DOI:10.34084/bshr.1352333 e-ISSN 2587-1641

Journal of Biotechnology and Strategic Health Research

Araştırma Makalesi /Research Article

http://dergipark.org.tr/tr/pub/bshr

Identification of Anaerobic Bacteria Isolated from Clinical Samples and Determination of Antibiotic Resistance Profiles

Klinik Örneklerden İzole Edilen Anaerop Bakterilerin Tiplendirilmesi ve Antibiyotik Direnç Profillerinin Belirlenmesi

Tünzala Asgarova, Filiz Kibar, Hatice Hale Gümüş

Cukurova University, Faculty of Medicine, Department of Medical Microbiology, Adana, Türkiye

ORCID ID: Tünzala Asgarova: https://orcid.org/0000-0003-4000-5095, Filiz Kibar: https://orcid.org/0000-0003-2983-2399, Hatice Hale Gümüş: https://orcid.org/0000-0001-9071-9606

***Sorumlu Yazar / Corresponding Author:** Hatice Hale Gümüş, **e-posta / e-mail:** hhaleag01@hotmail.com / hhgumus@cu.edu.tr

Geliş Tarihi / Received : 30-08-2023 **Kabul Tarihi / Accepted:** 16-09-2023 **Yayın Tarihi / Online Published:** 25-10-2023

Atıf Gösterimi/How to Cite: Asgarova T., Kibar F., Gümüş H.H. Identification of anaerobic bacteria isolated from clinical samples and determination of antibiotic resistance profiles. J Biotechnol and Strategic Health Res. 2023;7(3):157-165

Abstract

Aim In this study, it was aimed to identify anaerobic bacteria isolated from various clinical samples, and to determine their antibiotic resistance by gradient method (E-test).

Material and Method The study was carried out between January 15 and November 1, 2021. The 213 of 863 samples were included in the study. Anaerobic strains were isolated by conventional methods and identified by an automated system. Antimicrobial susceptibility was determined by the gradient method according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

Results Anaerobic bacteria were detected in 10.3% of the samples (n=22), aerobic/facultative anaerobic bacteria were detected in 34.8% (n=74), while growth was not observed in 54.9% (n=117) of the samples. The 76.9% of the samples (n=164) were abscess. The 72.7% (n=16) of anaerobic bacteria were Gram positive bacteria, and 27.3% (n=6) were Gram negative bacteria. The most common species were; Cutibacterium (22.7%, n=5), Actinomyces (18.3%, n=4), Prevotella (13.7%, n=3), Bacteroides (9.1%, n=2), Anaerococcus (9.1%, n=2), Clostridium species (9.1%, n=2). The antibiotic susceptibilities of all anaerobic bacteria were as following; moxifloxacin (95.5%, n=21), piperacillin-tazobactam (95.5%, n=21), amoxicillin-clavulanic acid (95.5%, n=21), cefoxitin (90.9%, n=20), meropenem (90.9%, n=20), clindamycin (77.3%, n=16), ampicillin (59.1%, n=13), and metronidazole (22.7%, n=5), respectively. The susceptibility rates of Gram positive bacilli were 91.7% (n=11) for ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, moxifloxacin, meropenem, and 75% (n=9) for clindamycin. In Gram positive cocci, susceptibility to ampicillin was 50% (n=2), susceptibility to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, clindamycin, moxifloxacin were 100% (n=4), and to meropenem was 75% (n=3). The susceptibility rates for Gram-negative bacilli were 0.0% (n=0) for ampicillin, 100% (n=6) for amoxicillin-clavulanic acid, piperacillin-tazobactam, moxifloxacin, meropenem, 83.3% (n=5) for metronidazole, 66.7% (n=4) for cefoxitin, and 50% (n=3) for clindamycin.

Conclusion In our study, it was observed that the sensitivity rates for especially, metronidazole and ampicillin were low among anaerobic bacteria. The resistance profile of many anaerobic bacteria has changed significantly over the past decade, making the antimicrobial susceptibility of anaerobic bacteria unpredictable. For this reason, revealing and documenting local data on this subject at regular intervals will constitute an important reference for both empirical treatment, public health, and surveillance studies.

Keywords Anaerobe, *Cutibacterium spp.*, antibiotic concentration gradient method, E-test, metronidazole.

Özet

Amaç *Bu çalışmada, çeşitli klinik örneklerden izole edilen anaerop bakterilerin tanımlanması ve gradiyent yöntemi (E-test) ile antibiyotik dirençlerinin belirlenmesi amaçlandı.*

Gereç ve Yöntem *Çalışma, 15 Ocak - 1 Kasım 2021 tarihlerinde gerçekleştirildi. 863 örneğin 213'ü çalışmaya dahil edildi. Anaerop suşlar konvansiyonel yöntemlerle izole edildi ve otomatize sistemle tanımlandı. Antibiyotik duyarlılıkları Clinical and Laboratory Standards Institute (CLSI) kriterlerine göre gradiyent yöntemi ile belirlendi.*

Bulgular *Örneklerin %10,3'ünde (n=22) anaerop bakteri, %34,8'inde (n=74) aerop/fakültatif anaerop bakteri tespit edilirken, %54,9'unda (n=117) üreme görülmedi. Örneklerin %76,9'u (n=164) apse materyaliydi. Anaerop bakterilerin %72,7'si (n=16) Gram pozitif bakteri, %27,3'ü (n=6) Gram negatif basildi. En yaygın türler; Cutibacterium (%22,7; n=5), Actinomyces (%18,3; n=4), Prevotella (%13,7; n=3), Bacteroides (%9,1; n=2), Anaerococcus (%9,1; n=2), Clostridium türleri (%9,1; n=2) idi. Sırasıyla, tüm anaerop bakterilerin antibiyotik duyarlılıkları; moksifloksasin (%95,5; n=21), piperasilin-tazobaktam (%95,5, n=21), amoksisilin-klavulonik asit (%95,5; n=21), sefoksitin (%90,9; n=20), meropenem (%90,9; n=20) klindamisin (%77,3; n=16), ampisilin (%59,1; n=13) ve metronidazol (%22,7; n=5) idi. Gram pozitif basillerin duyarlılık oranları ampisilin, amoksisillin-klavulanik asit, piperasilin-tazobaktam, sefoksitine, moksifloksasin, meropenem için %91,7 (n=11), klindamisin için %75'di (n=9). Gram pozitif koklarda ampisiline duyarlılık %50 (n=2), amoksisillin-klavulanik asit, piperasilin-tazobaktam, sefoksitin, klindamisin, moksifloksasine duyarlılık %100 (n=4) iken, meropeneme duyarlılık %75'di (n=3). Gram negatif basillerde duyarlılık oranları ampisilin %0,0 (n=0), amoksisillin-klavulanik asit, piperasilin-tazobaktam, moksifloksasin, meropenem için %100 (n=6), metronidazol %83,3 (n=5), sefoksitin için %66,7 (n=4) ve klindamisin için %50 (n=3) idi.*

Sonuç *Çalışmamızda anaerop bakterilerde özellikle metronidazol ve ampisilin için duyarlılık oranlarının düşük olduğu gözlemlendi. Birçok anaerop bakterinin direnç profilinin, son on yılda önemli ölçüde değişmesi, anaerop bakterilerin antimikrobiyal duyarlılıklarını fazla tahmin edilemez hale getirmiştir. Bu sebeple bu konudaki lokal verilerin belli aralıklarla ortaya çıkarılması ve dökümante edilmesi hem ampirik tedavinin şekillenmesi, hem halk sağlığı, hem de surveyans çalışmaları için önemli bir referans oluşturacaktır.*

Anahtar Kelimeler *Anaerop, Cutibacterium spp., antibiyotik konsantrasyon gradiyent yöntemi, E-test, metronidazol.*

INTRODUCTION

Anaerobic bacteria are commensal in the microbiota of different parts of the human body such as the gastrointestinal tract, genital tract and mouth. These microorganisms, which do not cause infection under normal conditions, can become pathogenic as a result of translocation of bacteria due to disruption of tissue integrity or overgrowth due to impaired blood circulation and decreased oxygenation. Following the identification of pathogenic anaerobic bacteria in the mid-19th century, anaerobes could be overlooked because it was technically difficult to obtain pure cultures of microorganisms. The use of inadequate anaerobic incubation techniques often only allowed the isolation of the most common anaerobic pathogens, members of the Bacteroides fragilis group or Clostridium perfringens, known as 'moderate' anaerobes, which survive at oxygen levels of 2-8%.[1-2] This may allow microbiologists to consider anaerobic culture techniques to be completely adequate because they can regularly isolate 'anaerobes'. Accurate and practical identification of anaerobic bacteria at the species level is challenging because it requires speed and precision at every stage, from the selection of the appropriate sample type, to sample collection, transfer to the laboratory and diagnostic procedures. Nowadays, there are a variety of commercial kits, specialized media and instruments for the isolation, typing and antimicrobial susceptibility testing of many anaerobic bacteria in microbiology laboratories.[1-4]

The resistance profile of anaerobic bacteria has changed significantly in the last decade, both within and between countries. This makes it difficult to predict the antimicrobial susceptibility of anaerobic bacteria.[5] Therefore, periodically collecting and documenting local data on this subject is an important reference for shaping empirical treatment, public health and surveillance studies. According to the international guidelines, antimicrobial susceptibility testing (AST) of anaerobic bacteria is very expensive, time-consuming and requires experienced laboratory personnel, and therefore cannot be performed for every isolate in routine laboratories. According to the international guidelines, the use of the disk diffusion method for AST of anaerobic bacteria is not recommended. The agar dilution method is currently the gold standard for AST of anaerobic bacteria. Standard broth microdilution method is difficult to standardize as there is no homogeneous growth of anaerobic bacteria except Bacteroides spp..[6] Antimicrobial concentration gradient method (E-test) is the most commonly used test for anaerobic AST in routine laboratories. Minimum inhibition concentration (MIC) values obtained by the E-test are considered reliable and correlate well with the reference method.[3,7]

In this study, we aimed to identify anaerobic bacteria isolated from various clinical specimens of patients with suspected anaerobic infection at species level, and to determine their antibiotic resistance profiles by antimicrobial concentration gradient method E-test (BioMerieux Inc, Marcy L'Etoile, France).

MATERIALS and METHODS

This is a prospective descriptive study conducted between January 15 and November 1, 2021 with the approval of the Cukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (TTU-2020-13333).

The study included clinical specimens such as tissues, aspirates, blood and other body fluids taken from the relevant foci of patients with suspected anaerobic infections such as diabetic foot ulcers, head and neck infections, breast abscess, brain abscess, psoas abscess, osteomyelitis, bacteremia, peritonitis in various clinics of Cukurova University Faculty of Medicine Balcalı Hospital. A total of the 863 clinical specimens sent to the microbiology laboratory, specimens that were not taken under sterile conditions, specimens that were not sent to the laboratory immediately after collection, specimens with missing patient information, specimens that were not suitable for anaerobic culture such as sputum, tracheal aspirate, bronchoalveolar

lavage, stool, midstream urine, skin swab specimens taken from body parts in contact with air were excluded (n=650) from the study. Abscess and body fluid samples were aspirated with a syringe, and tissue samples were taken in sterile saline in sterile small containers, and transported to the Microbiology Laboratory within 20 minutes without any delay. The samples were examined macroscopically for the presence of purulent, bloody mucus, foul odor and sulfur granules, and microscopically for the presence of polymorphonuclear leukocytes, pleomorphic staining, and spore formation by Gram staining and Giemsa staining.

For simultaneous anaerobic and aerobic cultures of the samples, 5% sheep blood Columbia Agar, Chocolate Agar PolyVitex, 5% sheep blood Schaedler agar, 5% sheep blood Schaedler Kanamycin Vancomycin Agar were used, and thioglycolate broth containing resazurin was used as enrichment media in the absence of growth (all media by BioMerieux Inc, Marcy L'Etoile, France). The seeded plates were placed in a 2.5 L anaerobic jar with Gas-Pak (GENboxanaer, BioMerieux Inc, Marcy L'Etoile, France) kit and an anaerobic medium indicator (Merck KGaA, Germany), and incubated at 35-37 °C for 48-72 hours. When the color of the indicator strip changed from blue to white, anaerobic environment was considered to be achieved. If no color change was observed on the strip within 1-2 hours, the procedures were repeated. After 48-72 hours of incubation, aerobic and anaerobic growths were examined and compared macroscopically, and evaluated microscopically by Gram staining. If colonies with the same morphologic structure grew in both media, facultative anaerobic bacteria were determined. When growth occurred only in anaerobic media, colonies were subjected to aerotolerance test.2 When anaerobic growth was observed in the test, the bacterium was considered obligate anaerobe. For identification, the automated diagnostic system VITEK 2 (BioMerieux Inc, Marcy L'Etoile, France) was used together with staining characteristics, morphology, susceptibility to colistin (10 µg), kanamycin (1000 µg) and vancomycin (5 µg) discs (Bioanalyse Inc., Ankara, Turkey).

Susceptibility tests of anaerobic bacteria identified at species level against ampicillin, amoxicillin clavulonic acid, piperacillin tazobactam, cefoxitin, meropenem, clindamycin, metronidazole, and moxifloxacin were performed using the antimicrobial concentration gradient method E-test (BioMerieux Inc, Marcy L'Etoile, France) with Brucella Blood Agar (BioMerieux Inc, Marcy L'Etoile, France) as media, and incubated at 35°C for 48 hours in an anaerobic atmosphere. All isolated and identified anaerobic bacteria were tested for beta-lactamase production by chromogenic Nitrocefin disk (Bioanalyse Inc., Ankara, Turkey). Bacteroides fragilis ATCC 25285 and Clostridium difficile ATCC 700057 standard strains were used as quality control strains. Antimicrobial susceptibility testing was interpreted according to the clinical breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012).[8]

RESULTS

Anaerobic bacteria were isolated in 10.3% (n=22) of 213 clinical samples cultured (Table 1). Of these, 17 (7.98%) grew as pure anaerobic bacteria and 5 (2.3%) as mixed with aerobic bacteria. Aerobic/facultative anaerobic bacteria growth was detected in 34.8% (n=74) of the cultures, including Staphylococcus spp. in 32, Escherichia coli in 19, Klebsiella spp. in 11, Streptococcus spp. in five, Pseudomonas spp. in three, Enterobacter spp. in three and Enterococcus spp. in one. No growth was observed in 54.9% (n=117) of the cultures.

The clinical samples from which anaerobic bacteria were isolated were abscess (n=11, 50%), blood (n=7, 31.8%), pleural fluid (n=2, 9.2%), cornea (n=1, 4.5%) and cerebrospinal fluid (n=1, 4.5%). Of the 5 samples with mixed growth, 4 were abscess samples and the associated aerobic bacteria were Escherichia coli (n=2), Proteus mirabilis in one sample, Staphylococcus epidermidis in one sample and Enterococcus avium in one sample.

Among the patients with anaerobic bacterial infection,

54.5% (n=12) were male and 45.5% (n=10) were female. Of these patients, 9% (n=2) were in the age group of 18 years and younger, 77.2% (n=17) were between 18-60 years, and 13.6% (n=3) were 60 years and older.

Table 1. Type of the clinical specimens and distribution of the isolated bacteria.

Sample type	Number of samples (n*, %)	Number of samples which only anaerobic bacteria were isolated (n, %)	Number of samples which aerobic/ facultative anaerobic bacteria were isolated (n, %)	Number of samples with mixed growth (n, %)
Abscess	164 (76.9)	7 (4.27)	58 (35.36)	4 (2.44)
Blood	10 (4.7)	7 (70)	2 (20)	None
Pleural fluid	12 (5.6)	2 (16.67)	8 (66.67)	None
Peritoneal fluid	7 (3.3)	None	5 (71.43)	None
Cornea	16 (7.6)	1 (6.25)	1 (6.25)	None
CSF**	1 (0.5)	None	None	1 (100)
Pericardial fluid	3 (1.4)	None	None	None
Total	213 (100)	17 (7.98)	74 (34.74)	5 (2.35)

* Number of patients
**CSF; Cerebrospinal fluid

Of the anaerobic bacterial isolates, 6 were Gram negative bacilli (27.3%) and 16 were Gram positive (72.7%), of which 12 were bacillus (54.54%) and 4 were cocci (18.18%). The most common anaerobes isolated were Propionibacterium/Cutibacterium species (22.7%, n=5), followed by Actinomyces species (18.3%, n=4), Prevotella species (13.7%, n=3), Bacteroides species (9.1%, n=2), Anaerococcus species (9.1%, n=2), Clostridium species (9.1%, n=2), Fusobacterium species (4.5%, n=1), Lactobacillus species (4.5%, n=1), Parvimonas micra (4.5%, n=1) and Peptoniphilus assacharolyticus (4.5%, n=1).

The β-lactamase positivity was detected in 4 of the anaerobic bacteria isolated (4/22, 18.2%). Two of the β-lactamase positive bacteria were Prevotella spp., one was Anaerococcus spp. and the other was Bacteroides spp (Table 2). Antimicrobial susceptibility profiles of the isolated anaerobic bacteria are summarized in Table 2. The most active antimicrobials were moxifloxacin (95.5%, n=21), piperacillin-tazobactam (95.5%, n=21) and amoxicillin-clavulonic acid (95.5%, n=21), followed by cefoxitin (90.9%, n=20), meropenem (90.9%, n=20), clindamycin (77.3%, n=16), ampicillin (59.1%, n=13) and metronidazole (22.7%, n=5). The susceptibilities of anaerobes to ampicillin were 91.7% (n=11) for Gram positive bacilli and 50% (n=2) for Gram positive cocci, while resistance was observed in all anaerobic Gram negative bacilli. For amoxicillin-clavulanic acid and piperacillin-tazobactam, anaerobic Gram positive bacilli showed 91.7% (n=11), Gram positive cocci 100% (n=4) and Gram negative bacteria 100% (n=6) susceptibility. The 91.7% (n=11) of anaerobic Gram positive bacilli, 100% (n=4) of Gram positive cocci and 66.7% (n=4) of Gram negative bacteria were susceptible to cefoxitin, while 33.3% (n=2) of Gram negative bacteria were moderately susceptible. The 75% (n=9) of anaerobic Gram positive bacilli, 100% (n=4) of Gram positive cocci and 50% (n=3) of anaerobic Gram negative bacilli were sensitive to clindamycin. While 83.3% (n=5) of anaerobic Gram negative bacilli were susceptible to metronidazole, 100% (n=16) resistance was observed in anaerobic Gram positive bacteria. Susceptibility to moxifloxacin was 91.7% (n=11) in anaerobic Gram positive bacilli and 100% in Gram positive cocci and Gram negative bacteria (n=10). Susceptibility to meropenem was 91.7% in anaerobic Gram positive bacilli, 75% (n=11) in Gram positive cocci and 100% (n=6) in Gram negative bacilli.

Table 2. Antimicrobial susceptibility profiles and β-lactamase test results of isolated anaerobic bacteria.

Isolated bacteria (Isolation number)	Ampicillin (µg/mL)	Amoxicillin-clavulanic acid (µg/mL)	Piperacillin-tazobactam (µg/mL)	Meropenem (µg/mL)	Metronidazole (µg/mL)	Clindamycin (µg/mL)	Moxifloxacin (µg/mL)	Cefoxitin (µg/mL)	β- lactamase
	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.002-32 µg/mL	MIC 0.016-256 µg/mL	MIC 0.016-256 µg/mL	MIC 0.002-32 µg/mL	MIC 0.016-256 µg/mL	
Gram positive anaerobes									
Anaerococcus prevotii* (5)	8 (R)	1.5 (S)	1.5 (S)	>32 (R)	>256 (R)	0.25 (S)	1.5 (S)	16 (S)	-
Anaerococcus prevotii* (43)	3 (R)	0.75 (S)	0.75 (S)	1.5 (S)	>256 (R)	0.125 (S)	0.125 (S)	12 (S)	+
Peptoniphilus asaccarolyticus (17)	0.023 (S)	0.032 (S)	0.032 (S)	0.012 (S)	>256 (R)	0.047 (S)	0.015 (S)	0.094 (S)	-
Parvimonas micra (208)	0.064 (S)	0.125 (S)	0.047 (S)	0.008 (S)	>256 (R)	0.064 (S)	0.125 (S)	4 (S)	-
Actinomyces naeslundii* (149)	0.032 (S)	0.023 (S)	0.123 (S)	0.004 (S)	>256 (R)	0.25 (S)	0.38 (S)	0.125 (S)	-
Actinomyces naeslundii* (190)	0.064 (S)	0.064 (S)	0.094 (S)	0.023 (S)	>256 (R)	>256 (R)	0.25 (S)	0.125 (S)	-
Actinomyces naeslundii* (27)	0.016 (S)	0.023 (S)	0.064 (S)	0.004 (S)	>256 (R)	0.047 (S)	0.125 (S)	0.032 (S)	-
Actinomyces naeslundii* (171)	0.032 (S)	0.023 (S)	0.064 (S)	0.032 (S)	>256 (R)	0.75 (S)	0.38 (S)	2 (S)	-
Cutibacterium acnes* (195)	0.032 (S)	0.023 (S)	0.125 (S)	0.016 (S)	>256 (R)	0.032 (S)	0.094 (S)	0.19 (S)	-
Cutibacterium acnes* (73)	0.047 (S)	0.047 (S)	0.19 (S)	0.47 (S)	>256 (R)	0.064 (S)	0.125 (S)	0.19 (S)	-
Cutibacterium acnes* (91)	0.047 (S)	0.032 (S)	0.125 (S)	0.008 (S)	>256 (R)	0.19 (S)	0.125 (S)	0.125 (S)	-
Cutibacterium gronulosum* (11)	0.25 (S)	0.025 (S)	1 (S)	0.125 (S)	>256 (R)	>256 (R)	0.047 (S)	0.75 (S)	-
Cutibacterium gronulosum* (84)	0.064 (S)	0.094 (S)	0.047 (S)	0.064 (S)	>256 (R)	0.032 (S)	0.064 (S)	0.19 (S)	-
Lactobacillus plantarum (103)	0.094 (S)	0.38 (S)	1 (S)	0.094 (S)	>256 (R)	0.016 (S)	1.5 (S)	>256 (R)	-
Clostridium group (142)	0.047 (S)	0.016 (S)	0.016 (S)	0.004 (S)	>256 (R)	0.023 (S)	0.094 (S)	0.047 (S)	-
Clostridium subterminale (211)	>256 (R)	>256 (R)	>256 (R)	>32 (R)	>256 (R)	>256 (R)	>32 (R)	>256 (R)	-
Gram negative anaerobes									
Bacteroides fragilis (32)	4 (R)	0.19 (S)	0.5 (S)	0.094 (S)	1 (S)	1 (S)	0.125 (S)	12 (S)	-
Bacteroides thetaiotamicron (61)	>256 (R)	4 (S)	16 (S)	0.38 (S)	0.38 (S)	2 (S)	1 (S)	24 (I)	+
Fusobacterium necrophorum (127)	>256 (R)	0.125 (S)	0.094 (S)	0.032 (S)	>256 (R)	>256 (R)	0.016 (S)	0.047 (S)	-
Prevotella bivia (205)	>256 (R)	4 (S)	8 (S)	0.125 (S)	0.25 (S)	>256 (R)	0.75 (S)	16 (S)	+
Prevotelle buccae (199)	>256 (R)	0.75 (S)	0.5 (S)	0.023 (S)	0.125 (S)	>256 (R)	0.019 (S)	0.5 (S)	+
Prevotelle oralis (74)	12 (R)	0.19 (S)	8 (S)	0.125 (S)	0.19 (S)	1.5 (S)	0.5 (S)	24 (I)	-

S: Sensitive, I: Moderately sensitive, R: Resistant, n: Number of patients, MIC: Minimum inhibitory concentration
*The same isolates were listed separately in the table, as their antibiotic susceptibilities were different.

DISCUSSION

Anaerobic bacteria constitute an important part of the human microbiome. They play an important role in various infections such as central nervous system, intraabdominal and foreign body infections, especially in polymicrobial infections.[9-11] Previous studies have shown that anaerobic bacteria isolated and their antimicrobial susceptibilities vary depending on the type of infection and hospital.[12-14] This situation shows the importance of local and national data in this regard.

Bacteroides, Prevotella, Propionibacterium/Cutibacterium species and Gram positive cocci are among the most frequently isolated anaerobic bacteria from clinical specimens.[11] In our study, pure anaerobic bacteria were isolated in 7.98% of 213 clinical specimens sent with the suspicion of anaerobic infection and cultured, of which 72.72% were Gram positive and 27.27% were Gram negative bacteria. The most frequently isolated anaerobic bacteria were Cutibacterium species (22.7%), Actinomyces species (18.1%) and Prevotella species (13.6%). In a few studies on anaerobic bacteria in our country, different rates of growth were found. In a study conducted in Afyon, a total of 4% anaerobic agents were reported in 5535 clinical samples sent for anaerobic culture in the three-year period between 2015-2017, of which 68% were Gram negative and 32% were Gram positive, and Propionibacterium/Cutibacterium acnes, Actinomyces spp and Clostridium spp. were the most common isolates.[15] In a study conducted in Diyarbakır, anaerobic bacteria were isolated from 73 (19.8%) of 368 clinical specimens; in 50 (13.6%) of these specimens, only anaerobic bacteria were isolated, and in 23 (6.3%) of these specimens, anaerobic bacteria as well as aerobic/facultative anaerobic agents were isolated.[16] In another study conducted in Konya, a total of 22 anaerobic bacteria were isolated from 14 of 100 clinical samples. In seven of these specimens, more than one anaerobic bacteria were found at the same time, while in eight samples anaerobic and facultative anaerobic bacteria were reported to grow together. The most frequently isolated bacteria were reported to be Bacteroides fragilis and Peptostreptococcus spp. And it was reorted that the two of six Bacteroides fragilis isolates were found to produce beta-lactamase enzyme, while the presence of beta-lactamase was not detected in other anaerobic strains.[17] In a study conducted in Sivas, no growth was observed in 409 (75.3%) of the samples, while various anaerobic bacteria were isolated in 134 samples (24.6%). And it was reported that Bacteroides spp. (29.9%), Peptopstreptococcus spp. (23.1%) and Propniobacterium spp. (20.2%) were the most common ones among the anaerobic bacteria isolated.[18] The reason why Bacteroides spp. were found to be the most common causative agent in the Konya and Sivas studies may be related to the higher number of intraabdominal samples. In a study conducted in Diyarbakır, Cutibacterium spp. were the most frequently isolated anaerobic bacteria in accordance with our study.[19] In a study conducted in Bulgaria, it was reported that Prevotella spp. were the most frequently isolated bacteria in abscess samples (22%).[20] These differences may be caused by the geographical location, age and other demographic characteristics of the patients, sample types and isolation methods.

In this study, anaerobic bacteria were mostly isolated from abscess materials. Of the cultured samples, 164 (77%) were abscesses, followed by blood (31.8%) and pleural fluid samples (9.1%). In other studies in which anaerobic bacteria were isolated, abscess specimens were reported most frequently, which is consistent with our study.[17-19,21]

It was previously reported that the antimicrobial resistance rates were increasing in anaerobic bacteria, which affected both treatment costs and mortality rates. And attention was drawn that regional susceptibility profiles were important in determining the empirical treatment of anaerobic infections.[11,22] In our study, 78% of the anaerobic bacteria for which AST was performed were resistant to metronidazole. And the resistance was mainly observed in Gram positive anaerobic bacteria (100%). This is indicative of intrinsic resistance found in most non-spore-forming

Gram positive anaerobic bacilli, especially Actinomyces, Propionibacterium/Cutibacterium and Lactobacillus species. Metronidazole showed good activity against most Gram negative bacilli (83.3%). In various countries of the world, metronidazole resistance rates of anaerobic bacteria have been reported in a wide and varied range, ranging between 1-58.3% in Gram positive and 1-50% in Gram negative bacilli.[11,21-22,23-26] Metronidazole resistance was reported as 96.2% for anaerobic Gram positive bacilli, 61.1% for Gram positive cocci and 33.3% for Gram negative bacilli in Diyarbakır, 0% for anaerobic Gram negative bacteria in Afyon and 94.9% for anaerobic Gram positive bacilli in Van.[15-16,27] In our study, the ampicillin resistance rate of anaerobic isolates that underwent AST was 40.9%, resistance was detected in 18.75% in Gram positive (8.3% in bacilli, 50% in cocci) and 100% in Gram negative isolates. In a study conducted in Malaysia, it was reported that the resistance rate of anaerobic Gram positive bacteria to ampicillin was 23.3% and that of Gram negative bacteria was 33.3%.[25] In various studies, penicillin resistance rates of anaerobic bacteria were reported as 30.8% in Gram positive bacilli, 19-50% in Gram positive cocci and 33.3-78.57% in Gram negative bacilli.[15-16,21,23,26] In almost all the studies, amoxicillin-clavulanic acid and piperacillin-tazobactam were reported to be the most susceptible antibiotics against anaerobic bacteria, consistent with our findings.[15,21,22,26,28] In this study, resistance to cefoxitin was found in 9.1%. The 12.5% of anaerobic Gram positive bacteria (bacilli 8.3%, cocci 0.0%) and 33.3% of Gram negative bacteria were resistant to cefoxitin. When domestic and foreign studies were analyzed, cefoxitin resistance was detected at quite different rates (3%-89%) in this bacterial group.[15-16,23,26,29-30] Variations in clindamycin susceptibility were also observed in the studies. In our study, clindamycin resistance was detected in 22.7%. The 18.7% of Gram positive bacteria (bacilli 25%, cocci 0.0%) and 50% of Gram negative bacilli were resistant to clindamycin. While these results were consistent with some studies,[23,26,28] our results were higher than the results of some other studies.[20,24] For example, in the study conducted in Bulgaria, which included mainly odontogenic abscess samples, Actiomyces spp. was most frequently isolated as Gram positive bacteria and Prevotella spp. as Gram negative bacteria, and resistance rates were tested by the agar dilution method. The resistance rates of isolates to clindamycin (2-3%) were significantly lower than this study.[20] The antimicrobial resistance profiles vary depending on geographical location, hospital centers, national antibiotic consumption, antimicrobials used for empirical therapy, diagnostic methods, bacterial species and sample types.[7,12]

In our study, susceptibility rates of anaerobic Gram positive bacteria were generally higher than those of Gram negative bacteria. The resistance to moxifloxacin was found at a rate of 4.5%. In addition, resistance was detected in 6.2% of Gram positives (8.3% of bacilli, 0.0% of cocci), while all Gram negatives were found to be susceptible to moxifloxacin. In contrast to this study, the studies conducted abroad, reported higher rates of resistance to moxifloxacin.[21,22-24,31] In a study conducted in Afyon, it was reported that no resistance to moxifloxacin was observed in Gram negative anaerobic bacteria in accordance with our results.[15] In this study, meropenem resistance of anaerobic bacteria was found to be 9.1%. It was found that 12.5% of Gram positive bacteria (bacilli 8.3%, cocci 25%) were resistant, and all Gram negative bacteria were susceptible. These results were compatible with the previous reports.[15,22-23,31]

Our study has some limitations. The molecular mechanism of resistance to antianaerobic drugs, risk factors that may cause resistance and their relationship with mortality have not been investigated. To understand the impact of antimicrobial resistance on patients and public health, it may be important to study the evolution and consequences of antimicrobial resistance in anaerobic bacteria together.

CONCLUSION

In our study, low susceptibility rates were observed especially for metronidazole and ampicillin in anaerobic bacteria. In particular, an alarming resistance rates of 77.3% to

metronidazole and 40.9% to ampicillin were detected. Due to the emergence of drug resistance in anaerobes, it would be useful to investigate newer and alternative options for patient management. It is time for susceptibility testing of anaerobic bacteria to become a routine service in microbiology laboratories. The data of this study can serve as a reference for monitoring resistance and determining empirical treatment, and can be used for periodic monitoring of resistance trends in surveillance studies.

Ethical Approval

The approval was obtained from the Cukurova University Faculty of Medicine Non-interventional Clinical Research Ethics Committee (No: 104, Date: 02.10.2020).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Consept: F.K., T.A., Design: F.K., T.A., H.H.G. Data Collection or Processing: F.K., T.A., H.H.G., Analysis and Interpretation: F.K., T.A., H.H.G., Literature Search: F.K., T.A., H.H.G., Writing: F.K., T.A., H.H.G.

Conflict of Interest

The authors declare no conflict of interest in relation to this article.

Funding

This study was conducted as a Specialization Thesis in Medicine and supported by the Cukurova University Scientific Research Projects with the project number TTU2020-13333.

References

1. Sood A, Ray P, Angrup A. Antimicrobial susceptibility testing of anaerobic bacteria: In routine and research. Anaerobe. 2022; 75: 102559. doi: 10.1016/j.anaerobe.2022.102559.
2. Winn WC, Koneman EW, Allen SD, et al. The Anaerobic Bacteria (6. Baskı). Konemans Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia: Lippincott Williams & Wilkins Publication, 2006: 877-944.
3. Nagy LE, Boyanova, Justesen US. How to isolate, identify and determine antimicrobial susceptibility of anaerobic bacteria in routine laboratories. Clin Microbiol Infect. 2018; 24(11): 1139-1148. doi: 0.1016/j.cmi.2018.02.008.
4. Hentges DJ. Anaerobes as normal flora. Anaerobic infections in humans, Finegold SM, George WL (Eds), San Diego, Elsevier Publications, 2012; p. 37e53.
5. Boyanova L, Kolarov R, Mitov I. Recent evolution of antibiotic resistance in the anaerobes as compared to previous decades. Anaerobe. 2015; 31: 4e10. doi: 10.1016/j.anaerobe.2014.05.004.
6. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria (9th Ed.), Document M11eA8, Wayne, PA, USA, 2019.
7. Byun JH, Kim M, Lee Y, et al. Antimicrobial Susceptibility Patterns of Anaerobic Bacterial Clinical Isolates From 2014 to 2016, Including Recently Named or Renamed Species. Ann Lab Med. 2019; 39: 190-199. doi: 10.3343/alm.2019.39.2.190.
8. Clinical and Laboratory Standards Institute (2012, January). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Second Informational Supplement, Document M100-S22, Clinical and Laboratory Standards Institute, Wayne PA, USA. https://m.ibric.org/miniboard/down.php?Board=exp_qna&filename=CLSI%20-%20M100%20S22E.pdf&id=531983&fidx=1 adresinden 30 Haziran 2023'de erişildi.
9. Brook I. Spectrum and treatment of anaerobic infections. J Infect Chemother. 2016; 22: 1e13. doi: 10.1016/j.jiac.2015.10.010.
10. Shah NB, Tande AJ, Patel R, et al. Anaerobic prosthetic joint infection. Anaerobe. 2015; 36: 1-8. doi:10.1016/j.anaerobe.2015.08.003.
11. Gajdács M, Spengler G, Urbán E. Identification and Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Rubik's Cube of Clinical Microbiology?. Antibiotics (Basel). 2017; 6(4): 25. doi:10.3390/antibiotics6040025.
12. Boyanova L, Kolarov R, Mitov I. Recent evolution of antibiotic resistance in the anaerobes as compared to previous decades. Anaerobe. 2015; 31: 4-10. doi:10.1016/j.anaerobe.2014.05.004.
13. Ananth-Shenoy P, Vishwanath S, Targain R, et al. Anaerobic infections in surgical wards: A two year study. Iran J Microbiol. 2016; 8: 181–186.
14. Byun JH, Kim M, Lee Y, et al. Antimicrobial Susceptibility Patterns of Anaerobic Bacterial Clinical Isolates From 2014 to 2016, Including Recently Named or Renamed Species. Ann Lab Med. 2019; 39(2): 190-199. doi:10.3343/alm.2019.39.2.190.
15. Demir C, Keşli R. Çeşitli klinik örneklerden izole edilen Gram-negatif anaerop basillerin tiplendirilmesi ve antibiyotik direnç profillerinin E-test yöntemi ile belirlenmesi [Identification of anaerobic Gram-negative bacilli isolated from various clinical specimens and determination of antibiotic resistance profiles with E-test methods]. Microbiyol Bul. 2018; 52(1): 72-79. doi:10.5578/mb.66175.
16. Özcan N, Saat N, Atmaca N. Klinik örneklerden soyutlanan anaerop bakterilerin in vitro antibiyotik duyarlılıkları. Flora. 2020; 25(2): 245-55. doi: 10.5578/flora.68705.
17. Doğan M, Baysal B. Çeşitli klinik örneklerden izole edilen anaerop bakterilerin tanımlanması ve antibiyotik duyarlılıklarının belirlenmesi. Microbiyol Bul. 2010; 44: 211-219.
18. Uysal BE, Çelik C, Alan Ç, et al. Klinik örneklerden izole edilen anaerobik bakteriler: yedi yıllık değerlendirme. Cumhuriyet Tıp Derg. 2014;36:327-31. doi: 10.7197/cmj.v36i3.5000034023.
19. Özcan N, Bacalan F, Çakır F, et al. Identification and antimicrobial susceptibility testing of anaerobic bacteria isolated from clinical samples. J Bacteriol Mycol Open Access. 2020; 8(1): 29-32. doi: 10.15406/jbmoa.2020.08.00269.
20. Boyanova L, Kolarov R, Gergova G, et al. Anaerobic bacteria in 118 patients with deep-space head and neck infections from the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria. J Med Microbiol. 2006; 55(Pt12): 1759-1760. doi:10.1099/jmm.0.46512-0.
21. Cobo F, Guillot V, Navarro-Marí JM. Breast Abscesses Caused by Anaerobic Microorganisms: Clinical and Microbiological Characteristics. Antibiotics (Basel). 2020; 9(6): 341. doi:10.3390/antibiotics9060341.
22. Byun JH, Kim M, Lee Y, et al. Antimicrobial Susceptibility Patterns of Anaerobic Bacterial Clinical Isolates From 2014 to 2016, Including Recently Named or Renamed Species. Ann Lab Med. 2019; 39(2): 190-199. doi:10.3343/alm.2019.39.2.190.
23. Wybo I, Van den Bossche D, Soetens O, et al. Fourth Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. J Antimicrob Chemother. 2014;69(1):155-161. doi:10.1093/jac/dkt344.
24. Badr MT, Blümel B, Baumgartner S, et al. Antimicrobial Susceptibility Patterns and Wild-Type MIC Distributions of Anaerobic Bacteria at a German University Hospital: A Five-Year Retrospective Study (2015-2019). Antibiotics (Basel). 2020; 9(11): 823. doi:10.3390/antibiotics9110823.
25. Ishak N, Abdul Wahab Z, Amin Nordin S, et al. Susceptibility patterns of anaerobes isolated from clinical specimens in tertiary Hospital, Malaysia. Malays J Pathol. 2020; 42(2): 245-252.
26. Naidoo S, Perovic O, Richards GA, Duse AG. Clinically significant anaerobic bacteria isolated from patients in a South African academic hospital: antimicrobial susceptibility testing. S Afr Med J. 2011;101(10):732-734.
27. 2Bozkurt H, Güdücüoğlu H, Bayram Y, et al. Klinik örneklerden izole edilen anaerob bakteriler ve antibiyotik duyarlılıkları. Van Tıp Derg. 2004; 11: 85-91.
28. Jeverica S, Kolenc U, Mueller-Premru M, et al. Evaluation of the routine antimicrobial susceptibility testing results of clinically significant anaerobic bacteria in a Slovenian tertiary-care hospital in 2015. Anaerobe. 2017; 47: 64-69. doi:10.1016/j.anaerobe.2017.04.007.
29. Katsandri A, Avlamis A, Pantazatou A, et al. In vitro activities of tigecycline against recently isolated Gram-negative anaerobic bacteria in Greece, including metronidazole-resistant strains. Diagn Microbiol Infect Dis. 2006; 55(3): 231-236. doi:10.1016/j.diagmicrobio.2006.01.022.
30. Akhi MT, Ghotaslou R, Beheshtirouy S, et al. Antibiotic Susceptibility Pattern of Aerobic and Anaerobic Bacteria Isolated From Surgical Site Infection of Hospitalized Patients. Jundishapur J Microbiol. 2015; 8(7): e20309. doi:10.5812/jjm.20309v2.
31. Lee Y, Park Y, Kim MS, et al. Antimicrobial susceptibility patterns for recent clinical isolates of anaerobic bacteria in South Korea. Antimicrob Agents Chemother. 2010; 54(9): 3993-3997. doi:10.1128/AAC.00481-10.