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Anaerobes in Focus: Two-Year Epidemiology and Resistance Trends in Osijek, Croatia

Anaerobi u fokusu: epidemiologija i antimikrobna osjetljivost anaeroba u dvogodišnjem periodu u KBC-u Osijek, Hrvatska

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Summary

Background: Although anaerobic bacteria are not often isolated from clinical specimens, they are an important cause of morbidity. The aim of this study was to determine the distribution and antimicrobial resistance profile of strictly anaerobic bacteria at the University Hospital Centre Osijek, Croatia over a two-year period.

Methods: Data collected at the Department of Clinical Microbiology and Hospital Infections, University Hospital Centre Osijek, Croatia over a two-year period were analysed. Samples sent to the laboratory for anaerobic cultivation were inoculated onto solid and/or liquid media and incubated in an anaerobic atmosphere. Culture media were inspected every 48 hours, and grown colonies were subjected to an aerotolerance test. Identification of strictly anaerobic isolates was performed using a MALDI-TOF mass spectrometer (MALDI Biotyper, Bruker, Germany). Susceptibility of isolates was tested by the breakpoint method with a commercially available test (ATB ANA EU, Biomerieux, France) and interpreted according to EUCAST guidelines.

Results: A total of 605 anaerobic isolates were detected, of which 274 were strictly anaerobic. The most abundant genus was *Bacteroides* spp., with 130 (47.45%) isolates, and *B. fragilis* was the predominant species with 71 (54.61%) isolates. The highest resistance among Gram-negative bacteria was to penicillin (80.17%). The highest resistance among Gram-positive bacteria was to metronidazole (38.46%).

Conclusions: *Bacteroides fragilis* was the most frequently isolated anaerobic bacterium during the two-year period at the University Hospital Centre Osijek, Croatia. Resistance to metronidazole, the first therapeutic choice, remains very low, but resistance to clindamycin is concerning. Further monitoring of the antimicrobial susceptibility profile of anaerobic bacteria is required.

Sažetak

Cilj: Iako ih se ne detektira često, anaerobne bakterije su iznimno važni uzročnici infekcija. Cilj ovog istraživanja bio je ispitati učestalost i antimikrobnu osjetljivost striktno anaerobnih bakterija u Kliničkom bolničkom centru Osijek, Hrvatska, u dvogodišnjem periodu.

Metode: U istraživanju su analizirani podatci prikupljeni u Zavodu za kliničku mikrobiologiju i bolničke infekcije Kliničkoga bolničkog centra Osijek u dvogodišnjem periodu. Uzorci pristigli u laboratorij sa zahtjevom za anaerobnu kultivaciju inokulirani su na kruta i/ili tekuća hranilišta potom inkubirana u anaerobnoj atmosferi. Hranilišta su vizualno pregledavana svakih 48 sati, a morfološki različite porasle kolonije podvrgnute testu aerotolerancije. Pomoću MALDI-TOF spektrometra masa (MALDI Biotyper, Bruker, Germany) identificirani su striktni anaerobi. Antimikrobna osjetljivost ispitana je metodom prijelomne točke pomoću komercijalno dostupnog testa (ATB ANA EU, Biomerieux, France), a dobiveni rezultati su interpretirani prema važećim smjernicama EUCAST-a.

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Rezultati: Ukupno je detektirano 605 izolata od kojih je 274 bilo striktnih anaeroba. Najzastupljeniji rod je *Bacteroides* spp., s ukupno 130 (47,45%) izolata, a *B. fragilis* sa 71 (54,61%) izolatom jest najčešće detektirana vrsta. Detektirane gram-negativne bakterije najčešće su bile rezistentne na penicilin (80,17%), a gram-pozitivne na metronidazol (38,46%).

Zaključak: U dvogodišnjem periodu *Bacteroides fragilis* je najčešće detektiran anaerob. Rezistencija na metronidazol koji je ujedno i prvi terapijski izbor i dalje je niska, ali istovremeno zabrinjava visoka stopa rezistencije na klindamicin. Daljnje praćenje antimikrobne osjetljivosti i pravovremeno ažuriranje smjernica iznimno je važno za empirijsku terapiju.

1. INTRODUCTION

Anaerobic bacteria are common human pathogens. Although they form an integral part of the normal flora of the mucous membranes of the oral cavity, gastrointestinal tract, and female genital tract, they can cause serious and life-threatening infections. Infections involving anaerobes are usually polymicrobial and often result from damage to mucosal surfaces, mainly due to surgery, trauma, or the presence of tumours. Their isolation requires appropriate methods of collection, transport, and cultivation, and due to their demanding growth conditions, they are often overlooked or neglected. *Bacteroides* spp., *Prevotella* spp., *Fusobacterium* spp., and *Clostridium* spp. are the genera most frequently isolated from clinical samples[1,2].

In general, successful treatment of anaerobic infections should include appropriate antimicrobial agents and surgical intervention if necessary. Antibiotics with the greatest activity against *Bacteroides* spp. include metronidazole, β-lactam/β-lactamase inhibitor combinations, and carbapenems. Antibiotic resistance among anaerobic microorganisms has increased significantly in recent years, but resistance rates vary widely between different geographic regions[2]. The aim of this research was to evaluate the epidemiology and antimicrobial susceptibility of strictly anaerobic bacteria over a two-year period at University Hospital Centre Osijek.

2. MATERIALS AND METHODS

2.1. Isolates

Data on isolated anaerobic bacteria collected at the Division of Clinical Microbiology and Hospital Infections, University Hospital Centre Osijek, Croatia from May 2020 to May 2022, were used in this research.

Samples sent to the Division's laboratory with a request for anaerobic cultivation were inoculated onto solid media (Columbia agar, Schedler Neomycin Vancomycin agar) and/or liquid media (thioglycolate broth) and incubated in a jar with a generator (Genebox anaerobic gas pack, Biomerieux, France) and an indicator (Anaerobic indicator, Biomerieux, France) of anaerobic atmosphere. Culture media were inspected every 48 hours, and morphologically distinct colonies were subjected to an aerotolerance test, i.e. subcultured and cultivated in appropriate atmospheric conditions (blood agar – aerobic atmosphere, chocolate agar – 3-5% CO2, Columbia agar – anaerobic atmosphere). Strictly anaerobic isolates were identified using a MALDI-TOF mass spectrometer (MALDI Biotyper, Bruker, Germany).

2.2 Susceptibility test

Antimicrobial susceptibility of the detected isolates was determined using the breakpoint method with a commercially available test (ATB ANA EU, Biomerieux, France) and interpreted according to current EUCAST guidelines[3].

The following antibiotics were tested: penicillin (PCN), amoxicillin (AMO), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), clindamycin (CLI), imipenem (IMI), and metronidazole (MTR).

3. RESULTS

During the study period, a total of 6,693 samples were submitted for anaerobic cultivation, as shown in Table 1. Of these, 556 (8.31%) samples were positive for anaerobic bacteria. A total of 605 isolates were identified, including 274 (4.09%) strictly anaerobic isolates.

The distribution of isolated facultative and strictly anaerobic bacteria among the cultivated samples is presented in Table 2.

As shown in Figure 1, 130 (47.45%) isolates belonged to the Bacteroides genus, making them the most frequently isolated pathogens. Among these, the most common species were *B. fragilis* at 54.61% (n = 71), *B. thetaiotaomicron* at 22.30% (n = 29), and *B. vulgatus* at 11.54% (n = 15). *Prevotella* spp. was the second most abundant genus with 89 isolates (32.48%). *P. denticola* accounted for 20.22% (n=18) of these isolates, while *P. bivia* and *P. buccae* each accounted for 19.10% (n = 17).

Table 1. Overview of the results of the performed tests
Tablica 1. Pregled rezultata izvršenih analiza

SAMPLE	TEST RESULTS	N*	%	P†
Skin and subcutaneous tissues	total tested samples	1091	16.30 %	< 0,001
	negative	927	84.97 %	
	positive	164	15.03 %	
Blood culture and cerebrospinal fluid	total tested samples	4343	64.89 %	
	negative	4081	93.97 %	
	positive	262	6.03 %	
Intraoperative samples	total tested samples	659	9.85 %	
	negative	572	86.80 %	
	positive	87	13.20%	
Aspirates	total tested samples	563	8.41 %	
	negative	526	93.43 %	
	positive	37	6.57%	
Other	total tested samples	37	0.55 %	
	negative	31	83.78 %	
	positive	6	16.22 %	
TOTAL		6693	100 %	

*number of samples, †chi-square test (χ 2)

Table 2. Distribution of isolated bacteria from positive samples according to metabolic oxygen requirements
Tablica 2. Distribucija detektiranih bakterija sukladno metaboličkoj ovisnosti o kisiku

SAMPLE		N*	%	P†
Skin and subcutaneous tissues	**Total isolates**	**175**	**28.93 %**	< 0,001
	Facultative anaerobes	16	2.64 %	
	Strict anaerobes	159	26.28 %	
Haemoculture and cerebrospinal fluid	**Total isolates**	**283**	**46.78 %**	
	Facultative anaerobes	276	45.62 %	
	Strict anaerobes	7	1.16 %	
Intraoperative samples	**Total isolates**	**99**	**16.36 %**	
	Facultative anaerobes	5	0.83 %	
	Strict anaerobes	94	15.54 %	
Aspirates	**Total isolates**	**42**	**6.94 %**	
	Facultative anaerobes	31	5.12 %	
	Strict anaerobes	11	1.82 %	
Other	**Total isolates**	**6**	**0.99 %**	
	Facultative anaerobes	3	0.50 %	
	Strict anaerobes	3	0.50 %	
TOTAL		**605**	100 %	

*Number of isolates, †chi-square test (χ 2)

Antimicrobial susceptibility testing was performed on 258 isolates, as shown in Table 3. There were 232 Gram-negative isolates, mainly *Bacteroides* spp. and *Prevotella* spp. Gram-negative anaerobic bacteria were susceptible to metronidazole, except for one *Bacteroides* spp. isolate and four *Prevotella* spp. isolates. Most were also susceptible to amoxicillin-clavulanic acid and piperacillin-tazobactam. All Gram-negative anaerobic pathogens were susceptible to imipenem, although two *Bacteroides* spp. isolates showed sensitivity only with increased exposure to the antibiotic (Table 4).

Gram-positive anaerobic bacteria were less common, with only 26 isolates, mainly from the genera *Cutibacterium* spp., *Peptoniphilus* spp., and *Clostridium* spp. (Table 5). All tested isolates were susceptible to amoxicillin-clavulanic acid, piperacillin-tazobactam, and imipenem (Table 6). Overall resistance rate of different anaerobes to clindamycin is shown in Table 7. No significant differences of clindamycin resistance across the years were detected (χ^2 = 0.8785; df = 2; P = 0.64), nor was a linear trend of increasing or decreasing resistance observed among all isolates

Table 3. Antimicrobial susceptibility of the most common Gram-negative anaerobes

Tablica 3. Antimikrobna osjetljivost najčešćih gram-negativnih anaeroba

ISOLATE (number of tested samples) AND ANTIBIOTIC	S* (%)	I† (%)	R‡ (%)
Bacteroides spp. (126)			
Penicillin	3.17	0.00	96.83
Amoxicillin	4.76	0.00	95.24
Amoxicillin-clavulanic acid	69.05	3.17	27.78
Piperacillin-tazobactam	83.33	5.56	11.11
Clindamycin	59.52	0.00	40.48
Imipenem	98.41	1.59	0.00
Metronidazole	99.21	0.00	0.79
Prevotella spp. (83)			
Penicillin	28.92	1.20	69.88
Amoxicillin	33.73	1.20	65.06
Amoxicillin-clavulanic acid	97.59	0.00	2.41
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	28.92	0.00	71.08
Imipenem	100.00	0.00	0.00
Metronidazole	95.18	0.00	4.82
Fusobacterium spp. (16)			
Penicillin	81.25	6.25	12.50
Amoxicillin	87.50	0.00	12.50
Amoxicillin-clavulanic acid	100.00	0.00	0.00
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	93.75	0.00	6.25
Imipenem	100.00	0.00	0.00
Metronidazole	100.00	0.00	0.00
Porphyromonas spp. (5)			
Penicillin	60.00	0.00	40.00
Amoxicillin	60.00	0.00	40.00
Amoxicillin-clavulanic acid	100.00	0.00	0.00
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	80.00	0.00	20.00
Imipenem	100.00	0.00	0.00
Metronidazole	100.00	0.00	0.00

*susceptible, †susceptible, increased exposure, ‡resistant

Table 4. Susceptibility of Gram-negative anaerobes to antimicrobial chemotherapeutics
Tablica 4. Zbirna antimikrobna osjetljivost gram-negativnih anaeroba

	S* N (%)	I† N (%)	R‡ N (%)
Penicillin	44 (18.97)	2 (0.86)	186 (80.17)
Amoxicillin	51 (21.98)	1 (0.43)	180 (77.59)
Amoxicillin- clavulanic acid	189 (81.47)	4 (1.72)	39 (16.81)
Piperacillin- tazobactam	210 (90.52)	7 (3.02)	15 (6.47)
Clindamycin	119 (51.29)	0 (0.00)	113 (48.71)
Imipenem	230 (99.14)	2 (0.86)	0 (0.00)
Metronidazole	227 (97.84)	0 (0.00)	5 (2.16)

*susceptible, †susceptible, increased exposure, ‡resistant

Table 5. Antimicrobial susceptibility of the most common Gram-positive anaerobes
Tablica 5. Antimikrobna osjetljivost najčešćih gram-pozitivnih anaeroba

ISOLATE (number of tested samples) AND ANTIBIOTIC	S* (%)	I† (%)	R‡ (%)
***Clostridium* spp. (6)**			
Penicillin	66.67	0.00	33.33
Amoxicillin	66.67	0.00	33.33
Amoxicillin-clavulanic acid	100.00	0.00	0.00
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	83.33	0.00	16.67
Imipenem	100.00	0.00	0.00
Metronidazole	100.00	0.00	0.00
Vancomycin	66.66	16.67	16.67
***Cutibacterium* spp. (8)**			
Penicillin	100.00	0.00	0.00
Amoxicillin	100.00	0.00	0.00
Amoxicillin-clavulanic acid	100.00	0.00	0.00
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	75.00	0.00	25.00
Imipenem	100.00	0.00	0.00
Metronidazole	0.00	0.00	100.00
Vancomycin	100.00	0.00	0.00
***Peptoniphilus* spp. (8)**			
Penicillin	75.00	0.00	25.00
Amoxicillin	100.00	0.00	0.00
Amoxicillin-clavulanic acid	100.00	0.00	0.00
Piperacillin-tazobactam	100.00	0.00	0.00
Clindamycin	75.00	0.00	25.00
Imipenem	100.00	0.00	0.00
Metronidazole	100.00	0.00	0.00
Vancomycin	100.00	0.00	0.00

*susceptible, †susceptible, increased exposure, ‡resistant

over the tested period (Cochran–Armitage trend test: $\chi^2 = 0.8781$; df = 1; P = 0.35). Contrary, clindamycin resistance in *Bacteroides* spp. showed a significant linear trend over the two-year period (Cochran-Armitage test: $\chi^2 = 4.855$; df = 1; P <0.05) although overall differences between years did not reach statistical significance ($\chi^2 = 5.468$; df = 2; P = 0.07) as shown in Figure 2. Data regarding clindamycin consumption in University Hospital Centre Osijek, Croatia in Daily Defined Dose (DDD) over years in comparison with resistance rate of Gram-positive and Gram-negative is presented in Table 8. Correlation of clindamycin consumption (oral and parenteral) to the overall resistance rate of detected anaerobes is shown in Figure 3.

Table 6. Susceptibility of Gram-positive anaerobes to antimicrobial chemotherapeutics

Tablica 6. Zbirna antimikrobna osjetljivost gram-pozitivnih anaeroba

	S* N (%)	I† N (%)	R‡ N (%)
Penicillin	22 (84.62)	1 (3.85)	3 (11.54)
Amoxicillin	24 (92.31)	0 (0.00)	2 (7.69)
Amoxicillin- clavulanic acid	26 (100.00)	0 (0.00)	0 (0.00)
Piperacillin- tazobactam	26 (100.00)	0 (0.00)	0 (0.00)
Clindamycin	21 (80.77)	0 (0.00)	5 (19.23)
Imipenem	26 (100.00)	0 (0.00)	0 (0.00)
Metronidazole	16 (61.54)	0 (0.00)	10 (38.46)
Vancomycin	24 (92.31)	1 (3.85)	1 (3.85)

*susceptible, †susceptible, increased exposure, ‡resistant

Table 7. Overview of overall resistance rates of different anaerobic bacteria to clindamycin

Tablica 7. Pregled rezistencije različitih anaeroba na klindamicin

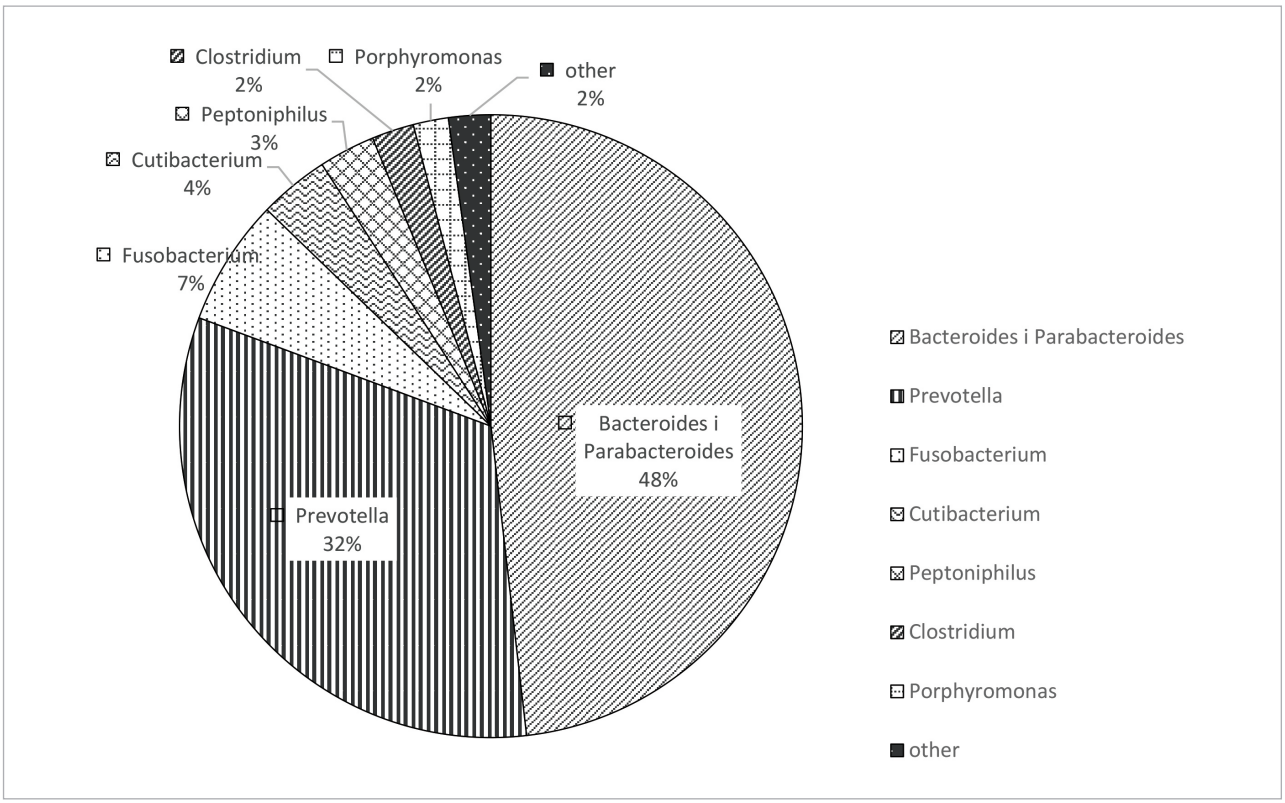
Genus	2020			2021			2022			TOTAL	
		R			R			R		R	
	tested	n	%	tested	n	%	tested	n	%	n	%
Bacteroides	40	22	55%	62	22	35%	27	8	30%	52	40%
Fusobacterium	4			11	1	9%	1			1	6%
Porphyromonas	3	1	33%	1			1			1	20%
Prevotella	28	18	64%	41	30	73%	15	11	73%	59	70%
Clostridium	4	1	25%				2			1	17%
Cutibacterium	3			5	2	40%				2	25%
Peptoniphilus	2			4	2	50%	2	1	50%	3	38%
GN	75	41	55%	115	53	46%	44	19	43%	113	48%
GP	9	1	11%	9	4	44%	4	1	25%	6	27%
TOTAL	84	42	50%	124	57	46%	48	20	42%	119	46%

GN-Gram-negative; GP-Gram-positive; R-resistant

Table 8. Clindamycin consumption and resistance rate of Gram-positive and Gram-negative anaerobes over years
Tablica 8. Potrošnja klindamicina i stopa rezistencije gram-pozitivnih i gram-negativnih anaeroba u ispitivanom periodu

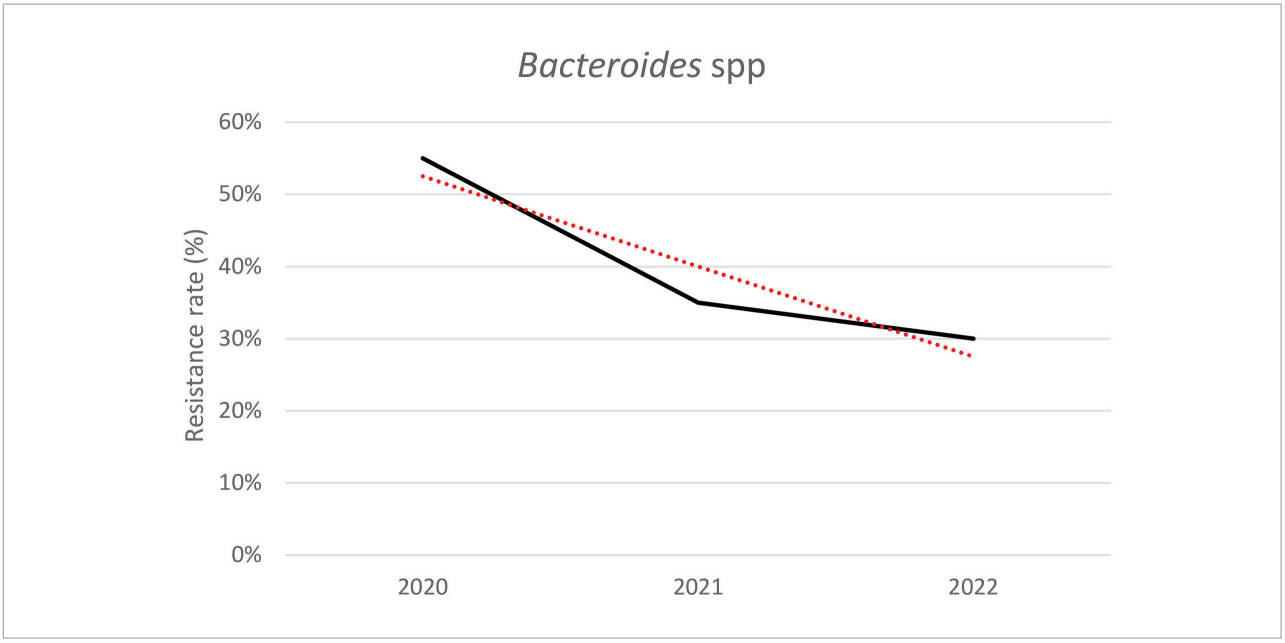
Year	Oral consumption (DDD/100PD)	Parenteral consumption (DDD/100PD)	Resistance rate Gram-negative(%)	Resistance rate Gram-positive (%)	Total resistance rate (%)	*P*-value
2020	4,63	17,6	55	11	50	0,64
2021	4,42	16,68	46	44	46	
2022	4,76	11,3	43	25	42	

DDD- daily defined dose; PD- Patient days



other-*Actinomyces* 1 (0,36%), *Anaerococcus* 2 (0,73%), *Bifidobacterium* 1 (0,36%), *Peptostreptococcus* 1 (0,36%), *Veillonella* 1 (0,36%).

Figure 1. Overview of anaerobic isolates by genera in a two-year period
Slika 1. Prikaz učestalosti detektiranih anaerobnih rodova u dvogodišnjem periodu



Cochran-Armitage test: $\chi^2 = 4.855$; P <0,05

Figure 2. Resistance rate and resistance trend of Bacteroides isolates to clindamycin in tested period

Slika 2. Stopa i trend rezistencije na klindamicin Bacteroides spp. u ispitivanom periodu

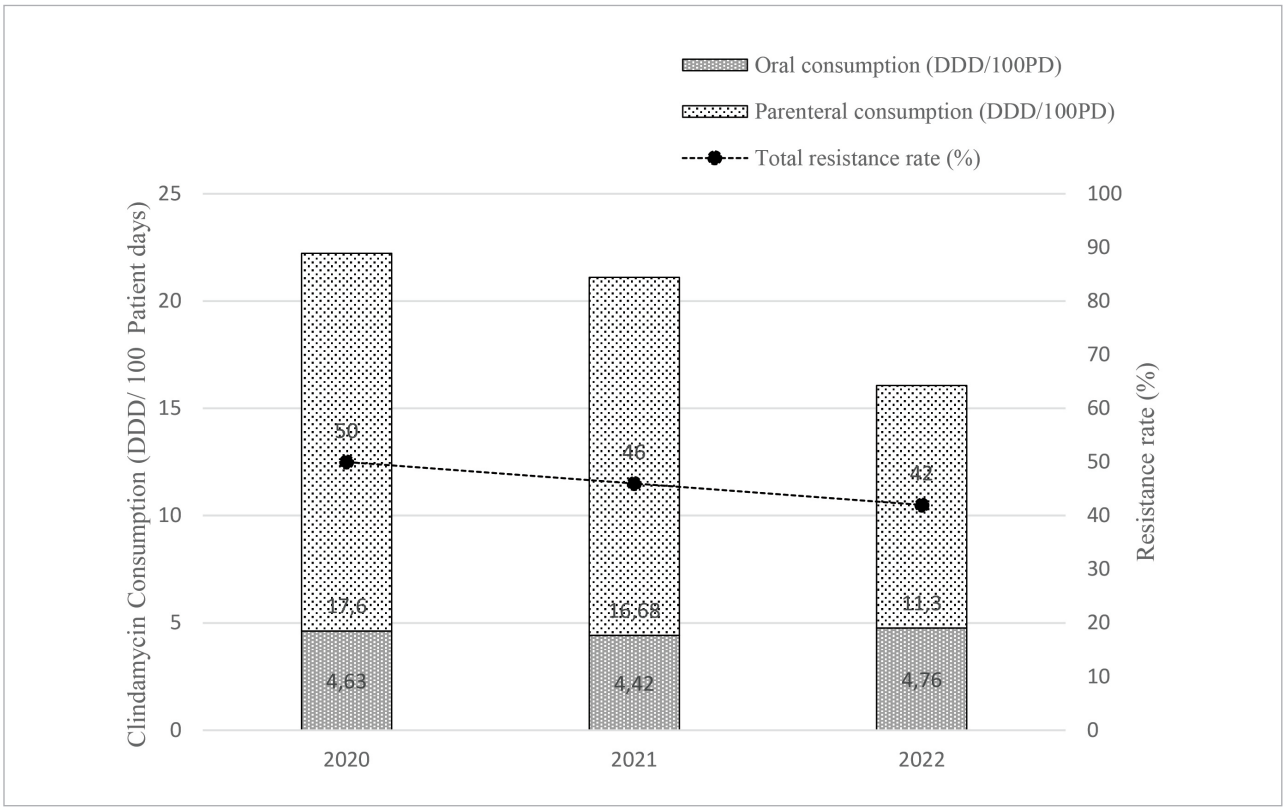


Figure 3. Correlation between clindamycin consumption and total resistance rate in Gram-positive and Gram-negative anaerobic bacteria

Slika 3. Korelacija potrošnje klindamicina i ukupne stope rezistencije gram-pozitivnih i gram-negativnih bakterija

4. DISCUSSION

In this study, the largest number of isolates was detected from blood culture and cerebrospinal fluid samples (46.78%), followed by skin and subcutaneous tissue samples (28.93%). Similar results were obtained in a reference laboratory in Canada, where most anaerobic bacteria (55.30%) were also isolated from blood cultures[4], as well as in Ireland (31%)[5]. In contrast, research conducted in Slovenia showed different results: 57% of the samples were from skin and tissue, and only 4% from blood cultures[6].

Furthermore, 89.42% of the isolates were Gram-negative bacteria, while only 10.58% were Gram-positive. A higher proportion of Gram-negative anaerobes, though lower than in this study, was also found at the University Hospital Centre Split (52 %)[7] and in Slovenia (57 %)[6]. The dominance of Gram-positive anaerobes was observed in Spain (54 %)[8].

The most frequently isolated genus was *Bacteroides* spp. (47.45%), followed by *Prevotella* spp. (32.48%). *Bacteroides* spp. was also the most common isolate in other studies, accounting for 16.7 – 31% of isolated anaerobes[4–9]. *Prevotella* spp., however, was less represented in these studies (7.75 – 14%) than in ours (32.48%). Among Gram-positive bacteria, the most common isolates were from the genus *Cutibacterium* spp. (formerly *Propionibacterium* spp.), which corresponds to results from other hospital institutions. It is important to note that the percentage of *Cutibacterium* spp. isolates was much higher in those studies than in ours[7–10].

The antimicrobial susceptibility of the tested isolates is consistent with previously recorded data on the susceptibility and resistance of the genus *Bacteroides* spp. Penicillin resistance in this study was 96.83%, with similar results observed at University Hospital Centre Split, in several European countries[6,8,11], and in Iran[12]. According to the literature, resistance to penicillins and cephalosporins is mediated by the *cepA* and *cfxA* genes[13]. In this study, 11.11% of the isolates were resistant to piperacillin-tazobactam, comparable to other studies where resistance ranged from 8.6% to 10%[7,11,12]. Resistance of *Bacteroides* spp. to carbapenems, specifically imipenem, was not detected in this study, but in 2013, one case was documented at University Hospital Centre Osijek[14]. Studies have shown a low rate of resistance (<2%) in Slovenia[15], Spain[16], and Australia[17]. However, an increase in resistance was recorded in China[18], and in one Japanese study it reached as high as 54%[19]. In this study, 40.48% of the isolates were resistant to clindamycin. On the other hand, at University Hospital Centre Split and in Slovenia, the figures are somewhat lower, around 28%[6,7]. Resistance to clindamycin is increasing, both globally and in Europe, and has reached a concerning 60% for certain *Bacteroides* species[20]. Recent studies suggest a gradient of resistance across Europe with higher resistance observed in the southern and eastern regions compared with northern and western Europe. Moreover, higher clindamycin resistance rate have been reported among non-fragilis *Bacteroides* spp. Compared with *B.fragilis*[21]. In our study we observed slight decrease in the overall resistance rate from 50 % to 42 % of all tested isolates, as well a reduction in clindamycin consumption in our hospital over the study period, although neither change reached statistical significance. We emphasise this decline is likely related to the restricted use of clindamycin, implemented to improve better control of *Clostridioides difficile* infection and to limit its administration to specific clinical situations. A statistically significant linear trend was observed only in clindamycin resistance among *Bacteroides* spp. isolates. These findings are consistent with published studies reporting that interventions aimed at limiting clindamycin use successfully lead to a reduction in resistance rates[22].

Resistance to metronidazole in this study was very low, detected in only one isolate. Analysing 25 papers from several European countries, the USA and South Africa from 1989 to 2017, Ghotaslou et al. found that resistance to metronidazole ranges from 0.5% to 7.8%, and is mostly mediated by the nim gene[23]. Although resistance rates in Western countries are very low, in India they are much higher, ranging from 31% to 55.5%[24,25].

We found that resistance of *Prevotella* spp. to penicillin (69.88%) in our institution was similar to that reported elsewhere (60-69%)[4,6,8]. Resistance to amoxicillin was high, but the combination of this antibiotic with clavulanic acid was much more effective, with resistance rates below 3%. We did not detect strains resistant to piperacillin-tazobactam, as was also observed in one hospital in the Netherlands[26]. There was no resistance to imipenem. In our study, clindamycin was the least effective antibiotic against *Prevotella* spp. In other studies, resistance ranged from 35% to 50%, but in the Netherlands, it was only 10%. Finally, resistance to metronidazole was below 5%, which is consistent with other studies[4,6,8].

Fusobacterium spp. were completely susceptible to four antibiotics: amoxicillin-clavulanic acid, piperacillin-tazobactam, imipenem, and metronidazole. Other studies reported similar results[4,6,8]. Resistance to penicillin and amoxicillin was higher in our study than in the aforementioned studies. Although *Fusobacterium* is considered to be very susceptible, results from a ter-

tiary care hospital in Korea showed that some *Fusobacterium* species were resistant to clindamycin, moxifloxacin, penicillin, and ceftriaxone[27].

Porphyromonas spp. was also fully susceptible to the same antibiotics as *Fusobacterium* spp. However, resistance to penicillin and amoxicillin was much higher (40%).

Cutibacterium spp. were susceptible to all tested antibiotics except clindamycin and metronidazole. Resistance to clindamycin was somewhat higher in this study (25%) than in other studies, where it ranged from 4.3% to 11.3%[4,6–8,10]. Results of susceptibility testing to metronidazole clearly show that *Cutibacterium* spp. are intrinsically resistant to this antibiotic.

Peptoniphilus spp. showed 25% resistance to both penicillin and clindamycin. Studies from Canada and Greece reported similar results for clindamycin, but all their *Peptoniphilus* strains were susceptible to penicillin[4,10]. In contrast, results from nine hospitals in France showed higher resistance to clindamycin at 47%[28]. In Slovenia and Spain, resistance to penicillin was up to 6% and to clindamycin around 30%[6,8].

Clostridium spp. isolates were susceptible to metronidazole in many studies, including ours, but a study by Maraki et al. found resistance in 8.1% of their isolates[10]. Notably, at University Hospital Centre Split, all tested antibiotics were effective against *Clostridium* spp. except clindamycin, to which 50% of isolates were resistant[7]. In some studies, resistance to penicillin and clindamycin was approximately 30% and 18%, respectively[4,6]. We obtained similar results. No resistance was found to amoxicillin-clavulanic acid, piperacillin-tazobactam, or imipenem. Other researchers also reported very low resistance to these antibiotics, around 1%[4,6,7,10].

5. CONCLUSION

Bacteroides fragilis was the most frequently isolated anaerobic bacterium during a two-year period at the University Hospital Centre Osijek, Croatia. Given the prevalence of *Bacteroides* spp. isolates in laboratories worldwide, it is important to assess their antimicrobial susceptibility. Analysis of the results indicates low resistance to metronidazole, which remains, depending on the site of infection, the first therapeutic choice. Numerous studies, including this one, have shown that resistance to clindamycin is high, and it is no longer recommended as empiric therapy for anaerobic infections. Ongoing surveillance of the antimicrobial susceptibility profiles among *Bacteroides* spp. and other obligate anaerobes, is crucial for the timely implementation of both empiric and targeted antimicrobial therapy.

In conclusion, this study provides important insights into the antimicrobial susceptibility patterns of obligate anaerobes at the University Hospital Centre Osijek, Croatia, underscoring the need for continuous resistance surveillance and regularly updated local therapeutic guidelines.

Summary

Strictly anaerobic bacteria were analysed over two years in Osijek, revealing *Bacteroides fragilis* as the most common isolate and notable resistance to penicillin and clindamycin, while metronidazole resistance remained low, underscoring the need for continued antimicrobial-susceptibility surveillance.

Conflict of interest

Authors do not have an association that might pose a conflict of interest.

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