

# Identification and distribution of anaerobic bacteria isolated from clinical specimens in a University Hospital: 4 years' experience

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## ABSTRACT

Anaerobes, which are components of microbiota, can cause life-threatening infections. Because of their fastidious nature, they are difficult to isolate and are often overlooked. The goal of this study was to identify the anaerobic bacteria isolated from clinical specimens at the Central Laboratory of Hacettepe University Hospital in 2015-2018 and to evaluate the distribution of the isolated bacterial species among the different specimen types. The anaerobic bacteria isolated from the specimens were identified by the conventional methods and MALDI-TOF MS.

Overall, 15,300 anaerobic cultures were studied. Of these, 14,434 (94.3%) were blood samples and 866 (5.7%) were other clinical specimens. A total of 138 anaerobic bacteria were isolated: 62 (44.9%) were isolated from blood samples and 76 (55.1%) from other specimens. The most isolated anaerobes from blood cultures were *Bacteroides* spp. (41.9%), followed by *Cutibacterium acnes* (25.8%) and *Clostridium* spp. (9.7%). The most isolated anaerobes from the other specimens were Gram-negative bacilli, including *Bacteroides* spp. (15.8%), *Fusobacterium* spp. (14.5%), *Prevotella* spp. (14.5%), and *Porphyromonas* spp. (2.6%). Anaerobic *Fingoldia magna* represented the major species among the isolated Gram-positive bacteria (10.5%). Anaerobic growth was observed in 0.4% of all the blood cultures and in 5.8% of the positive blood cultures. The results of our study showed that the incidence of anaerobic bacteremia was stable during the 2015-2018 period.

**Keywords:** Anaerobic culture, *Bacteroides*, blood culture, anaerobes

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## INTRODUCTION

Anaerobic bacteria represent a significant portion of human microbiota. They can cause opportunistic infections if they are displaced and/or move to other sites of the body that do not have normal microflora. Anaerobic infections are usually known to be endogenous and polymicrobial. These infections are significant as they may be severe and life-threatening in some cases (e.g., brain abscess, bloodstream infections, endocarditis) [1-3].

In many clinical microbiology laboratories, the analyses of anaerobic culture specimens are not routinely performed because anaerobic microbiology is difficult, time-consuming, and requires special equipment and qualified staff. In addition, errors in the choice, collection, and transport of clinical specimens of anaerobic culture negatively affect the recovery rates of anaerobic bacteria from specimens. As a result, anaerobic infections are usually overlooked [1-4].

The objective of the current study was to investigate the genus/species of anaerobic bacteria that were isolated from patients and identified at the Central Laboratory of Hacettepe University Hospital between 2015 and 2018 and to evaluate the distribution of the anaerobic bacterial species among the different specimen types.

## MATERIALS AND METHODS

### Specimens and patients

Blood specimens and other acceptable specimens (e.g., tissue, pus, pleural fluid) with an anaerobic culture request collected between 2015 and 2018 were analyzed. The specimens were collected from a total of 8,153 patients.

### Analysis of the specimens

Blood culture bottles were placed into a continuous-monitoring blood culture system without delay. The BacT/ALERT (BioMérieux, France) blood culture system was used to incubate the blood culture vials during the period 2015-2017. The BD BACTEC™ FX (BD, USA) blood culture system was used in 2018. Other specimens were cultured on Schaedler agar (Oxoid, UK), chocolate agar (Oxoid, UK), sheep blood agar (Oxoid, UK), EMB agar (Oxoid, UK), and in thioglycolate broth (Oxoid, UK). Specimens on Schaedler agar and chocolate agar were incubated in an anaerobic atmosphere and in an atmosphere of 5-10% CO<sub>2</sub>, respectively, for 48 h. The specimens on other media (Sheep blood agar and EMB agar) were incubated aerobically for 18-24 h. All of the samples were incubated at 37°C. The primary culture plates were evaluated after 48 h of incubation. The colony morphologies on primary plates were examined and aerotolerance testing was performed on each colony type. The bacteria that grew only in anaerobic conditions were considered anaerobes.

### Bacterial identification

Conventional methods (Gram staining, catalase test, indole test, susceptibility to special potency antibiotic disks (vancomycin 5 µg, kanamycin 1,000 µg, colistin 10 µg)) as well as Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (VITEK MS v3.0, BioMérieux, France (between January 2015 and November 2017)) and MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) (from November 2017) were used for the identification of isolates [5, 6].

### Statistical analysis

For the comparison of anaerobic growth rates in blood, tissue, and pus cultures over time, the  $\chi^2$ -test for the

linear trend was used. Fischer's exact test was used for the analysis of the association between anaerobic bacterial species and the types of specimens. Analyses were conducted with the IBM SPSS version 25.0 and R Radiant package. The differences were considered statistically significant at  $p < 0.05$ .

## RESULTS

In total, we studied 15,300 specimens with an anaerobic culture request in 2015-2018. Blood cultures (n=14,434) represented 94.3% of all the specimens. Other samples (n=866, 5.7%) included specimens isolated from tissue, pus, peritoneal fluid, pleural fluid, cerebrospinal fluid (CSF), cyst fluid, bile, bone marrow, synovial fluid, pericardial fluid, and dialysis fluid (Table 1).

Anaerobic bacterial growth was observed in 0.4% (62 of 14,434) of blood specimens, whereas aerobic bacterial growth was observed in 6.9% (999 of 14,434) of blood cultures. Among 999 aerobic/facultative anaerobic microorganisms, 512 (51.3%) were only isolated from cultures cultivated in anaerobic conditions. In total, microbial growth was detected in 7.4% (1,061 of 14,434) of blood cultures.

Anaerobic and aerobic growth was observed in 8.8% (76 of 866) and in 25.5% (221 of 866) of specimens other than blood, respectively. In total, bacterial growth was observed in 34.3% (297 of 866) of specimens other than blood.

The most frequently isolated anaerobes, being found in 13 of 1,415 anaerobe-positive specimens and accounting for 0.9% were isolated in 2016, followed by 0.4% (42 of 9,569) in 2018, 0.2% (7 of 3,308) in 2017, and 0% (0 of 142) in 2015. The anaerobic bacterial growth rate in blood specimens collected between 2015 and 2018 years did not differ significantly ( $p=0.609$ ) according to the  $\chi^2$  for the linear trend test (Table 1). Out of the 62 anaerobic bacteria that were growing in the blood cultures, 30 (48.4%) were Gram-negative bacteria, whereas 32 (51.6%) were Gram-positive bacteria. The rate of anaerobic bacterial growth among the positive blood cultures was also the highest (7.5%, 13 of 174) in 2016, followed by 5.7% (42 of 737) in 2018 and 2017 (7 of 123), and 0% (0 of 27) in 2015. On average, the rate of anaerobic bacterial growth among the positive blood cultures was 5.8% (62 of 1,061).

The rate of anaerobic bacterial growth in tissue specimens was 22.5% (23 of 102) in 2015, 20.7% (12 of 58) in 2016, 6.5% (3 of 46) in 2017, and 11.1% (3 of 27) in 2018 and did not differ significantly ( $p=0.737$ ) (Table 1). The anaerobic bacterial growth rate in pus cultures comprising 4.2% (2 of 48) in 2015, 15.6% (10 of 64) in 2016, 7.5% (4 of 53) in 2017, and 12.9% (9 of 70) in 2018 was also

comparable ( $p=0.067$ ) (Table 1). We used aerobic and anaerobic blood culture bottles for blood collecting as a routine set since the second half of 2015. Fig. 1 shows the impact of this implementation on the results of blood cultures analysis.

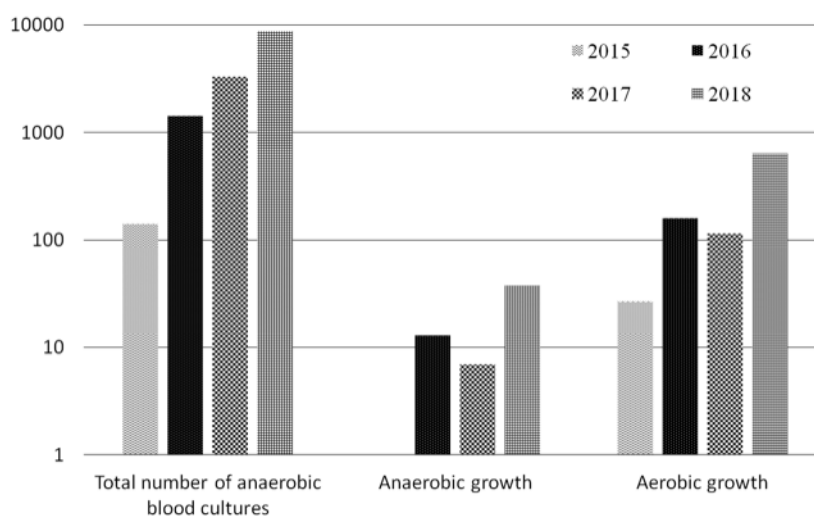
The distribution of anaerobic bacterial species according to the specimen types is shown in Table 2. Among the 138 anaerobic isolates, 62 (44.9%) were from blood

samples and 76 (55.1%) from other than blood specimens. Among the anaerobic bacteria from specimens other than blood, 53.9% (41 of 76), 31.6% (24 of 76), 9.2% (7 of 76), 3.9% (3 of 76), and 1.3% (1 of 76) were isolated from tissue, pus, pleural fluid, bile, and cerebrospinal fluid, respectively.

*Bacteroides* spp. were the most frequently isolated anaerobic organisms (27.5%, 38 of 138) from all of the

**Table 1.** The number of samples with aerobic and anaerobic growth obtained from different specimens with anaerobic culture request in 2015-2018 period

Specimens	2015 (n=346)			2016 (n=1620)			2017 (n=3480)			2018 (n=9854)		
	No growth	Aerobic growth	Anaerobic Growth	No growth	Aerobic growth	Anaerobic growth	No growth	Aerobic growth	Anaerobic growth	No growth	Aerobic growth	Anaerobic growth
Blood	115	27	-	1241	161	13	3185	116	7	8832	695	42
Tissue	39	40	23	26	20	12	32	11	3	19	5	3
Pus	22	24	2	33	21	10	36	13	4	41	20	9
Peritoneal fluid	12	5	-	17	10	-	19	9	-	51	5	-
Pleural fluid	10	3	2	18	8	3	17	4	-	51	3	2
CSF	8	-	-	6	1	-	6	-	-	16	1	1
Cyst fluid	5	-	-	-	-	-	-	-	-	3	-	-
Bile	2	1	1	5	3	-	-	1	1	8	4	-
Bone marrow	3	-	-	3	2	-	6	-	-	19	-	-
Synovial fluid	2	-	-	2	1	-	9	1	-	13	2	-
Pericardial fluid	-	-	-	2	2	-	-	-	-	7	1	-
Dialysis fluid	-	-	-	-	-	-	-	-	-	1	-	-
TOTAL	218	100	28	1353	229	38	3310	155	15	9061	736	57



**Fig. 1.** The number of anaerobic blood culture requests and blood culture results per year.

**Table 2.** Distribution of anaerobic bacterial species isolated in a period 2015-2018 according to specimen types

Organism	Blood	Tissue	Pus	Bile	Pleural fluid	CSF	Total
<i>Bacteroides</i> spp.							38
<i>B. fragilis</i>	24**	5	5				
<i>B. thetaiotaomicron</i>	2		1				
<i>B. vulgatus</i>				1			
<i>Fusobacterium</i> spp.							13
<i>F. nucleatum</i>	2	6**	2		2		
<i>F. necrophorum</i>			1				
<i>Porphyromonas</i> spp.							3
<i>P. asaccharolytica</i>	1	1	1				
<i>Prevotella</i> spp.							11
<i>P. buccae</i>		6**	1				
<i>P. denticola</i>		1					
<i>P. disiens</i>			1				
<i>P. intermedia</i>		1					
<i>P. melaninogenica</i>		1					
Unidentified anaerobic gram-negative bacilli	1						1
<i>Veillonella parvula</i>		9**	1		1		11
<i>Clostridium</i> spp.							8
<i>C. perfringens</i>	5**						
<i>C. ramosum</i>		1					
<i>C. tertium</i>	1						
<i>C. sporogenes</i>			1				
<i>Actinomyces</i> spp.							10
<i>A. odontolyticus</i>		2	1		2		
<i>A. viscosus</i>		1			1		
<i>A. europaeus</i>	1						
<i>A. neuii</i>	1						
<i>A. oris</i>	1						
<i>Bifidobacterium</i> spp.		3	1				4
<i>Cutibacterium acnes</i>	16**	2					18
<i>Propionibacterium avidium</i>	1					1	2
Unidentified anaerobic gram-positive bacilli	2			2			4
<i>Finegoldia magna</i>	4	1	6**		1		12
<i>Peptostreptococcus anaerobius</i>			2				2
Unidentified anaerobic gram-positive cocci		1					1
Total	62	41	24	3	7	1	138

The sign (\*\*\*) indicates the statistically significant difference in the number of positive samples isolated from particular specimen as compared to the other specimens ( $p < 0.01$ ), according to Fischer's exact test.

specimen types. *Bacteroides* spp. were also the most common anaerobic isolates (41.9%, 26 of 62) from the blood cultures, followed by *Cutibacterium acnes* (formerly *Propionibacterium acnes*) (25.8%, 16 of 62) and *Clostridium* spp. (9.7%, 6 of 62) ( $p < 0.01$ ) (Table 2). *Bacteroides fragilis* was the most frequently isolated species of the *Bacteroides* spp. genus. *Fusobacterium* spp., *Prevotella* spp., and *Veillonella parvula* were most frequently isolated from tissue cultures ( $p < 0.01$ ). *Finegoldia magna* isolates dominated in pus cultures ( $p < 0.01$ ).

The most common anaerobes isolated from the specimens other than blood were Gram-negative bacteria including *Bacteroides* spp. (15.8%, 12 of 76), *Fusobacterium* spp. (14.5%, 11 of 76), *Prevotella* spp. (14.5%, 11 of 76), *Veillonella parvula* (14.5%, 11 of 76), and *Porphyromonas* spp. (2.6%, 2 of 76). The most frequently isolated anaerobic Gram-positive bacteria were *Finegoldia magna* (10.5%, 8 of 76), *Actinomyces* spp. (9.2%, 7 of 76), and *Bifidobacterium* spp. (5.3%, 4 of 76).

## DISCUSSION

Anaerobic bacteria can cause serious and life-threatening infections, such as bloodstream infections and intracranial infections in humans. These microorganisms are usually isolated from the nidus of infection located in the patient's head and neck, skin, and soft tissues. They also are isolated from the patients with pleuropulmonary, intra-abdominal, and gynecological infections. Most clinical microbiology laboratories have limited capabilities in terms of anaerobic bacteriology because the isolation and identification of anaerobic bacteria is time-consuming, costly, and often associated with technical difficulties [4, 7, 8].

The most important factor that causes anaerobic infections is the introduction of anaerobic members of normal microflora into the body sites that do not have microflora because of injury. As a result, most of the bacteria that were isolated from patients with anaerobic infections originated from the endogenous bacterial flora.

In this retrospective study, 138 anaerobic isolates from various clinical specimens were analyzed. The anaerobic bacteria were isolated from blood (44.9%, 62 of 138), tissue (29.7%, 41 of 138), pus (17.4%, 24 of 138), pleural fluid (5.1%, 7 of 138), bile (2.2%, 3 of 138), and cerebrospinal fluid (0.7%, 1 of 138). These data are in accordance with other studies [1, 2, 4].

Gram-negative bacilli, namely *Bacteroides* spp., *Fusobacterium* spp., *Porphyromonas* spp., and *Prevotella* spp., are known to be the most isolated organisms from anaerobic infections [1, 8-12]. In our study, 76 anaerobic bacteria were isolated from specimens other than blood and approximately half (47.4%) of these isolates were

Gram-negative bacilli. *Bacteroides* spp., *Fusobacterium* spp., and *Prevotella* spp. constituted the major group of anaerobic Gram-negative bacilli, which is consistent with the previous studies [1, 9-12]. Among the Gram-positive bacteria, anaerobic species *Finegoldia magna* (formerly *Peptostreptococcus magnus*) were the most frequently isolated (10.5%) from specimens other than blood.

The most isolated anaerobes from blood were *Bacteroides* spp. The genus/species of bacteria isolated from blood differ from the bacteria isolated from specimens other than blood. However, in our study, *Bacteroides* spp. were the most frequently isolated anaerobic bacteria from both blood samples and specimens other than blood.

Although the incidence of bacteremia due to anaerobic bacteria is low, anaerobic bacteremia is associated with a high mortality rate (14-60%) [9, 13-18]. According to the literature data, anaerobic bacteria are isolated from 0.5-20% of positive blood cultures and comprise 0.5-1% of all blood cultures [13, 14, 16, 17, 19-21]. These rates vary by geographic location and institutions as well as the age and other demographic characteristics of hospitalized patients [14, 16, 19, 22].

In the present study, the rate of positive blood cultures in the period 2015-2018 was 7.4%. The rate of anaerobic bacterial growth among all the blood cultures was 0.4%. The anaerobic bacterial growth rate among the positive blood cultures was 5.8%. The rates observed in our study correspond to those reported by other authors [13, 15, 16, 19, 23-25].

The annual rate of anaerobic bacterial growth among all the blood cultures in our study varied between 0% and 0.9%. In previous studies, some authors reported an increase [22, 26, 27] or decrease [16, 28] in the incidence of anaerobic bacteremia, while others reported no significant changes in the incidence of anaerobic bacteremia over years [15, 29]. According to our data, the incidence of anaerobic bacteremia was relatively stable over time.

Anaerobic bacteria that most often cause bacteremia belong to the *B. fragilis* group and are responsible for approximately half of all anaerobic bacteremia cases [13, 15, 19, 24]. Other frequent causative agents of anaerobic bacteremia include *Clostridium* spp., *Peptostreptococcus* spp., *Prevotella* spp., *Fusobacterium* spp., and other gram-negative bacilli [13, 15, 16]. Similarly, in our study, bacteria that belong to the *B. fragilis* group were the most frequently isolated anaerobic microorganisms (41.9%) from blood cultures followed by *Bacteroides thetaiotaomicron* – the second most common member of the *B. fragilis* group, which is in accordance with the results of Kim et al. and Keukeleire et al. [9, 16]. Other anaerobic



organisms frequently isolated from blood cultures in our study were *Cutibacterium acnes* (25.8%) and *Clostridium* spp. (9.7%). *Clostridium* spp. were also frequently isolated from blood cultures according to previous reports [9, 15, 16, 19, 24]. However, the isolation rate of *C. acnes* in our study was higher than that reported by Vena et al. [15]. One reason for this may be the contamination of blood cultures with skin flora because of the improper collection of blood specimens. On the other hand, it is known that *C. acnes* may cause bacteremia especially in patients with vascular catheters or shunts [13].

Currently, the use of a blood culture set, including one aerobic and one anaerobic bottle, is recommended for routine practice in clinical microbiology laboratories. The use of an anaerobic blood culture bottle is important not only for the recovery of anaerobic bacteria but also for the recovery of facultative anaerobes that grow better under anaerobic conditions. The use of an anaerobic culture bottle also reduces the time to detection of microbial growth [23, 30-32]. Our data confirm these findings. Between 2015 and 2018, 512 aerobic/facultative anaerobic organisms were isolated from the anaerobic culture bottles only. Educational programs and practices

of blood culture collection were initiated at the Hacettepe University Hospital in 2015. As a result, the number of discovered anaerobic blood cultures has significantly increased.

The growing number of immunocompromised patients as well as the advent of more sophisticated methods for bacterial identification have led to a change in the number and distribution of isolated anaerobic bacteria. Furthermore, the antimicrobial resistance profiles of many anaerobic bacteria have changed in recent decades. Antimicrobial resistance profiles are known to vary by geographic location, hospital centers, national antibiotic consumption, bacterial species, and type of specimens [2, 33]. As the number of requests for anaerobic culture analysis in our hospital has increased over the years, we have started to analyze the clinical isolates of anaerobic bacteria from blood cultures for antimicrobial susceptibility.

In order to provide efficient treatment for patients diagnosed with infectious diseases, it is important to follow the epidemiological changes, distribution, and antimicrobial resistance profiles of the causative agents of anaerobic infections.

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