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**RESEARCH ARTICLE**

# Identification and detection of antibiotic susceptibility of the most common anaerobes causing infection in Surgical hospital, faculty of Medicine Zagazig University, Egypt.

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## *Abstract*

**Objectives**:

Anaerobic infections are considered to be the most difficult organisms to be identified in the microbiology laboratory. It requires strict conditions, proper sampling, long time and laboratory skills. In addition most of them are mixed infections having both aerobic and anaerobic organisms. Choice of the proper antibiotic for treating these anaerobes is live saving for the patient.

**Methods**:

Identification of anaerobic organisms using MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) as a recent tool for identification together with API 20A (Analytical Profile Index) (as a reference method). Antibiotic susceptibility test was done for the anaerobic isolates using Agar Dilution Method. With the most commonly used antibiotics in our hospital, which are Amoxacillin/Clavulonic acid, clindamycin, metronidazole and Imipenem.

**Results**:

Anaerobic infections constitutes 21.7% of total 249 specimen from different surgical departments. Bacteroids spp. (41%) was the most prevalent anaerobic organism followed by peptostreptococcus (26.9%). MALDI TOF MS system and API achieved 100% agreement for identification of porphoryomonas spp. and fusobacterium, while near results were obtained for other isolates. Bacteroid spp. shows the highest rate of resistance to clindamycin (69%). Excellent results were obtained for Imipenem and metronidazole. Most of resistance to Amoxacillin/Clavulonic acid is related to Bacteroid spp. and fusobacterium spp.

**Conclusions**:

MALDI TOF MS System is a useful tool for identification of Anaerobes. Higher rates of resistance are recorded by anaerobic organisms to commonly used antibiotics thus detection of resistant strains is vital for proper selection of antibiotics.



## INTRODUCTION

Anaerobes are common cause of bacterial infections. Anaerobic bacteria are very sensitive organisms that require special methods for collection, transportation and cultivation. As a result, most of anaerobic infections are not properly diagnosed [1]

Treatment of anaerobic infections is a major concern, not only because they are usually overlooked during diagnosis, but also due to the progressively rising resistance rates among anaerobic genera [**2].** Continuous surveillance of anaerobic sensitivity is thus essential to detect changes in susceptibility patterns **[3][4].**

Because laboratory diagnosis of anaerobes require special techniques, extensive experience, and they consume much time and expenses, so there is always a search for newer diagnostic options [5]**.** Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry **(MALDI-TOF MS)** is a rapid and inexpensive technology used nowadays for identification for most bacterial strains [6]**.**

This study aimed to identify the most common anaerobic organisms that cause infection in the surgical hospital, Zagazig University, Egypt. Comparison between MALDI-TOF MS and API 20A techniques that are used for routine anaerobes identification and to detect the antibiotic sensitivity patterns for the isolated organisms using the standard agar dilution method.

## Methodology:

Samples were taken from 249different lesions suspected to have anaerobic infections. Consents from all patients were obtained prior to sampling. Laboratory investigations were carried out in the Clinical Pathology Department in Surgical Hospital, Zagazig University in the period between December, 2013 and March, 2015.

**Inclusion criteria**: Patient admitted to surgical hospital, Zagazig University with infection that clinically suggested anaerobic infection like: deep infection, bad odor, foul Discharge and crepitation. The quality of the obtained sample was assessed according to the Algorithms for Wound Specimens and Q score described by **[7].**

**Exclusion criteria:** Lesions that don't show previous manifestations of anaerobic infections and failure to obtain proper consent.

Specimens were colleted as described by Sinha et al., 2007 **[8]**. Briefly, for **diabetic foot infection:** After full laboratory investigation**,** X- ray on the foot was done to check presence or absence of osteomyelitis. All procedures were done in the operating room, under complete aseptic condition. Cases are insensate due to peripheral neuropathy so only sedatives were given. Samples included purulent discharge, necrotic infected tissues and infected bone parts. **Appendicular abscess:** During exploration of abdomen and under general anesthesia, aspiration of peritoneal fluid in sterile syringe was done before any surgical steps were done. **Psoas abscesses:** under local anesthesia and complete aseptic conditions, ultrasound guided aspiration of pus in sterile syringe followed by either ultrasound guided drainage or open surgical drainage was done. **Surgical sites infection (SSIs):** the area was wiped with sterile saline then 70% alcohol. From the wound site, material from the wound was collected by aspiration and nectrotic tissue was excised **[8].**

Specimens were transported to the lab within 2 hours. Tissue specimens were homogenized using vortexer Bead Beating. Grinding stainless steel beads (>2 mm) were added to the sample to disturb the tissue, and then was repeatedly vortexed. To overcome excessive heat produced, the vortexer was interspersed with cooling on ice **[9].**

All samples were examined by Gram stain, cultures were done on non-selective blood agar for aerobic ulture and on neomycin blood agar for anaerobic culture. Test. A single colony of each morphotype was examined microscopically using Gram stain and was subcultured for aerotolerance testing on chocolate agar, which was incubated in carbon dioxide and anaerobic blood agar was incubated anaerobically **[10]**. Bacteroids fragilis ATCC 25285 for gram negative anaerobes and Eubacterium lentum ATCC43055 for gram positive anaerobic bacteria were included as control strains.

### MALDI-TOF MS identification:

***Samples Preparation:***

A portion of a single colony was applied directly to a disposable target slide (product no. 11111149BM; bioMérieux, Marcy l'Etoile, France) composed of a polypropylene carrier with a stainless steel layer, using a 1-l loop and was lysed by direct application. One ùl of matrix solution (3.1% [wt/vol] _-cyano-4- hydroxycinnamic acid, product no. 1002317170; bioMérieux) was applied and allowed to dry at room temperature prior to mass spectrometric analysis.

Isolates were prepared for mass spectrometric analysis at the Vitek MS preparation station, and the isolate information was transferred to the Vitek MS acquisition station using Myla v2.4 middleware. The total sample preparation time was approximately 1 min per isolate.

Samples were then analyzed using the Vitek MS MALDI-TOF mass spectrometer in linear positive-ion mode, across the mass-to-charge ratio range of 2,000 to 20,000 Da. Each spot was irradiated with 500 laser shots at 50 Hz. Target plates were calibrated and quality controlled both before and after data acquisition by using Escherichia coli ATCC8739. A sample containing matrix only (negative control) were assayed for quality control purposes.

After the acquisition of spectra, data were transferred from the Vitek MS acquisition to the Vitek MS analysis server and identification results were displayed using Myla v2.4 middleware. The total processing and data analysis time was approximately 20 min for a single isolate.

***Data Anaysis***: The Vitek MS identification system is based on comparison of the characteristics of the spectra obtained with the Vitek MS v2.0 database. This database was built using spectra for known strains for each claimed species. Based on this representative data collection, a weight is assigned to each peak for each species according to its specificity. A single identification is displayed with a confidence value from 60.0 to 99.9.

**API 20A:** Identification of microorganisms was done according to the manufacture protocol *(BioMerieux SA, France)*. Results of MALDI-TOF MS and API were categorized as: 1) identical identification to the species level or identical identification to the genus level (if either or both techniques identified to the genus level only), 2) discrepant results, 3) unreliable.

***Antibiotic sensitivity testing:*** We selected the four most commonly used antimicrobials to treat clinically suspected anaerobic infections in our hospital. These antibiotics were Amxacillin/Clavulonic acid, Clindamycin, Mitronidazole and Imipenem.

**The Agar dilution Method**

The method was done according to the Clinical Laboratory Standard Institute (CLSI) recommendation for testing anaerobic bacteria. For the antibiotic sensitivity discs, Brucella agar *(Difco, Becton Dickinson, Sparks MD21152, USA)* supplemented with 5% lysed sheep blood, 5 mg/L haemin and 1 mg/L vitamin K was used. Briefly, appropriate dilutions of antimicrobial solutions were added to Brucella blood agar that had been allowed to equilibrate in a water bath to 50–55°C. The agar and antibiotic solution were mixed thoroughly, and the mixture was poured into Petri dishes on a level surface to result in an agar depth of 3–4 mm. Each bacterial culture was adjusted to a turbidity equivalent to that of a 0.5 McFarland standard (~1–9×$10^8$ CFU/mL for most species) and was then diluted 1:10 in sterile Mueller–Hinton broth. A 5 µL aliquot of each diluted bacterial suspension containing ~$10^4$ CFU was spotted onto the agar surface using an automatic pipette within 15 min of preparation. All plates were incubated in an anaerobic jar for 48 h. MIC for all isolates was interpreted using the The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI break points **[11]**.

## Results

According to MALDI-TOF results, out of 249 lesions 50 (20.1%) were sterile, 145 (58.2%) showed growth of aerobic organisms only, and only 54 (21.7%) revealed anaerobic organisms upon culturing. Those 54 lesions were distributed as follows: 27 from diabetic foot (that represents 18% of all diabetic foot lesions), 14 surgical wound aspirates (that represents 25% of all surgical wound aspirates), 8 appendicular abscess (26.6% of all appendicular abscess aspirates) and 5 psoas abcess aspirates (38.4% of all psoas abscess aspirates).

The polymicrobial nature of anaerobic infection was greatest in psoas abscess aspirates as ratio of isolates number to the cases was (1.6), followed by diabetic foot (1.5) and surgical wound aspirates which was (1.4), lastly appendicular abscess aspirates (1.25). Four different anaerobic genera were cultured from different clinical samples. The most common anaerobic isolates were *Bacteroides spp.* 32 *(41%)* and *Peptostreptococcus* spp. 21 *(26.9%)*. All genera and species identified by MALDI-TOF have confidence level 85–90% **(Table 1).**

Comparison between API 20 and MALDI-TOF for identification of different anaerobic genera revealed 100% agreement in identification of *Porphoryomonas spp* and Fusobacterium spp. However, It was *98%* for *Bacteroid ssp, 94%* for *Peptostreptococcus*, and only *79%* for *Prevotella* **(Table 2)**.

**Table (1) Anaerobes distribution among the different surgical infections categories**

| | Surgical infection categories | | | | |
|---|---|---|---|---|---|
| | Diabtic foot | Surgical sites | Appendicular abcess | Psoas Abcess | Total |
| ***Bacteroides spp. NO. (%)*** | 12 (30%) | 8 (40%) | 7 (70%) | 5 (62.5%) | 32 (41%) |
| *B. fragilis* | 8 | 8 | 6 | 5 | |
| *B.thetaiotaomicron* | 3 | 0 | 0 | 0 | |
| *B.vulgatus* | 1 | 0 | 1 | 0 | |
| ***Peptostreptococcus spp. NO. (%)*** | 18 (45%) | 0 | 0 | 3 (37.5%) | 21 (27%) |
| *P.asaccharolyticus* | 16 | | | 2 | |
| *P. anaerobius* | 2 | | | 1 | |
| ***Porphorymonas spp. NO. (%)*** | 2 (5%) | 7 (35%) | 3 (30%) | 0 | 12 (15.4%) |
| *P. asaccharolytica* | 2 | 5 | 3 | | |
| *P. uneonis* | 0 | 2 | 0 | | |
| ***Prevotella spp. NO. (%)*** | 5 (12.5%) | 4 (20%) | 0 | 0 | 9 (11.5%) |
| *P.melaninogenica* | 4 | 4 | | | |
| *P. bivia* | 1 | 0 | | | |
| ***Fusobactrium . spp. NO. (%)*** | 3 (7.5%) | 1 ( 5%) | 0 | 0 | 4 (5%) |
| **F.Nucleatum** | 3 | 1 | | | |
| Total | 40 | 20 | 10 | 8 | 78 |

**Table (2) Comparison between results of API 20 and MALD-TOF in identification of anaerobic isolates**

| Anaerobic organisms | Identified by API 20 | Identified by MALDI TOF | Kappa (P-value) |
|---|---|---|---|
| ***Bacteroides spp.*** | 31 | 32 | 0.98 *(<0.001)* |
| ***Peptostreptococcus spp.*** | 19 | 21 | 0.94 *(<0.001)* |
| ***porphorymonas spp.*** | 12 | 12 | 1.0 *(<0.001)* |
| ***Prevotella spp.*** | 6 | 9 | 0.79 *(<0.001)* |
| ***Fusoacterium spp.*** | 4 | 4 | 1.0 *(<0.001)* |

The antibiotic susceptibility pattern and antibiotics MICs of the anaerobes were shown in **(Tables 3 and 4 respectively)**. Bacteroide spp. was most sensitive to metronidazole *(94%)*. *Peptostreptoccus* spp. were *(100%)* sensitive to imipenium and amoxicillin-clavulonic acid. The most effective antibiotics for *Porphoryomonas spp* were imipenen and amoxicillin-clavulonic acid *(100%)*. *Prevotella spp*.were most sensitive *(100%)* to metronidazole and amoxicillin-clavulonic acid. Imipenium and metronidazole were *(100%)* active against *Fusobacterium spp***. (Table 3).**

Antibiotic susceptibilities for metronidazole and imipenem were the highest among all antibiotics *(94.9%)* and *(93.6%)* respectively. However, only 45 *(57.7%)* isolates were susceptible to clindamycin with Bacteroids non-fragilis showing the highest resistance (four out of five). 70 *(89.7%)* isolates were susceptible to amoxicillin-clavulonic acid **(Table 3)**

The MICs of tested antibiotics are listed in **(Table 4)**. MICs 50 and MIC 90 were determined for all strains. MICs 50/ MIC 90 of clindamycin were the highest, as MIC90 of bacteroids, prevotella and fusibacterium exceeds 256 ug/ml . Bacteroids showed high level of resistance against both amoxicillin/clavulonic acid and clindamycin. Metronidazole is the most active antibiotic MIC 90 didn't exceed 2ug/ml for any strain.

**Table 3: Susceptibility pattern of anaerobic isolates from different surgical infection categories**

| | Amoxacillin /clavulonic acid | | | Clindamycin | | | Metronidazole | | | Imipenem | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R | S | I | R |
| ***Bacteroides spp NO.* %)** | 25 (78) | 2 (6) | 5 (16) | 10 (31) | 0 | 22 (69) | 30 (94) | 0 | 2 (6) | 29 (90.6) | 0 | 3 (9.3) |
| *Bacteroid fragilis* | 20 | 2 | 5 | 9 | | 18 | 25 | | 2 | 24 | | 3 |
| *Non-Bacteroid Fragilis* | 5 | 0 | 0 | 1 | | 4 | 5 | | 0 | 5 | | 0 |
| ***Peptostreptococcus spp* NO. (%)** | 21 (100) | 0 | 0 | 18 (86) | 0 | 3 (14) | 21 (100) | 0 | 0 | 21 (100) | 0 | 0 |
| ***Porphorymonas spp.* NO. (%)** | 12 (100) | 0 | 0 | 9 (75) | 0 | 3 (25) | 10 (83) | 0 | 2 (17) | 12 (100) | | 0 |
| ***Prevotella spp* NO. (%)** | 9 (100) | 0 | 0 | 6 (67) | 0 | 3 (33) | 9 (100) | 0 | 0 | 7 (78) | 0 | 2 (22) |
| ***Fusobactrium spp* NO. (%)** | 3 (83) | 0 | 1 (17) | 2 (50) | 0 | 2 (50) | 4 (100) | 0 | 0 | 4 (100) | 0 | 0 |
| Total NO. (%) | 70 (89.7) | 2 (2.5) | 6 (7.7) | 45 (57.7) | 0 | 33 (42.3) | 74 (94.9) | 0 | 4 (5.1) | 73 (93.6) | 0 | 5 (6.4) |

**Table 4: MICs levels of different antibiotics tested on anaerobic isolate**

| Organism / Antibiotics | Amoxacillin clavulonic acid MIC range ( MIC50/ $MIC_{90}$) µg/ml | Clindamycin MIC range (MIC50/ $MIC_{90}$) µg/ml | Metronidazol MIC range (MIC50/ $MIC_{90}$) µg/ml | Imipenem MIC range (MIC50/ $MIC_{90}$) µg/ml |
|---|---|---|---|---|
| ***Bacteroides spp*** | | | | |
| *Bacteroid fragilis* | <0.06->256 (0.5/ 32) | <0.06->256 (>256/>256) | 0.25-16 (0.5/1) | 0.125- 16 (0.125/2) |
| | 0.25-2 (<0.06/0.5) | | 0. 5- 16 (<0.06/1) | |
| *Non-Bacteroid Fragilis* | | 0.06- >256 (>256/>256) | | 0.125- 16 (0.5/2) |
| ***Peptostreptococcus spp.*** | <0.06 -4 (0.125/2) | 0.06-8 (0.25/16) | 0.25-4 (0.5/1) | 0.06-2 (<0.06/0.5) |
| ***Porphorymonas spp.*** | <0.06 - 4 (0.125/1) | 0.06-8 (1/8) | 0.06-4 (0.125/2) | 0.06-2 (0.125/0.5) |

| | | | | |
|---|---|---|---|---|
| ***Prevotella spp*** | <0.06-2 (<0.06/1) | 0.25- >256 (1/ >256) | <0.06-2 (.125/0.5) | <0.06->256 (0.25/>32) |
| F.Nucleatum *Spp* | <0.06->32 (0.125/>32) | 0.5->256 (1/>256) | <0.06->2 (0.5/1) | <0.06- 2 (0.06/0.5) |

## Discussion:

Anaerobic bacteria are part of the human flora; however, they can cause variety of life threatening infections. Culture and identification of anaerobes in the microbiology laboratory is difficult and require strict conditions , long time, and laboratory skills for isolation. Also, traditional methods do not always capable of differentiation between closely related species **[12].** The alternative recent techniques as Mass Spectrometry and molecular techniques such as real-time polymerase chain reaction , sequencing and microarrays provide faster and accurate diagnostic tools. However, Molecular techniques are not applied as a routine tool as they are expensive, and need technical expertise **[13].**

Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a useful tool for identification of different micro-organisms including anaerobes **[12].**

The identification of anaerobes by MALDI-TOF MS offers several advantages in comparison with the conventional routine method. Most importantly, reducing the period required to identify an organism from days to few minutes that will improve the patiet clinical outcome **[14]**. Also, it has a great significance in identification of biochemically inert, fastidious and slow growing anaerobic cocci **[15].**

The result of our work demonstrated that infections caused by anaerobic bacteria constitutes 21.7% of different surgical infection categories in surgical department, and 52% of these anaerobic infections were caused by mixed aerobic and anaerobic bacteria. This high frequency of mixed aerobic and anaerobic infection is explained by symbiotic relationship with aerobic or facultative bacteria as these species may consume oxygen to the level that allow the anaerobes to survive and exert their virulence to cause anaerobic infection **[16].**

Our study revealed that MALDI-TOF diagnosis of different surgical specimens identified ten species within five genera. This is nearly the same result obtained by study of **Jamal et al., 2013 [17],** who identified fourteen species within five genera of anaerobic clinical isolates. In this study, *Bacteroides spp*. was the most frequent species (41%) isolated from different surgical infection. This result was also demonstrated by studies performed by **Knoester and his colleagues ,2012 [18]** and **Jamal etal., 2013 [17]** results, which revealed that *Bacteroides* species constitutes more than one-third of the isolates that were identified by MALDI-TOF MS.

The second prevalent genus isolated by our work was *peptostreptococcus spp* (26.9%). However, **Knoester and his colleagues ,2012 [18]** demonstrated that *Propionibacterium* (15%), *Prevotella* (13%) are the second frequently isolated genera. In contrast, the study of (12)Scola et al., 2011demonstrated that the most common anaerobes were *Propionibacterium spp*. (12%), followed by *Fusobacterium spp*. (6%) and *Bacteroides spp.* These difference in anaerobic species may be due to different categories of anaerobic infections in these different studies and different bacterial flora that cause these infections in the case of presence of risk factors,

There are several predisposing factors that favour anaerobic bacterial infection in diabetic patients as metabolic and physiological disturbance, vascular occlusive disease and peripheral neuropathy **[19].** In addition, immune deficient mechanisms as defective leukocyte chemotaxis, phagocytosis, and intracellular killing are important risk factors **[20]**.

In agreement with the study done by **El-Tahawy, 2000 [20]**, The diabetic anaerobic infection was polymicrobial as 40 bacterial isolates were cultured from 27 cases resulting in an average of 1.5 organisms per lesion. In our study, anaerobic isolates in diabetic foot constitutes 18 % of the diabetic foot infections. However, **Ng et al., 2008 [21]** isolation rate of anaerobes was 79% which is far more than that of the present study . Also, **Edmiston et al., 2002 [19]** *concluded that a*naerobic bacteria were recovered from 87% of diabetic foot infections. This finding differences most probably due to different sampling methods, type of transport media, different transportation time of samples.

The anaerobic genera isolated by our work from diabetic foot infections are in line with other studies done by **Ng et al., 2008 [21] and Lipsky 1997 [22],** which demonstrated that *peptostreptococci* was the predominant isolates. However, **El-Tahawy, 2000 [20]** found that *Bacteroides fragilisn were respon*sible for 92% of anaerobic diabetic foot infections. In contrast, **Edmiston et al., 2002 [19]** found that *Bacteroides* and *Peptostreptococcus* representing the predominant anaerobic isolates. This discrepent frequency of anaerobic species isolation could be due to different ranges of diabetic soft-tissue infections from mild ulcer and cellulitis to chronic osteomyelitis .

Surgical site infections (SSIs) infection is the infection of skin or/and soft tissues at the surgical incision site that occurs within 30 days after the operation **[23].** Surgical infections are the third frequent nosocomial infections reported and responsible for a quarter of all nosocomial infections **[24].**

In the present study, positive culture fot anerobes in patients with surgical site infection was (25%) higher than that obtained by studies of **Rao et al., 2013 [24]** and **Reddy, 2012 [25],** which found that anaerobic infection of SSI was rare (3.4%), While we detected the Polymicrobial nature of these infections in 50% of the cases, **Rao et al., 2013 [24]** found that 35.2% of lesions were polymicrobial n nature.

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The predominant anaerobic bacteria isolated from SSI and in line with study done by **Reddy, 2012 [25]** was *Bacteroides spp.* While **Rao etal., 2013 [24]** result revealed that *Peptostreptococcus* species (2%) was the most frequently isolated species. However,the predominance of bacilli and anaerobes contradicts previous reports that aerobic cocci are the primary contributor to SSI **.** Also, The importance of anaerobes such as *Peptostreptococcus, Prevotella, Finegoldia* and *Peptoniphilus in causing anaerobic* SSI has been reported in other studies **[26][27].** These discrepant result may due to the various bacterial flora responsible for surgical site infections and different categories of surgical wounds that include clean, contaminated and dirty lesions **[25].**

Complicated intra-abdominal infection is a common problem, with appendicitis alone affecting more than 300,000 patients/year and consuming 11 million hospital days **[28].** In our study, 26.6% of appendicular abcesses cases were due to anaerobic infection. In association with the result obtained by study of Solomkin et al., 2010 the major pathogen isolated by our work from appendicular abcess cases was *Bacteroides spp*. (70%), followed by *porphorymonas spp.* (30%).

In our study, anaerobic infection was demonstrated in 38.4% of the patients with psoas abcess and *Bacteroides spp*. was the most frequently isolated pathogen as it is responsible for 62.5% of these infection, followd by *peptostreptococcus spp*. (37.5%). However, **Adelekan etal., 2004(29)** found that *clostridium difficil* was the most commom anaerobic pathogen isolated from psoas abcess cases. This means that bacterial flora are responsible for theses two types of infection in this study.

In agreement with result obtained by studies of **Knoester etal., 2012 [18], Jamal etal., 2013 [17], and Veloo et al. (2011) [30]**, we demonstrated that all isolates (100% ) could be identified to the species level with MALDI-TOF MS system. In addition, **Garner etal., 2014 [31]** study revealed that Vitek MS provided correct identification for 92% isolates to species level, 94% isolates to the genus level. However, **Justesen et al. (2011) [32],** found that the species level identification with the MALDI-TOF MS system was 43.8–49% .
However, **Li et al., 2014 [33]** and **Scola et al., 2011 [12]** found that MALDI-TOF MS system was effective for certain common species or genera, with 100% identification level for *Bacteroides fragilis* and 80% for *Prevotella spp* but identification levels were above 50% for *Propionibacterium spp*., and 21.6% for *Fusobacterium spp.* This could be explained by absence of reference spectra of unidentified isolates in the system database **[34].**

The agreement between MALDI-TOF MS system and API in identification varies with different anaerobic genera or species. In this study, both tests achieved 100% agreement (Kappa; 1.0) for identification of *Porphoryomonas spp*. and *Fusobacterium spp.* In addition, the comparison between both tools for identification of *Bacteroides spp*. and *Peptostreptococcus spp*, demonstrated very good agreement (kappa; 0.9). However, the least degree of agreement between both techniques was in identification of *Prevotella spp*. (kappa; 0.7). This finding is in accordance with previous reports of this technique's efficacy in identifying anaerobes which demonstrated that MALDI-TOF MS system is more accurately and quickly than conventional commercial techniques **[35], [36], [14].**

In this study there was a discrepancy between MALDI-TOF MS system and API in identification of 8% of all isolates (33% of *Prevotella spp*., 9.5% of *Peptostreptococcus spp,* and 3% of *Bacteroides spp*) . Also, **Knoester**

**etal., 2012 [18]** demonstrated that the discrepant result was found in 11% of the isolates. The isolates with discrepant results in the previous study were subjected to identification by 16S rRNA gene sequencing, and revealed that MALDI-TOF MS did not result in major errors **[18]**. However, the limitation of our study is the small number of anaerobic genera and species that were isolated and tested from different surgical infections.

The fact that anaerobes are fastidious in nature and thus difficult to be isolated and diagnosed makes them often overlooked. As a result, treatment of anaerobic infections is usually empirical. Although the type of anaerobic bacteria causing certain infection can be suspected, resistance of anaerobes to antibacterial drugs is a continuously growing problem and may even develop, while the patient is receiving therapy **[37]**. Reports around the world are reporting an increase in anaerobes resistance to antimicrobial **[38]**.

MIC distribution of the antimicrobial agents tested is in **Table (4)**, in our hospital, these four drugs are the antibiotics of choice to treat clinically suspected anaerobic infection.

***Susceptibility to Clindamycin:*** Clindamycin was considered the gold standard for anaerobic infection treatment scince 1960, However, resistance to clindamycin has steadily increased among anaerobes in the last 15 years **[39].** According to our result, one third of all the isolates were resistant to clindamycin. *Bacteroid* strains shows the highest rate of resistance (69%) especially *Bacteroid fragilis.* Many multicentric studies have shown that resistance to clindamycin is as high as 44% . While, one third of *Prevotella spp.*in this study were resistant to clindamycine, other studies showed that *Prevotella spp* resistance to clindamycin ranges between (31%-70%) **[40][41]**. In this study, 25% of *Porphorymonas* were resisatnce to clindamycin, compared to 1% only, which was reported in other studies **[42][43]**.

Half of *Fusobacterium isolated* by our work were resistant to clindamycin. However, resistance of *Fusibacterium* to clindamycin has been detected in other places of the world to be in the range of 0-20%, this could be explained by the difference in geographical distribution and pattern of antibiotic usage in our hospital, **[44][45]**. *Peptostreptococcus* species resistance to clindamycin is 14%, near to the resistance of 11% detected in a study in Taiwan Hospital **[3]**.

***Imipenem***: Our results shows that *Peptostreptococcus, Porphoryomonas,* and *Fusibacterium* have excellent sensitivity to imipenem with 100% sensitivity among the isolated strains. These results matches the results of **Al-Jebouri1 and Al-Hadeethy 2014 [46]** in Iraq. About 10% of *Bacteroid* strains, however were resistant to Imipenem. Resisatnce of bacteroids to imipenium has been also reported in earlier works done by **(Hecht, 2004) [39]** and **(liu et al 2008 ) [3].** Resistance of provetella rised to 25% in another study performed by **l-Jebouri1 and Al-Hadeethy 2014 [46].**

***Metronidazole resistance*.** Metronidazole has shown to be an excellent antimicrobial activity among most of anaerobes in our study, this was supported by the study of **Liu et al., 2008 [3]**. However, resistantance of *Bacteroid. fragilis* has been reported in several countries **[47][48][4].**

***Amoxacillin/clavulonic acid:*** Our results show that all *Fusobacterium*, *Porphoyromonas*, *Peptostreptococcus* and *Prevotella* isolates remain sensitive to Amoxacillin/clavulonic acid. However, only 78% of *Bacteroids* are sensitive. In a study done by **Jamal et al., 2015 [49]** showed that the drug gives excellent activity against *Fusobacterium*, *Porphoyromonas,* and *Peptostreptococcus.*

*Bacteroid fragilis* MIC50/MIC90 in this study for amoxicillin/clavulonic acid (0.5/32), clindamycin (>256/>256) and Imipeniem (0.125/2) were higher than that detected in Kuwait (0.75- 8), (4 >256) and (0.125-1) respectively, and these values were much higher than MIC50/MIC 90 for amoxicillin/clavulonic acid (0.016-0.5) and clindamycin (0.016->256) in Netherland**.** While MIC50/MIC90 for metronidazole (0.5/1) were lower than (0.75-2) in Kuwait both values however, are much higher than that in Netherland (0.064-0.75)**.** MIC50/MIC90 for *Non-Bacteroid Fragilis* were characteristically high for clindamycin (>256/>256) indicating higher level of resistance than elsewhere, while values for other drugs were within given ranges **[44] [49]**.

*Peptostreptococcus* showed the best sensitivity profile, MIC50/MIC90 for all drugs has been reserved within acceptable ranges in relation to other studies **[49].**

*Prevotella* in our study showed high level of resistance to clindamycin (1/ >256) and imipenium (0.25/>32). This high resistant level to clindamycin has been detected before, while better sensitivity to imipenim is the same with **[45]**, **[44]**, **[49]**.

**F. Nucleatum** *spp* MIC50/MIC 90 were (0.125/>32) for amoxicillin calvulonic acid, (1/ >256) for clindamycin, (0.5/1) for metronidazole and (0.06/0.5)for imipenim. Our results for *Porphorymonas spp.* MIC50/MIC 90 were (0.125/1) for amoxicillin calvulonic acid, (1/8) for clindamycin, (0.125/2) for metronidazole and (0.125/0.5) for imipenim. Values for **F.Nucleatum** *spp* and *Porphorymonas spp.* were higher than previous studies **[44][49]**.

Analysis of MIC50/MIC 90 values for this study reveals that in general they are much higher than other studies and this can be explained in view of the following: 1) resistance is a continuously growing problem and as more recent studies are introduced, the more incidence of resistance can be detected. 2) Chosen drugs are the most commonly used drugs in the hospitals and high level of resistance is expected to be detected. 3) There is no clear antibiotic policy for the hospital till now and antibiotic are unfortunately misused which lead to existence of highly resistant strains and appearance of severe infection.

We conclude that anaerobes are common causes of infection, unfortunately there is increasing tendency toward developing resistance in many species due to different factors, thus routine testing of antibiotic sensitivity is a must to treat affected patients. We also recommend continuous monitoring for different patterns of resistance in our hospitals and elsewhere.

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