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ORIGINAL RESEARCH

Antibiotic Susceptibility Patterns of Gram-Negative Anaerobic Bacteria Isolated from Clinical Samples

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Alican BİLDEN[1a*], Selahattin ATMACA[2b], Nida ÖZCAN[2c]

[1] Ahi Evran University Faculty of Medicine, Department of Parasitology, Kırşehir, Turkey
[2] Dicle University Faculty of Medicine, Department of Microbiology, Diyarbakır, Turkey[a]
[a]ORCID: 0000-0003-1119-3859,
[b]ORCID: 0000-0002-2730-5790,
[c]ORCID: 0000-0002-9808-4366,

Abstract:

Aim: *Although anaerobic bacteria are the normal flora of the body, they can cause infection with the weakening of the immune system. Isolation and identification of these bacteria are difficult and not performed in most laboratories. For this reason, anaerobic bacteria can often be ignored. As a result, antibiotic resistance situations that develop cannot be detected. We carried out this study to reveal that anaerobic bacteria are important, that knowing antibiotic resistance profiles is necessary for the course of treatment, and that antibiotic susceptibility tests should be performed at certain periods.*

Method: *This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. These samples were first inoculated on Brucella blood agar for bacteria isolation. After identifying the isolated bacteria with MALDI-TOF MS, the E-test method was used to determine the antimicrobial susceptibility profiles.*

Results: *Antibiotics with the highest antimicrobial resistance were determined as 100% penicillin (P), 41.2% clindamycin (CM) and 35.3% amoxicillin-clavulanic acid (AMC), respectively in 17 isolated Bacterioides species. Antibiotics with the highest antimicrobial resistance in the 22 isolated Prevotella species were determined as 45.5% penicillin (P) and 27.3% moxifloxacin (MX). Again, only metronidazole (MZ) resistance was observed in 1 Dialister pneumosintes bacteria, whereas resistance was observed against penicillin (P), imipenem (IP) and piperacillin-tazobactam (TPZ) antibiotics in 1 Veillonella parvula bacteria. Finally, resistance was observed against any antibiotic in 4 Fusobacterium species isolated.*

Conclusion: *It was observed that the majority of the Gram-negative anaerobic bacteria isolated in our study developed resistance to penicillin, clindamycin, moxifloxacin, metronidazole and amoxicillin-clavulanic acid, or had an increased resistance to these antibiotics, and were also sensitive to the remaining antibiotics.*

Key Words: *Gram-Negative Anaerobic Bacteria, Antibiotic Sensitivity, MIC, MALDI-TOF MS*

Corresponding author: E-mail: alicanbilden@gmail.com

Özet:

Amaç: *Anaerop bakteriler vücudun normal flora elemanı olmasına karşın, bağışıklık sisteminin zayıflamasıyla enfeksiyona neden olabilirler. Bu bakterilerin izolasyonları ve identifikasyonları güç olduğu için çoğu laboratuvarda yapılmamaktadır. Bu sebeple çoğu zaman anaerop bakteriler göz ardı edilebilmektedir. Bunun sonucu olarak da gelişen antibiyotik direnç durumları tespit edilememektedir. Çalışmamız anaerop bakterilerin antibiyotik direnç profillerinin bilinmesinin tedavinin seyri açısından gerekli olduğunu ve belirli periyodlarda antibiyotik duyarlılık testlerinin yapılması gerektiğini ortaya koymaktır.*

Metod: *Çalışmaya, Dicle Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji AD. Bakteriyoloji/Kültür laboratuvarına, çeşitli klinik birimlerden anaerop kültür istemiyle gönderilmiş 372 numune dahil edilmiştir. Bu numuneler, ilk olarak bakteri izolasyonu için Brucella kanlı agara ekildi. İzole edilen bakterilerin identifikasyonları MALDI-TOF MS ile yapıldıktan sonra antimikrobiyal duyarlılık profillerinin belirlenmesi için E – test yöntemi kullanıldı.*

Bulgular: *İzole edilen 17 Bacterioides türünde antimikrobiyal direncin en yüksek olduğu antibiyotikler sırasıyla % 100 oranında penisilin (P), % 41,2 oranında klindamisin (CM) ve % 35,3 oranında ise amoksisillin-klavulanik asid (AMC) olarak belirlendi. İzole edilen 22 Prevotella türünde ise antimikrobiyal direncin en yüksek olduğu antibiyotikler % 45,5 oranında penisilin (P) ve % 27,3 oranında moksifloksasin (MX) olarak belirlendi. Yine izole edilen 1 Dialister pneumosintes bakterisinde sadece metronidazol (MZ) direnci gözlemlenirken, 1 Veillonella parvula bakterisinde ise penisilin (P), imipenem (IP) ve piperasillin-tazobaktam (TPZ) antibiyotiklerine karşı direnç gözlemlenmiştir. Son olarak da izole edilen 4 Fusobacterium türünde ise hiçbir antibiyotiğe karşı direnç gözlemlenmemiştir.*

Sonuç: *Çalışmamızda izole edilen Gram-negatif anaerop bakterilerin büyük çoğunluğunun penisilin, klindamisin, moksifloksasin, metronidazol ve amoksisillin-klavulanik aside karşı bir direnç geliştirdiği veya bu antibiyotiklere karşı artan bir dirence sahip olduğu görülmüştür.*

Anahtar Sözcükler: *Gram-Negatif Anaerop Bakteriler, Antibiyotik Duyarlılığı, MIC, MALDI-TOF MS*

Introduction

For a long time, humanity has thought that oxygen is absolutely necessary for living things to maintain their vitality. As a result of the studies carried out by scientists, especially since the 18th century, they have shown that oxygen is not absolutely necessary for some microorganisms, or that these microorganisms can continue their vitality even if it is at very low levels. On the contrary, it has been demonstrated that oxygen has a toxic effect on these microorganisms.[1–4] Towards the end of the 19th century, Louis Pasteur, one of the founders of modern microbiology, divided microorganisms into 'aerobic' and 'anaerobic' according to their oxygen needs.[5–7]

In recent times, there has been a notable rise in global antimicrobial resistance, posing a heightened risk of severe clinical outcomes and mortality. [8] Despite numerous global studies on the resistance of anaerobic bacteria to antimicrobial agents [9], the existing data are incomplete due to technical challenges associated with isolating bacteria and conducting susceptibility testing. This process is not only expensive and time-consuming but also demands expertise. Furthermore, the absence of

universally applied guidelines for susceptibility testing and interpretation criteria adds to the challenge of accurately assessing the resistance of anaerobic bacteria to eradication therapy.[10]

The widespread antibiotic resistance seen recently in anaerobic bacteria seriously delays treatment, especially in polymicrobial infections. For this reason, it becomes important to identify isolated anaerobic bacteria and perform antibiotic sensitivity tests. However, since this process is laborious and time-consuming, it is recommended to be done in some special cases.[11] First of all, in anaerobic infections, surgical procedures such as abscess drainage, debridement of necrotic tissues, surface antisepsis, contact of the infection area with oxygen, elimination of foreign bodies and removal of obstructions should be performed. Antibiotics should be used as a continuation of treatment. Since bacterial isolation and identification in anaerobic infections is time-consuming and troublesome, patient treatment primarily begins with empirical treatment. For this reason, antibiotic susceptibility results of identified anaerobic bacteria are important for empirical treatment. The high antibiotic resistance in anaerobic bacteria seen recently requires antibiotic resistance monitoring at certain periods, especially for the success of empirical treatment. Therefore, reporting the antibiotic susceptibility results of anaerobic bacteria provides guidance in the treatment of serious infections such as endocarditis, recurrent and resistant bacteremia, lung abscess, pulmonary hypertension, osteomyelitis, which require long-term treatment, or in the treatment of infections that do not respond to empirical treatment. While sensitivity to commonly used antibiotics such as nitroimidazoles, carbapenems, chloramphenicol, beta lactams combined with beta lactamase inhibitors, penicillin, cefoxitin and clindamycin is gradually decreasing, resistance genes against these antibiotics have also been identified. These resistance genes can be carried between bacteria via genetic material transfer mechanisms.

Our aim in this study is to demonstrate that determining the antibiotic resistance profiles of anaerobic bacteria is necessary for the course of treatment and that antibiotic sensitivity tests should be performed at certain periods.

Materials and Methods

Collection of Samples

This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. Within this set of samples, we included one urine sample obtained through suprapubic aspiration and two tissue/abscess samples collected from the genital area through surgical procedures in the category of genital system samples. Additionally, two pleural fluid

samples obtained under sterile conditions were categorized as respiratory system samples and included in our study. The samples were delivered to the laboratory in anaerobic transport media and thioglycollate liquid media. Cultivation, purification and identification of all samples arriving at the laboratory were carried out without wasting time. All identified Gram-negative anaerobic bacteria were stored at – 80 °C in skim milk broth until the time of the study.

Cultivation of Samples

All suitable samples were cultured on brucella blood agar and thioglycollate liquid media. Thioglycollate broth used as return fluid was incubated at room temperature, and brucella blood agar plates were incubated in an anaerobic environment at 35-37°C for 24-72 hours. The morphological appearance and pigment formation of all colonies that appeared to grow at the end of incubation were evaluated and passaged for purification. The passaged plates were incubated again in an anaerobic environment at the appropriate temperature and time, and the identification phase began.

Identification of Isolated Anaerobic Bacteria

Plates were evaluated after 24-72 hours of incubation. Different techniques, devices, and materials were utilized to create an anaerobic culture environment. Foremost among these are the Anoxomat Jar Systems. In this system, where the anaerobic environment is automatically provided and controlled, an anaerobic atmosphere was established in jars connected to the system, containing anaerobic plates, with a gas mixture of 80-90% N_2, 5-10% CO_2, and 5% H_2. In addition to this, conventional methods such as anaerobic sealed bags, 2,5-liter anaerobic jars/jars, and standard incubation container systems were also employed. The incubation period of plaques without growth was extended to 5 days when necessary. Species-level identification of all different colonies growing in anaerobic plates was made with the MALDI-TOF MS. All anaerobic bacteria identified at species level were inoculated onto brucella blood agar. If the MALDI-TOF MS bacterial identification result scores were below the minimum species identification score (2,000 = min - 3,000 = max), the identification procedures were repeated. After the incubation period, 1-2 bacterial colonies taken from the passaged plates were transferred to the MALDI-TOF MS 96 sample working plate, dried, and 1µl of 70% formic acid was dropped on it and the steel plate was left to dry again. Finally, 1µl of matrix solution was dropped onto the steel plate, allowed to dry, and loaded into the device.

Determination of Antimicrobial Resistance of Isolated Anaerobic Bacteria

The E-test method was used to determine the antimicrobial resistance of Gram-negative anaerobic bacteria, purified and identified at species level, to 9 antibiotics. According to the European

Committee on Antimicrobial Susceptibility Testing (EUCAST Version 10.0: 2020) antibiotic susceptibility testing guide, 8 antibiotics (P, AMC, TPZ, IP, MP, CM, MZ, CL), Clinical Laboratory and Standard Institute (CLSI M100-ED30: 2020).) MIC values of 1 antibiotic (MX) were determined according to antibiotic susceptibility testing guidelines. The McFarland turbidity standard value of the purified and isolated Gram-negative anaerobic bacteria was set as 1.00. All bacterial suspensions, with the McFarland standard value set as 1.00, were inoculated onto the brucella blood agar surface with a 120° rotation at least three times with the help of a sterile swab. Before using the E-test strips, they were removed from –20 °C and allowed to reach 25 °C. With the help of sterile forceps, the strips were carefully placed on the agar surface, parallel and opposite to each other. A total of 9 antibiotic strips and 5 brucella blood agar plates were used for each bacterium. This process was done separately for each bacteria and was completed within a maximum of 15 minutes. Subsequently, the plates were transferred to an anaerobic environment and incubated at 37 ° C for 24-72 hours. As a result of incubation, the plates were evaluated and the point where the formed elliptical inhibition zone intersected with the antibiotic strip was accepted as the MIC value for that antibiotic. Thus, the determined MIC values were interpreted according to reference guidelines and it was determined whether the bacteria had antibiotic resistance.

Ethical Procedures

The Project titled as " Antibiotic susceptibility patterns of gram-negative anaerobic bacteria isolated from clinical specimen" planned by Selahattin ATMACA, Nida ÖZCAN and Alican BILDEN has been approved by the Ethics Committee of Dicle University Faculty of Medicine.

Results

This study included 372 samples sent from various clinical units to the Bacteriology/Culture Laboratory of Dicle University Faculty of Medicine Faculty of Medicine Department of Medical Microbiology. The samples were sent to the bacteriology laboratory. While 188 (50.5%) of 372 patients were female and 184 (49.5%) were male, the average age of these patients was 37 and the age range was 0 - 90. In addition, 260 (69.8%) of the samples were abscess, 95 (25.5%) were tissue/wound, 6 (1.6%) were blood, 5 (1.3%) were joint fluid, 4 (1)% were pleural/peritoneal fluid and 2 were urine. Of the 260 abscess samples, 33 (12.6%) were Gram-negative anaerobes, 15 (5.7%) were Gram-positive anaerobes, 2 (0.7%) were ARB (Acide Resistant Bacilli), 83 (9%). Facultative aerobic/anaerobic bacteria were 83 (% 31,9) of them. There was no growth in 127 (48.8%). Of the 95 tissue/wound samples, 7 (7.3%) grew Gram-negative anaerobic, 2 (2.1%) grew Gram-positive anaerobic, and 33 (34.7%) grew facultative aerobic/anaerobic bacteria. There was no

growth in 53 (55.7%) cases. Gram-positive anaerobic bacteria grew in 4 (66.6%) of 6 blood samples, and facultative aerobic/anaerobic bacteria grew in 1 (16.6%). There was no growth in 1 (16.6%). Of the 5 synovial fluid samples, 1 (20%) grew Gram-positive anaerobic bacteria and 2 (40%) grew facultative aerobic/anaerobic bacteria. There was no growth in 2 (40%) cases. Only 1 (25%) of 4 pleural/peritoneal fluid samples grew facultative aerobic/anaerobic bacteria. There was no growth in 3 (75%) cases. Gram-positive anaerobes grew in 1 of 2 urine samples (50%). There was no growth in 1 (50%). Skin, soft tissue and respiratory tract infection rates were lowest, and *Veillonella parvula* and *Dialister pneumosintes* were the least isolated species. Details of bacterial distribution according to isolation region are given in Table 1.

Table 1. *Distribution of Gram-Negative Anaerobic Bacteria by Isolation Region*

Isolation Region	*B. fragilis*	*Bacterioides spp.*	*Prevotella spp.*	*Fusobacterium spp.*	*Veillonella spp.*	*D. pneumosintes*	Total
Skin and soft tissue	0	1	0	0	1	0	2 (% 4,4)
infection	3	1	1	1	0	0	6 (% 13,3)
Intra-abdominal infection	1	0	2	0	0	0	3 (% 6,6)
Genital system infection	3	1	10	3	0	1	18 (% 40)
Head and neck infection	0	0	2	0	0	0	2 (% 4,4)
Respiratory tract infection	1	2	1	0	0	0	4 (% 8,8)
Bone and joint infections	3	1	6	0	0	0	10 (% 22,2)
Other infection							
Total	11	6	22	4	1	1	45 (% 100)

While the penicillin resistance of all isolated *Bacteroides* (17) species was determined to be 100%, 100% sensitivity to no antibiotic was observed. However, the antibiotics with the lowest antimicrobial resistance in *Bacteroides* species were determined to be imipenem (IP) and chloramphenicol (CL), with a rate of 5.9%. On the other hand, the antibiotics with the highest antimicrobial resistance were determined as penicillin (P) at 100%, clindamycin (CM) at 41.2% and amoxicillin-clavulanic acid (AMC) at 35.3%, respectively. In this study, some of the antibiotics with the lowest antimicrobial resistance of *Bacteroides* species were imipenem (IP) (5.9%), meropenem (MP) (17.7%), and metronidazole (MZ) (17.7%). However, although the antimicrobial sensitivity to these antibiotics was high, an increasing resistance was observed with the detection of bacterial colonies growing in the inhibition zone areas. While 100% antimicrobial resistance was

not observed against any applied antibiotic in *Prevotella* (22) species, 100% antimicrobial susceptibility was observed against imipenem (IP) and chloramphenicol (CL). On the other hand, the antibiotics with the highest antimicrobial resistance were determined as penicillin (P) at 45.5% and moxifloxacin (MX) at 27.3%. Although the antimicrobial susceptibility of some species to imipenem (IP) is 100%, an increasing resistance has been observed with the detection of bacterial colonies growing in the inhibition zone areas. In this study, only *Veillonella parvula* was isolated from *Veillonella* species, and resistance was observed against penicillin (P), imipenem (IP) and piperacillin-tazobactam (TPZ) antibiotics, while resistance was observed against amoxicillin-clavulanic acid (AMC), meropenem (MP), clindamycin (CM), no resistance was observed to the antibiotics moxifloxacin (MX), metronidazole (MZ), and chloramphenicol (CL). Similarly, while only metronidazole (MZ) resistance was observed in the isolated *Dialister pneumosintes* bacteria, penicillin (P), imipenem (IP), piperacillin-tazobactam (TPZ), amoxicillin-clavulanic acid (AMC), meropenem (MP), clindamycin (CM), no resistance to the antibiotics moxifloxacin (MX) and chloramphenicol (CL) was observed.

Table 2. *Distribution of Gram-Negative Anaerobic Bacteria*

Microorganisms	Number of Bacteria / %
Bacterioides spp.	17 (% 37,7)
Bacterioide fragilis	11
Bacteroides ovatus	3
Bacteroides thetaiotaomicron	2
Bacteroides vulgatus	1
Prevotella spp.	22 (%48,8)
Prevotella bivia	3
Prevotella disiens	2
Prevotella intermedia	1
Prevotella oris	2
Prevotella melaninogenica	1
Prevotella buccae	7
Prevotella denticola	3
Prevotella nigrescens	2
Prevotella baroniae	1
Fusobacterium nucleatum	4 (% 8,8)
Veillonella parvula	1 (% 2,2)
Dialister pneumosintes	1 (% 2,2)
Total	45 (% 100)

Conclusion

Since the identification of anaerobic bacteria and antimicrobial susceptibility tests are expensive and time-consuming, these procedures cannot be performed routinely in most laboratories. Various antibiotics used in the treatment of anaerobic infections over time and increasing bacterial resistance to them vary from region to region.[12–15] In our study, 48 anaerobic bacteria were isolated from 260 abscess samples, 9 anaerobic bacteria from 95 tissue/wound samples, 4 anaerobic bacteria from 6 blood samples, 1 anaerobic bacteria from 5 joint fluid samples, and 1 anaerobic bacteria from 2 urine samples. It has been stated in studies that the anatomical regions and clinical samples from which anaerobic bacteria are isolated may vary. Our study revealed that the sample processing procedure was compatible with previous studies. In our study, of the 45 Gram-negative anaerobic bacteria isolated from the infection sites, 11 (24.4%) were *Bacteroides fragilis* and 6 (13.3%) were other *Bacteroides* species, for a total of 17 bacterial species. According to EUCAST and CLSI MIC limit values, all of these bacteria were susceptible to penicillin (P), 7 (41.2%) to clindamycin (CM), 6 (35.3%) to amoxicillin-clavulanic acid (AMC), 4 (23%) to amoxicillin-clavulanic acid (AMC), 4 (% 236) to moxifloxacin (MX), 3 (17.7%) to meropeneme (MP) and metronidazole (MZ), 2 (11.7%) to piperacillin-tazobactam (TPZ), 1 (5%) to piperacillin-tazobactam (TPZ). While 1 (%5,9) were resistant to chloramphenicol (CL) and imipenem (IP), 1 (6%) was moderately sensitive to piperacillin-tazobactam (TPZ) and 3 (17.6%) was moderately sensitive to moxifloxacin (MX). However, 11 (64.7%) were susceptible to amoxicillin-clavulanic acid (AMC), 16 (94.1%) to imipenem (IP) and chloramphenicol (CL), and 14 (82.3%) to meropenem (MP), piperacillin-tazobactam (TPZ) and metronidazole (MZ), 10 (58.8%) to clindamycin (CM) and moxifloxacin (MX). In this study, the antibiotics to which *Bacteroides* species showed the highest resistance can be listed as penicillin (100%), clindamycin (41.2%), amoxicillin-clavulanic acid (35.3%) and moxifloxacin (23.6%). Neriman S. isolated 5 *Bacteroides* species in her thesis study in 2018. [16] According to EUCAST and CLSI MIC limit values, of these bacteria 4 (80%) were susceptible to penicillin (P), 3 (60 to chloramphenicol (CL), 1 (20%) to imipenem (IP) and metronidazole (MZ) was found to be resistant. In this study, while the results of penicillin and metronidazole were almost exactly compatible with the results of our study, they were also incompatible with the results of chloramphenicol and imipenem.

In our study, 22 (48.8%) of 45 Gram-negative anaerobic bacteria isolated from infection sites were *Prevotella* species. According to EUCAST and CLSI MIC limit values, 10 (45.5%) of these bacteria were susceptible to penicillin (P), 6 (27.3%) are susceptible to moxifloxacin (MX), and 5 (22.8%) wer susceptible to clindamycin (CM), 3 (13.7%) to metronidazole (MZ), 2 (9%) to

piperacillin-tazobactam (TPZ), 1 (4.5%) to amoxicillin-clavulanic acid (AMC) and meropenem (MP). Resistance to imipenem (IP) and chloromphenicol (CL) was not observed in any of these bacteria. In addition, 1 (4.5%) of these bacteria was moderately resistant to penicillin (P), and 2 (9%) were moderately resistant to piperacillin-tazobactam (TPZ). However, 11 (50%) were susceptible to penicillin (P), 21 (95.5%) to amoxicillin-clavulanic acid (AMC) and meropenem (MP), 18 (82%) to piperacillin-tazobactam (TPZ), 17 (77.2%) to clindamycin (CM), 16 (72.2%) to moxifloxacin (MX), and 19 (86.3%) to metronidazole (MZ). In our study, the antibiotics to which *Prevotella* species showed the highest resistance can be listed as penicillin (45.5%), clindamycin (22.8%), metronidazole (13.7%) and moxifloxacin (27.3%). Neriman S. isolated 3 *Prevotella* species in her thesis study in 2018. [16]. According to EUCAST and CLSI MIC limit values, 2 (66.6%) of these bacteria were resistant to penicillin (P) and chloramphenicol (CL), while 3 (100%) were sensitive to imipenem (IP) and metronidazole (MZ). All results in this study were incompatible with the results of our study. Bacalan et al. of the 69 anaerobic bacteria isolated in his study, 19 (27.5%) were *Prevotella* species.[17] According to CLSI MIC limit values, susceptibility results for only 23 anaerobic bacteria could be detected in this study. Among the *Prevotella* species for which results were obtained, penicillin (P) resistance was observed in only 2 species. These results were incompatible with the results of our study. In this study, according to CLSI and EUCAST MIC values, the antibiotics to which *Prevotella* species were resistant penicillin (77%), clindamycin (40%), amoxicillin-clavulanic acid (0%), imipenem (0%), meropenem (0%), metronidazole (%17. 7), piperacillin-tazobactam (3%) and moxifloxacin (30%). Again, according to the results of this study; In our study, the resistance rates to penicillin and clindamycin were low, while the resistance rates to amoxicillin-clavulanic acid, piperacillin-tazobactam, meropenem and metronidazole were found to be high, and they were also compatible with the resistance rates to imipenem and moxifloxacin. In our study, 4 (8.8%) of the 45 Gram-negative anaerobic bacteria isolated from infection sites were *Fusobacterium* species, 1 (2.2%) was *Veillonella parvula* and 1 (2.2%) was *Dialister pneumosintes*. Among these anaerobic bacteria, *Fusobacterium* species were sensitive to all antibiotics. However, *Veillonella* species were resistant to penicillin, piperacillin-tazobactam and imipenem, and *Dialister pneumosintes* was resistant only to metronidazole. Neriman S. in her thesis study in 2018, 2 *Fusobacterium*, 1 *Veillonella parvula* and 1 *Dialister pneumosintes* bacteria were isolated.[16] 1 Metronidazole resistance was observed in *Fusobacterium* and *Dialister pneumosintes*, penicillin resistance was observed in *Dialister pneumosintes* and *Veillonella parvula*, and chloromphenicol resistance was observed in *Veillonella parvula*, and these results were not compatible with the results of our study. Bacalan et al. Of the 69 anaerobic bacteria

isolated in his study, 14 (20.3%) were *Veillonella* and 3 (2.2%) were *Fusobacterium*. [17] According to CLSI MIC limit values, susceptibility results for only 23 anaerobic bacteria could be detected in this study. In this study, while 2 of the *Fusobacterium* were resistant to penicillin, no penicillin, clindamycin, imipenem and metronidazole resistance was observed in any of the *Veillonella*. These results were not compatible with the results of our study. In various studies conducted in different parts of the world, the majority of anaerobic bacteria isolated from clinical samples are *Bacteroides* species.[18–20] However, in different studies, the superiority of *Prevotella* species in number has been reported. [18,21] There are some changes in the resistance profile of anaerobes, particularly Bacteroides fragilis, to β-lactam antibiotics, as previously reported. [21–23] Although carbapenems are the most effective drugs against anaerobes, the emergence of resistance to them has been reported. Imipenem resistance due to metallo-β-lactam has been reported since 1986. [24] *Bacteroides* species are potentially resistant to most antibiotics, and this antibiotic resistance can also vary greatly between geographic regions and even healthcare facilities within the same area. Resistance rates can also vary greatly between different strains of the *Bacteroides fragilis* group.[25] In different studies, it has been reported that the variable resistance profiles of anaerobic bacteria against certain antibiotics may be related to the differences in the determined EUCAST and CLSI MIC limit values. [26]

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