Iranian Journal of Microbiology
Volume 14 Number 1 (February 2022) 24-30

ORIGINAL ARTICLE

Distribution of anaerobic bacteria and their sensitivity pattern to several antibiotics at the clinical microbiology laboratory of school of medicine, universitas Indonesia, Jakarta in 2019-2020

Conny Riana Tjampakasari*, Dimas Seto Prasetyo, Ika Ningsih, Ariyani Kiranasari

Department of Microbiology, School of Medicine, Universitas Indonesia, Jakarta, Indonesia

Received: April 2021**, Accepted:** June 2021

ABSTRACT

Background and Objectives: Anaerobic bacteria are a common cause of endogenous infections, some of which can be life threatening. These bacteria are not easily cultured and isolated and often cannot even found from infected sites. Delayed or inappropriate treatment of these microorganisms can lead to failure in eradicating these infections. The purpose of this study was to determine the diversity of anaerobic bacteria at present and their pattern of sensitivity to several antibiotics.
Materials and Methods: A retrospective study was conducted over a period of two years on various specimens. Specimens derived from body fluids are inoculated on a BacT/Alert (bioMérieux). Anaerobic isolates were identified by Gram staining and continued identification using Vitek 2® automated system. Antibiotic sensitivity examination was carried out using ATBTM ANA (bioMérieux).
Results: A total of 440 specimens were received in microbiology laboratory for anaerobic culture from patients with multiple infections from 13 hospitals in Jakarta. Our research was able to identify 18 species on anaerobic bacteria, consisting 52.5% Gram positive and 47.5% Gram negative bacteria. The most common bacteria found were *Clostridium perfringens* (15%) from Gram positive and *Provetella bivia* (10%) from Gram negative. The sensitivity pattern shows that antibiotic piperacilline-tazobactam is 100% effective against anaerobic bacteria, while metronidazole as the drug of choice is only 75% effective. Against Gram positive, several antibiotics such as piperacilline-tazobactam, ticarcilin-clavunic acid, cefoxitin, cefotetan, imipenem and chloramphenicol were 100% effective, however metronidazole occupied the lowest position (61.9%). Meanwhile against Gram negative antibiotics piperacilline-tazobactam is 100% effective and chloramphenicol in the second position (94.75%).
Conclusion: *Clostridium perfringens* and *Provetella bivia* are the most common bacteria found. The antibiotics piperacilline-tazobactam is 100% effective against both Gram positive and negative. The accuracy of specimen management, isolation, identification and sensitivity examination will determine the successful microbiological investigations.

Keywords: Anaerobic bacteria; Gram negative; Gram positive; Antibiotic sensitivity; Jakarta

INTRODUCTION

Anaerobic bacteria do not use oxygen for growth and metabolism, but still get energy from fermentation reactions (1). The presence of oxygen causes these bacteria to stunt their growth. This happens because the H_2O_2 formed in these conditions is toxic (2). Anaerobic bacteria are divided into 2 groups,

*Corresponding author: Conny Riana Tjampakasari, Ph.D, Department of Microbiology, School of Medicine, Universitas Indonesia, Jakarta, Indonesia. Tel: 62-21-3160491 Fax: 62-21-3100810 Email: connyrianat@yahoo.com

namely obligate anaerobes which cannot grow at all in the presence of oxygen and facultative anaerobes, which are bacteria that can still live in low oxygen conditions (3).

Anaerobic bacteria are common cause of infections, some of which are serious and life-threatening. These bacteria are a common cause of endogenous infections because they are a major component of the normal flora of the skin and mucous membranes (4). Infection by anaerobic bacteria occurs when these bacteria are in a place that should be sterile in the body (4, 5).

These bacteria are not easily cultured and isolated and often cannot even be found from infected sites. Delayed or inappropriate treatment of these organisms can lead to failure in eradicating this infection. The isolation of anaerobic bacteria requires adequate methods for specimen management and clinical specimen culture (6). Management of infection is often difficult because of the slow growth of anaerobic organisms. Other factors that can delay the identification of these bacteria are the frequent polymicrobial nature of these infections and increase in their resistance to antibiotics (7).

Metronidazole is an antibiotic that is classified as nitroimidazoles with heterocyclic chemical components, works by stopping the growth of bacteria and protozoa (8). The antibiotic is a low molecular weight compound that diffuses across the cell membrane of anaerobic microorganisms as a produg and is activated in the cytoplasm of bacteria or certain organelles in protozoa. Metronidazole molecules are converted to nitroso free radicals by intracellular reduction, which includes the transfer of electrons to the nitro drug group. The form of the drug becomes cytotoxic and can interact with DNA molecules causing loss of the DNA helix structure and breaking of the DNA strands, resulting in inhibition of DNA synthesis and cell death. This drug is active against bacteria only by anaerobic metabolisms (9). Susceptibility is still very high in *Fusobacterium, Prevotella* and all *Bacteroides* species according to research conducted by previous researchers (10).

Ticarcillin's antibiotic properties arise from its ability to prevent cross-linking of peptidoglycan during cell wall synthesis, when the bacteria try to divide, causing cell death. Ticarcillin, like penicillin, contains a β-lactam ring that can be cleaved by beta-lactamases, resulting in inactivation of the antibiotic and is used in combination with clavulanic as ticarcilin + acid clavunic. This antibiotic is effective against Gram-negative bacteria (11). Cefoxitin's spectrum of *in vitro* antimicrobial activity includes a broad range of Gram-negative and Gram-positive bacteria, including anaerobes and classified as second generation cephalosporins (12).

Chloramphenicol works to inhibit bacterial protein synthesis. The drug easily enters the cells by a facilitated diffusion process. The drug binds reversibly to the 50S ribosome unit, thereby preventing the bonding of an amino acid containing the aminoacyl t-RNA end with one of its binding sites on ribosome. The formation of the peptide bond is inhibited as long as the drug binds to the ribosome. Chloramphenicol can also inhibit mitochondrial protein synthesis in mammalian cells because the mitochondrial ribosomes are similar to bacterial ribosomes (12, 13).

In vivo resistance of Gram-negative bacteria to chloramphenicol is due to presence of special plasmids obtained at conjugation (13). There is a special acetyl transferase which inactivates the drug by using acetyl coenzim A as the acetyl group donor. Loss of sensitivity to chloramphenicol due to enzymatic degradation, can also be caused by decreased permeability of the walls of microorganisms or due to ribosome mutations. Resistance to chloramphenicol is relatively low and is slow compared to tetracycline (14, 15).

Resistance to chloramphenicol among anaerobes, as reported in most antimicrobial susceptibility surveys, remains uncommon probably due to its relatively infrequent use in areas where such surveillance studies have been conducted. Two different classes of chloramphenicol resistance genes have, however, been reported in *Bacteroides* spp. Both result in drug inactivation, either through acetylation or by nitro-reduction at the p-nitro group of the benzene ring. The chloramphenicol acetyltransferase gene is transferable and plasmid-mediated (9).

Currently, metronidazole is the antibiotic of choice for anaerobic infections. This antibiotics show antibacterial activity against all anaerobic cocci and anaerobic Gram-negative bacilli, including various *Bacteroides* species, as well as spore-forming anaerobic Gram-positive bacilli (11), however whether their effectiveness is still valid today is questionable. This study aims to determine the diversity of anaerobic bacteria today and how sensitivity to several antibiotics.

MATERIALS AND METHODS

The study was conducted at the Microbiology Laboratory, specimens were obtained during 2019-2020, patients demographic information was obtained from medical records. Specimens were collected and placed according to the procedure and sent to laboratory under anaerobic condition within 30 minutes.

Specimens derived from body fluids, such as blood and bartholin fluid are inoculated on a BacT/Alert (bioMérieux) bottle and incubated on a BacT/Alert, an automated microbial detection system based on the colorimetric detection of CO_2 produced by growing microorganisms. Results of an evaluation of the media, sensor, detection system and detection algorithm indicate that the system reliably grows and detects a wide variety of bacteria (16). Specimens with a positive signal were cultured according to the standard for anaerobic bacteria as well as other specimens (17).

Specimens other than body fluids, such as tissue and pus transported to the laboratory using amies transport medium (Merck) and then were processed for Gram staining (Becton Dickinson) and anaerobic cultures were done following standard techniques on 5% sheep blood agar, brucella agar and brucella kanamycin agar (Merck) with metronidazole discs (5U) (Becton Dickinson). The remainder of the specimen is inoculated on the Thioglycolate as a backup culture.

The plates were incubated in anaerobic jar equipped with anaerobic gas pack (OXOID), incubated at 35°C in CO_2 incubator (Thermo) and inspected daily for anaerobic growth. Formation of an inhibitory zone on metronidazole indicates the presence of anaerobic bacterial growth. Anaerobic isolates were identified by Gram staining.

The next step is identification using the Vitek 2® automated system (bioMérieux). Using the DensiCHECK™ instrument (bioMérieux), the bacterial isolate was added to 3 ml of NaCl 0,45% pH 5,0 (bioMérieux) and the turbidity was equalized until reached McFarland 2.7-3.3. The tube containing the bacterial suspension was connected through a hose into the ANC card. The card inserted into Vitek 2® incubator (18).

Antibiotic sensitivity examination were carried out using ATB ANA (bioMérieux). The ATB ANA system is a freeze-dried panel with large wells. The ATB ANA strip consists of 16 pairs of cupules. The first pair does not contain any antibiotic and serves as a positive growth control. The next 12 pairs contain antibiotics at two concentrations (corresponding to NCCLS M11-A3 M100-S6 breakpoints): benzylpenicillin, 0.5 and 2 mg/liter; amoxicillin, 2 and 4 mg/liter; co-amoxiclav, 4/2 and 8/4 mg/liter (throughout this paper, for combination drugs the pair x/y refers to the concentrations of the two drugs in the combination); piperacillin, 32 and 64 mg/liter; ticarcillin-clavulanic acid, 32/2 and 64/2 mg/liter; piperacillin-tazobactam, 32/4 and 64/4 mg/liter; cefoxitin, 16 and 32 mg/liter; cefotetan, 16 and 32 mg/liter; imipenem 4 and 8 mg/liter; clindamycin, 2 and 4 mg/liter; chloramphenicol, 8 and 16 mg/liter; and metronidazole, 8 and 16 mg/liter. Three wells, containing amoxicillin at 16 mg/liter, co-amoxiclav at 16/2 mg/liter (a fixed concentration of 2 mg of clavulanic acid per liter in France), and metronidazole at 4 mg/liter, were added when CA-SFM and NCCLS breakpoints for resistance were not identical. We conformed strictly to the recommendations of the manufacturer, as follows. Colonies from brucella blood agar (after 24 to 48 h of growth) were picked up with a swab and introduced into the suspension medium to produce a turbidity to match the McFarland no. 3 standard (9×10^8 CFU/ml). Two hundred microliters of this suspension was introduced into 7 ml of antibiotic S medium, and 135 µl was further delivered with an automatic pipette (bioMérieux) into each well of the ATBTM ANA device. Incubation was carried out in an anaerobic gas pack with CO_2 incubator (Thermo), 24-48 hours. Unless adequate growth is achieved, susceptibility testing cannot be done. The device was read visually by two well-trained technicians as follows: susceptible, no growth; intermediate, growth only at a low concentration; and resistant, growth in both wells of the pair (19, 20). We selected 9 antibiotics to observe in our study, that is antibiotics that can be used for both Gram positive and negative bacteria.

RESULTS

Over a period 2019-2020, a total of 440 specimens were received in microbiology laboratory for anaerobic culture from patients with various infections from 13 hospitals in Jakarta. Significant growth of pathogenic anaerobic bacteria was noted in 40 patients (9.09%) from 5 hospitals in the age range less than 61-70 years, with most ages ranging from 21-30 years. The proportions of gender between men and woman were 21 of 40 (52.5%) and 19 of 40 (47.5%) respectively. Clinical specimens for this anaerobic infection came from blood 22 (55%), tissue 9 (22.5%), pus 8 (20%) and bartholin fluid 1 (2.50%).

Our study succeeded in identifying 18 species on anaerobic bacteria, consisting of 52.5% (21 of 40) Gram-positive and 47.5% (19 of 40) Gram-negative bacteria. The most bacteria found were *Clostridium perfringens* (6 of 40, 15%) from Gram-positive and *Provetella bivia* (4 of 40, 10%) from Gram-negative (Table 1).

The pattern of sensitivity of anaerobic bacteria to several antibiotics, such as piperacillin, piperacillin -tazobactum, ticarcillin-clavulanic acid, cefoxitin, cefotetan, imipenem, clindamycin, chloramphenicol and metronidazole are presented in Table 2. The result indicated that piperacilline - tazobactam 100% effective, while metronidazole as the drug of choice is only 75% effective.

The pattern of anaerobic Gram-positive sensitivity show antibiotics such as piperacilline - tazobactam, ticarcilin + clavunic acid, cefoxitin, cefotetan, imipenem and chloramphenicol are 100% effective, while metronidazole occupies the lowest position (61.90%). However, the pattern of anaerobic Gram-negative sensitivity to show that piperacilline -tazobactam is 100% effective while chloramphenicol is a second place along 94.75%.

DISCUSSION

In our study, we found the majority of infections in the age range of 21-30 years (20%) and male gender predominance was noted (52.5%) in accordance with other studies (21-23). We found anaerobic Gram-positive (52.5%) are the predominantly isolated even this result is consistent with studies conducted by other researchers (22-24). The most common bacteria we found were *Clostridium perfringens* from Gram-positive and *Prevotella bivia* from Gram-negative, these study differed slightly from study of Ananth et al. (2016), where Gram-positive bacteria *Peptostreptococcus anaerobius* as predominant. For Gram negative our result were also different, where the investigators reported *Bacteroides*

Table 1. Distribution of anaerobic bacteria frequency by type of specimen

Isotes	Blood (%)	Tissue (%)	Pus (%)	Bartholin fluid (%)
Anaerobic Gram positive cocci				
Peptoniphilus asaccharolyticus	2 (5)			
Anaerobic Gram positive bacilli				
Atopobium vaginae	4 (10)			
Bividobacterium sp	2 (5)			
Clostridium clostridioforme		1 (2.5)	1 (2.5)	
Clostridium innocuum			1 (2.5)	
Clostridium perfringens	6 (15)			
Propionibacterium acnes	1 (2.5)	1 (2.5)	1 (2.5)	
Propionibacterium granulosum		1 (2.5)		
Anaerobic Gram negative bacilli				
Bacteroides fragilis	1 (2.5)		2 (5)	
Bacteroides stercoris	2 (5)		1 (2.5)	
Bacteroides thetaiotaomicron		1 (2.5)		
Bacteroides vulgaris	1 (2.5)			
Fusobacterium mortiferum	1 (2.5)		1 (2.5)	
Fusobacterium necrophorum		1 (2.5)		
Prevotella melanonogenica	1 (2.5)			
Prevotella bivia	1 (2.5)	2 (5)		1 (2.5)
Prevotella disiens		2 (5)	1 (2.5)	
Prevotella oralis				
Total	22 (55)	9 (22.5)	8 (20)	1 (2.5)

Table 2. The pattern of sensitivity of anaerobic bacteria to several antibiotics

Antibiotics	Gram positive and negative (%)	Gram-positive (%)	Gram-negative (%)
Piperacilline	70	95.23	41.11
Piperacilline - tazobactam	100	100	100
Ticarcilin -clavunic acid	97.5	100	94.74
Cefoxitin	97.5	100	94.74
Cefotetan	90	100	78.95
Imipenem	95	100	89.47
Clindamycin	70	90.48	47.37
Chloramphenicol	97.5	100	94.75
Metronidazole	75	61.90	89.47

spp. as the most frequently isolated microorganisms (25). Garg et al. (2014) in India found *Peptostreptococcus* sp. as the dominant Gram positive bacteria, while Gram negative bacteria were found to be *Prevotella, Veilonella* and *Bacteroides* similar to our study. Akhi et al (2015) from Iran found the same things as our study, *Clostridium perfringens* was the dominant Gram positive bacteria found while *B. fragilis* was dominant Gram negative fragilis (23, 24).

Bacterial growth is in influenced by biotic and abiotic factors. Abiotic factors that affect microbial life are influenced by the surrounding environment such as nutrition, temperature, pH, humidity, osmotic pressure, availability of oxygen and chemical substances. Biotic factors are interactions between bacteria and other organisms in a population, in this case with humans. The entry of bacteria as pathogens is influenced by the degree of virulence of the bacteria, the number and condition of the host's immunity. Thus, bacterial differences in each country may differ from one another because apart from climate and weather factors, the immune condition of the host also play role (26).

The most specimens from our study were blood samples which indicated that these patients had bacteremia. The incidence of anaerobic in bacteremia was various (5%-15%) (27). Generally anaerobic bacteremia patients are immunosuppressed patients with the most common isolate being *Bacteroides fragilis* group (more than 75% anaerobic isolate) (27, 28), which are Gram negative bacteria, consistent with our study where *Prevotella bivia* is the common Gram-negative found followed by *Bacteroides fragilis* and *Bacteroides stercoris.* The types of bacteria involved in bacteremia are strongly influenced by the entrance to the infection and the underlying disease. *Prevotella bivia* usually associated with infections of the female genital tract and can cause preterm premature rupture of membranes. It is occasionally associated with oral infections (29- 31). The most common Gram-positive bacteria found in our study was *Clostridium perfringens*, this bacteria show evidence of tissue necrosis, bacteremia, emphysematous cholecystitis and gas gangrene. *C. perfringens* can participate in polymicrobial anaerobic infections (31, 32).

Our study shows piperacilline-tazobactam is 100% effective against anaerobic bacterial isolates, this is in accordance with studies conducted by other researchers who state that piperacillin -tazobactam are β-lactam antibiotics with a broad spectrum of activity used for the treatment of mixed infections in which anaerobic and aerobic bacteria (22). Other antibiotics such as ticarcilin-clavunic acid, cefoxitin, cefotetan, imipemem and chloramphenicol achieved sensitivity above 90% , while metronidazole was 75% and the lowest was piperacilline and clindamycin (70%) respectively. The result were similar to Garg et al. (2015), where all antibiotics examined 100% sensitive but metronidazole was only 84% showed effective (23).

In Gram-positive, almost all antibiotics show sensitivity (above 90%), except Metronidazole (61.90%). This is an important note because as is well known, metronidazole is used as first-line therapy for various diseases caused by *Clostridium difficile* (33). Our study is similar to Akhi et al. (2015) where Cefoxitin reached 100% sensitivity, but differed from clindamycin and metronidazole which reached 100% sensitivity (24).

Resistance of anaerobic Gram-positive cocci is rare, and resistance of non sporulating bacilli is common. Microaerophilic streptococci, *P. acnes*, and *Actinomyces* spp. are almost uniformly resistant. Aerobic and facultative anaerobes, such as coliforms, are usually highly resistant. Over 90% of obligate anaerobes are susceptible to less than 2 µg/ml metronidazole (26).

In anaerobic Gram-negative bacteria other than piperacilline-tazobactam, metronidazole, ticarcilin - clavunic acid, cefoxitin and chloramphenicol play a very good role (above 90%). Metronidazole seems to compete with imipenem (89.47%). Meanwhile, susceptibility to cefotetan was 78.95%, but the rate of susceptibility to clindamycin and piperacilline were 47.37% and 41.11% respectively. In contrast to our study, other investigators found low rates of sensitivity to cefoxitin, chloramphenicol and metronidazole, while clindamycin was similar to our research (22, 24).

Although rare, resistance to metronidazole among *B. fragilis* group isolates has been observed worldwide (28, 29). Resistant *B. fragilis* group isolates carry one of nine known *nim* genes (nimA-I) on either the chromosome or a mobilizable plasmid that seems to encode a nitroimidazole reductase, which converts 4- or 5-Ni to 4- or 5-aminoimidazole, preventing the formation of toxic nitroso residues necessary for the agent's activity. These *nim* genes were found in 50/206 (24%) *Bacteroides* species isolates and resulted in MICs of 1.5 to 256 g/ml for metronidazole, including 16 isolates with MICs of 32 g/ml (15). These findings suggested incomplete mobilization of *nim* gene-associated resistance. Mechanisms of resistance can occur and that prolonged exposure to metronidazole may select them. The mechanism of metronidazole resistance for non-*Bacteroides* anaerobes is unknown (9, 15).

Anaerobic infection is not only associated with single pathogen. This occurs due to inaccurate identification and susceptibility testing as well as polymicrobial nature of anaerobic bacteria itself. Clinical failure has been shown to be closely related to antibiotic resistance in anaerobic bacteria (6, 17). Metronidazole resistance was considered to be a major factor in treatment failure. The high frequency use of this antibiotic causes resistance not to certain bacteria but because of its well-known safety and efficacy in clinical practice, metronidazole is still the main basis for the management of anaerobic of anaerobic infections worldwide.

CONCLUSION

Eighteen species from a total of 440 specimens were identified. *Clostridium perfringens* and *Prevotella bivia* are the most common bacteria found. The sensitivity examination to several antibiotics demonstrated piperacillin - tazobactam as 100% effective compared to other antibiotics. Against Gram-positive bacteria, almost all antibiotics are 100% effective except metronidazole (61.9%) however against Gram-negative, metronidazole and imipenem both were effective against 89.47% of isolates, this is contrast to piperacillin that showed lowest activity against Gram-negative organism in this study.

REFERENCES

1. Leber AL (2016). Clinical microbiology procedures handbook. 4th ed. ASM press. Washington DC.
2. Begum S, Roy S, Yusuf MA. Anaerobic bacteria: infection and management. *IOSR J Dent Med Sci* 2015; 14: 69-72.
3. Shenoy PA, Vishwanath S, Gawda A, Shetty S, Anegundi R, Varma M, et al. Anaerobic bacteria in clinical specimens - frequent, but a neglected lot: a five year experience at a tertiary care hospital. *J Clin Diagn Res* 2017; 11: DC44-DC48.
4. Brook I (2007). Anaerobic infections: diagnosis and management (infectious disease and therapy). 1st ed. CRC Press. United States.
5. Brook I. Spectrum and treatment of anaerobic infections. *J Infect Chemother* 2016; 22: 1-13.
6. Nagy E. Anaerobic infections: update on treatment considerations. *Drugs* 2010; 70: 841-858.
7. Seiler JS, Herold RW. The use of systemic antibiotics in the treatment of aggressive periodontal disease. *Gen Dent* 2005; 53: 155-159; quiz 160, 145-146.
8. Gajdács M, Spengler G, Urbán E. Identification and antimicrobial susceptibility testing of anaerobic bacteria: Rubik's cube of clinical microbiology? *Antibiotics (Basel)* 2017; 6: 25.
9. Schaumann R, Petzold S, Fille M, Rodloff AC. Inducible metronidazole resistance in nim-positive and nim-negative *Bacteroides fragilis* group strains after several passages metronidazole containing columbia agar plates. *Infection* 2005; 33: 368-372.
10. Jenkins SG. Infections due to anaerobic bacteria and the role of antimicrobial susceptibility testing of anaerobes. *Rev Res Med Microbiol* 2001; 12: 1-12.
11. Schuetz AN. Antimicrobial resistance and susceptibil-

ity testing of anaerobic bacteria. *Clin Infect Dis* 2014; 59: 698-705.
12. Brook I, Wexler HM, Goldstein EJ. Antianaerobic antimicrobials: spectrum and susceptibility testing. *Clin Microbiol Rev* 2013; 26: 526–546.
13. Liu CY, Huang YT, Liao CH, Yen LC, Lin HY, Hsueh PR. Increasing trends in antimicrobial resistance among clinically important anaerobes and *Bacteroides fragilis* isolates causing nosocomial infections: emerging resistance to carbapenems. *Antimicrob Agents Chemother* 2008; 52: 3161-3168.
14. Katsandri A, Papaparaskevas J, Pantazatou A, Petrikkos GL, Thomopoulos G, Houhoula DP, et al. Two cases of infections due to multidrug-resistant *Bacteroides fragilis* group strains. *J Clin Microbiol* 2006; 44: 3465-3467.
15. Gal M, Brazier JS. Metronidazole resistance in *Bacteroides* spp. carrying nim genes and the selection of slow-growing metronidazole-resistant mutants. J *Antimicrob Chemother* 2004; 54: 109-116.
16. Li Z, Liu S, Chen H, Zhang X, Ling Y, Zhang N, et al. Comparative evaluation of BACTEC FX, BacT/ALERT 3D, and BacT/ALERT VIRTUO-automated blood culture systems using simulated blood cultures. *Acta Clin 2*022;77:71-78.
17. Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis* 2010; 50 Suppl 1: S16-23.
18. Microbiology with confidence VITEK 2 results you can trust. Available from: www.biomerieux-usa.com
19. Microbiology with confidence ATB ANA. Available from: www.biomerieux.com
20. Cherkaoui A, Fischer A, Azam N, Riat A, Schrenzel J. A comparison of sensititreTM anaerobe MIC plate with ATB ANA® test for the routine susceptibility testing of common anaerobe pathogens. *Eur J clin Microbiol Infect Dis* 2018; 37: 2279-2284.
21. Jamal W, Al Hashem G, Rotimi VO. Antimicrobial resistance among anaerobes isolated from clinical specimens in Kuwait hospitals: comparative analysis of 11-year data. *Anaerobe* 2015; 31: 25-30.
22. De A, Gogate A. Prevalence of gram negative anaerobic bacilli in routine clinical specimens. *Indian J Pathol Microbiol* 2001; 44: 435-438.
23. Garg R, Kaistha N, Gupta V, Chander J. Isolation, identification and antimicrobial susceptibility of anaerobic bacteria: a study re-emphasizing its role. *J Clin Diagn Res* 2014; 8: DL01-2.
24. Akhi MT, Ghotaslou R, Beheshtirouy S, Asgharzadeh M, Pirzadeh T, Asghari B, 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.
25. Ananth-Shenoy P, Vishwanath S, Targain R, Shetty S, Sunil-Rodrigues G, Mukhopadhyay C, et al. Anaerobic infections in surgical wards: a two year study. *Iran J Microbiol* 2016; 8: 181-186.
26. Aldridge KE, Ashcraft D, Cambre K, Pierson CL, Jenkins SG, Rosenblatt JE. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella, Fusobacterium, Porphyromonas,* and *Peptostreptococcus* species. *Antimicrob Agents Chemother* 2001; 45: 1238-1243.
27. Brook I. The role of anaerobic bacteria in bacteremia. *Anaerobe* 2010; 16: 183-189.
28. Nguyen MH, Yu VL, Morris AJ, McDermott L, Wagener MW, Harrell L, et al. Antimicrobial resistance and clinical outcome of *Bacteroides bacteremia*: findings of a multicenter prospective observational trial. *Clin Infect Dis* 2000; 30: 870-876.
29. Lepivert JC, Guinet V, Auquit-Auckbur I. Necrotizing fasciitis of the hand and wrist due to *Prevotella bivia*. *J Hand Surg Eur Vol* 2015; 40: 757-758.
30. Knoester M, Lashley LE, Wessels E, Oepkes D, Kuijper EJ. First report of *Atopobium vaginae* bacteremia with fetal loss after chorionic villus sampling. *J Clin Microbiol* 2011; 49: 1684-1686.
31. Valéria Dos Santos K, Diniz CG, Coutinho SC, Apolônio ACM, Geralda de Sousa-Gaia L, Nicoli JR, et al. *In vitro* activity of piperacillin/tazobactam and ertapenem against *Bacteroides fragilis* and *Escherichia coli* in pure and mixed cultures. *J Med Microbiol* 2007; 56: 798-802.
32. Brook I. Microbiology and management of joint and bone infections due to anaerobic bacteria. *J Orthop Sci* 2008; 13: 160-169.
33. Keating GM, Perry CM. Ertapenem: a review of its use in the treatment of bacterial infections. *Drugs* 2005; 65: 2151-2178.