55
	Imipenem	86	58	43	20	40	57
	Moxifloxacin	68	55	14	20	10	45

Discussion and conclusions

Metronidazole and vancomycin are still considered as first option for treatment of CDI [7]. In many diagnostic laboratories worldwide, *Clostridioides difficile* isolates are not routinely tested for their antibiotic susceptibility, which means that these two antimicrobials are applied empirically. Although our results showed no resistance among the isolates to these two antibiotics, which favors the practice mentioned before, there is still place for concern. In few studies [8-10] it is noted that resistance to metronidazole and vancomycin in *Clostridioides difficile* isolates can be present, especially among those belonging to the ribotype 027. This suggests that introducing the regular susceptibility testing to all isolates to these two antibiotics can help in preventing the therapeutic failures which could be expected in our hospitals in the future. This practice can also trigger the introduction of new therapeutic drugs for CDI in our hospitals such as fidaxomicin[10], which unfortunately are still not available.

Of the other six antibiotics tested in this study, only tetracycline showed a good action against *Clostridioides difficile* isolates. Resistance of the isolates to tetracycline was only 1.25%, which means that this drug has low potential for CDI. In some articles, it is also mentioned as a possible therapeutic option for CDI[11].

Clindamycin was considered as a very high-risk antimicrobial for inducing CDI in the past and that resulted in reduction of its use[12]. In our study, 49% of the isolates showed resistance to clindamycin, which is in the frame of the world average[7]. Especially high resistance showed the isolates belonging to the hyper-virulent ribotype 017 and the dominant ribotype 001/072. Isolates originating from the surgery clinics showed a higher resistance to clindamycin than those from the other locations.

The resistance of the isolates to erythromycin was 55%, which means that using this antibiotic brings almost the same risk of CDI as clindamycin, probably as a result of the same resistance mechanisms[7]. Especially high resistance to erythromycin was shown by the isolates belonging to the hyper-virulent ribotypes 017 and 027 and the dominant ribotype 001/072. Isolates originating from the surgery clinics showed a higher resistance to erythromycin than those from the other locations.

Although imipenem had not been mentioned as a risk for CDI in the past, it should be taken into consideration now. Out of the 80 isolates in this study, 57% showed resistance to imipenem, a much higher percentage than in most of the European countries[8]. In our opinion, excessive use of imipenem in our hospitals contributed to the wide distribution of such resistant strains. As with clindamycin, the isolates belonging to the hyper-virulent ribotype 017 and the dominant ribotype 001/072 showed the highest resistance. Isolates originating from the surgery clinics showed a higher resistance to imipenem than those from the other locations.

Acquiring a resistance to fluoroquinolones is considered a key moment in the evolution of the hyper-virulent ribotype 027[13]. Currently, the application of ciprofloxacin is considered to be the greatest risk for CDI, considering the 100% resistance of the strains in this study, as well as in many others from all over the world. However, the application of moxifloxacin is considered not as risky as ciprofloxacin. Forty-five percent of the isolates showed resistance to moxifloxacin. This is the case especially among the isolates belonging to the hyper-virulent ribotypes 017 and 027, and the dominant ribotype 001/072. This resistance is very rare among other ribotypes. Like in all previous cases in terms of antibiotics with variable action against *Clostridioides difficile,* isolates originating from the surgery clinics showed a higher resistance to moxifloxacin.

Finally, we would like to conclude that the excessive use of a particular antibiotic plays a major role in selecting and multiplying resistant clones of *Clostridioides difficile* strains. Acquiring such characteristics contributes subsequently to the distribution of the ribotypes, but also contributes to the originating of new *Clostridioides difficile* ribotypes. Surveillance of such genotypic and phenotypic characteristics of the *Clostridioides difficile* isolates can be of great value in controlling this modern epidemic of CDI.

Conflict of interest statement. None declared.

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