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IDENTIFICATION AND DETECTION OF ANTIBIOTIC SUSCEPTIBILITY OF THE MOST COMMON ANEROBES 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 life 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 (as a reference method). Antibiotic susceptibility test was done for the anaerobic isolates using Agar Dilution Method. With the the most commonly used antibiotic in our hospital which are Amoxicillin/Clavulonic acid, clindamycin, metronidazole and Imipenem.

Results: Anaerobic infections constitutes 21.7% of total 249 specimen from different surgical departments. *Bacteroids spp.* (41%) were the most prevalent anaerobic organisms 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 Amoxicillin/Clavulonic acid is related to *Bacteroid spp.* and *Fusobacterium spp.*

Conclusion: MALDI TOF MS System is a useful tool for identification of. Anaerobes are showing higher rates of resistance to commonly used antibiotics thus detection of resistant strains is vital for proper selection of antibiotics.

Key words: Anaerobes, MALDI TOF System, API 20, Agar Dilution Method, Zagazig.

L'IDENTIFICATION ET LA DETECTION DE SENSIBILITE AUX ANTIBIOTIQUES DES AENEROBIES LES PLUS COURANTES QUI CAUSENT L'INFECTION A L'HOPITAL CHIRURGICAL, FACULTE DE MEDECINE, L'UNIVERSITE DE ZAGAZIG, L'EGYPTE.

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RESUME :

Objectifs : Des infections anaérobies sont considérées d'être les plus difficiles à identifier dans le laboratoire microbiologie. Elle nécessite des conditions strictes, l'échenillage appropriée, un long temps et compétences de laboratoire. En outre, la plupart d'entre eux sont des infections mixtes ayant à la fois les organismes aérobies et anaérobies. Le choix d'antibiotique appropriée pour le traiter ces anaérobies est un choix de sauvetage pour le patient.

Méthodes : L'identification des organismes anaérobies utilisant MALDI TOF (Méthode d'identification de micro-organismes au moyen de la spectrométrie de masse Matrix assisté Laser Désorption Ionisation) comme un outil récent pour l'identification ainsi que API 20 A (en tant qu'une méthode de référence). Le test pour la sensibilité à un antibiotique a été fait pour les isolats anaérobies en utilisant Méthode Agar de dilution. Avec l'antibiotique la

plus couramment utilisée dans notre hôpital qui sont Amoxicilline/ Acide clavulanique, clindamycine, métronidazole et l'imipénème.

Résultats : Les infections anaérobies constituent 21,7% du nombre total de 249 échantillons des départements chirurgicaux différents. *Bacteroides* spp (41%) étaient les organismes anaérobies les plus prévalents suivi par *peptostreptococcus* (26,9%). Le système MALDI TOF MS et API ont été d'accord pour l'identification de *Porphyromonas* spp et *Fusobacterium*, alors que les résultats peu près ont été obtenus pour les autres isolats. *Bacteroides* spp. a démontré le taux le plus élevé de la résistance au clindamycine (69%). Les résultats excellents ont été obtenus pour imipénème et métronidazole. Plus de résistance à Amoxicilline/ acide clavulanique est liée à *Bacteroides* spp. et *Fusobacterium* spp.

Conclusion : Le système MALDI TOF MS est un outil utile pour l'identification d'anaérobies montrant des taux plus élevés de la résistance aux antibiotiques les plus couramment utilisés ainsi la détection de souches résistantes est essentielle pour la sélection d'antibiotiques.

Mots - clés : Anaérobies, Le système MALDI TOF, API 20, Méthode Agar de Dilution, Zagazig.

INTRODUCTION

Anaerobes are considered as common cause of bacterial infections. Anaerobic bacteria is 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 raising resistance rates among anaerobic genera (2). Continued surveillance of anaerobic sensitivity is thus essential to detect changes in susceptibility patterns (3)(4).

Because the laboratory diagnosis of anaerobes requires special techniques, extensive experience, and they consume much time and expenses, 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 surgical hospital, Zagazig University, Egypt, to compare MALDI-TOF MS and API 20A technique that are used for routine anaerobes identification and to detect the antibiotic sensitivity patterns for the isolated organisms using the standard agar dilution method.

MATERIALS AND METHODS

Consent: Consents for all patients were obtained prior to sampling.

Inclusion criteria: Patient admitted to surgical hospital, Zagazig University with an 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 the study of Sharp (1997) (7).

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

Specimen collection

Specimens were collected as described by Sinha, 2007 (8). For **diabetic foot infection:** After full laboratory investigation, X-ray of the foot was done to check the presence or absence of osteomyelitis. All procedures were done in the operating room, under complete aseptic condition. Sedatives were given and samples included purulent discharge, necrotic infected tissues and infected bone parts. **Appendicular abscess:** During exploration of the abdomen and under general anesthesia, aspiration of peritoneal fluid in sterile syringe was done before any surgical steps. **Psoas abscesses:** Under local anesthesia and complete aseptic condition, ultrasound guided aspiration of pus in sterile syringe was performed. **Surgical site infection:** The area was wiped with sterile saline then with 70% alcohol. Material from the wound was collected by aspiration and necrotic tissue were excised (8).

Specimens were transported to the lab within 2 hours. Tissue specimens were homogenized using a vortex 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 specimens were interspersed with cooling on ice (9).

All samples were examined by Gram stain, cultures were done on non-selective blood agar for aerobic culture and on neomycin blood agar for anaerobic culture. A single

colony of each morphotype was examined microscopically by Gram stain preparations, evaluation of enzyme catalase production and aero-tolerant test. Aero-tolerance testing was done on chocolate agar and incubated in carbon dioxide(10).

Bacteroids fragilis ATCC 25285 for gram negative anaerobes and *Eubacterium lentum* ATCC43055 for gram positive bacteria was included as a control strain in each run.

MALDI-TOF MS identification

Samples Preparation

A portion of a single colony was applied directly to a disposable target slide (bioMérieux, Marcy-L'Etoile, France) composed of a polypropylene carrier with a stainless steel layer and was lysed by direct application. One μl of matrix solution (3.1% cyan-4-hydroxycinnamic acid, bioMérieux) was applied and allowed to dry at room temperature prior to mass spectrometric analysis.

Isolates were prepared for mass spectrometry 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) was 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 Analysis

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.

Results of MALDI-TOF MS and API 20A 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.

API 20A: Identification of microorganisms was done according to the manufacture protocol (BioMérieux SA, France).

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 ($\sim 1-9 \times 10^8$ 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 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. MICs for all isolates were 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%) 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 following; 27 from diabetic foot (that represented 18% of all diabetic foot lesions), 14 surgical wound aspirates (that represented 25% of all surgical wound aspirates), 8 appendicular abscess (26.6% of all appendicular abscess aspirates) and 5 psoas abscess aspirates (38.4% of all psoas abscess aspirates). Of the 54 lesions, 28 (52%) showed mixed aerobic anaerobic organisms

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%) as shown in fig.1 and *Peptostreptococcus spp.* 21 (27%). All genera and species were identified by MALDI-TOF with a score of 85-90% (table 1).

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

The antibiotic susceptibility pattern and antibiotics MICs of the anaerobes were

determined as shown in Tables 3 and 4; respectively. *Bacteroides spp.* were the most sensitive to metronidazole (94%). *Peptostreptococcus spp.* were the most sensitive with (100%) sensitivity to imipenem, metronidazole and amoxicillin-clavulonic acid. The most effective antibiotics for *Porphyromonas spp* were imipenem and amoxicillin-clavulonic acid (100%). *Prevotella spp.* was 100% sensitive to metronidazole and amoxicillin-clavulonic acid. However, *Fusobacterium spp.* Showed 100% sensitivity to imipenem and metronidazole (table 3).

Antibiotic sensitivity to 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 *Bacteroides non-fragilis* showing the highest resistance (four out of five). Seventy (89.6%) isolates were susceptible to amoxicillin-clavulonic acid (table 3).

The MICs of tested antibiotics were listed in table 4. MIC₅₀ and MIC₉₀ were determined for all strains. MIC₅₀/ MIC₉₀ of clindamycin were the highest, as clindamycin MIC₉₀ for *bacteroids*, *prevotella* and *fusobacterium* exceeded 256 ug/ml. *Bacteroids* showed high level of resistance against both amoxicillin clavulonic acid and clindamycin. Metronidazole was the most active antibiotic as MIC₉₀ didn't exceed 2ug/ml for any strain.

TABLE 1: ANAEROBES DISTRIBUTION AMONG THE DIFFERENT SURGICAL INFECTIONS CATEGORIES

	Surgical infection categories				Total
	Diabetic foot	Surgical sites infection	Appendicular abscess	Psoas Abscess	
<i>Bacteroides spp.</i> NO. (%)	12 (30%)	8 (40%)	7 (70%)	5 (62.5%)	32 (41%)
<i>B. fragilis</i>	8	8	6	5	
<i>B. thetaiotaomicron</i>	3	0	0	0	
<i>B. vulgatus</i>	1	0	1	0	
<i>Peptostreptococcus spp.</i> NO. (%)	18 (45%)	-	-	3 (37.5%)	21 (27%)
<i>P. asaccharolyticus</i>	16			2	
<i>P. anaerobius</i>	2			1	
<i>Porphyromonas spp.</i> NO. (%)	2 (5%)	7 (35%)	3 (30%)	-	12 (15.5%)
<i>P. asaccharolytica</i>	2	6	3		
<i>P. uneeonis</i>	0	1	0		
<i>Prevotella spp.</i> NO. (%)	5 (12.5%)	4 (20%)	-	-	9 (11.5%)
<i>P. melaninogenica</i>	4	4			
<i>P. bivia</i>	1	0			
<i>Fusobacterium . spp.</i> NO. (%)	3 (7.5%)	1 (5%)	-	-	4 (5%)
<i>F. Nucleatum</i>	3	1			
Total	40	20	10	8	78

TABLE (2) COMPARISON BETWEEN RESULTS OF API 20 AND MALD-TOF IN IDENTIFICATION OF ANAEROBIC ISOLATES

	Identified by API 20 (NO.)	Identified by MALDITOF (NO.)	Kappa (P-value)
<i>Bacteroides spp.</i>	31	32	0.98 (<0.001)
<i>Peptostreptococcus spp.</i>	19	21	0.94(<0.001)
<i>porphyromonas spp.</i>	12	12	1.0(<0.001)
<i>Prevotella spp.</i>	6	9	0.79(<0.001)
<i>Fusoacterium spp.</i>	4	4	1.0(<0.001)

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
<i>Bacteroides spp</i> NO.(%)	25 (78)	2 (6)	5 (16)	10 (31)	-	22 (69)	30 (94)	-	2 (6)	29 (90.6)	-	3 (9.3)
<i>Bacteroid fragilis</i>	20	2	5	9		4	25		2	24		3
<i>Non-Bacteroid Fragilis</i>	5	0	0	1			5		0	5		0
<i>Peptostreptococcus spp</i> NO. (%)	21 (100)	-	-	18 (86)	-	3 (14)	21 (100)	-	-	21 (100)	-	-
<i>Porphyromonas spp.</i> NO. (%)	12 (100)	-	-	9 (75)	-	3 (25)	10 (83)	-	2 (17)	12 (100)		-
<i>Prevotella spp</i> NO. (%)	9 (100)	-	-	6 (67)	-	3 (33)	9 (100)	-	-	7 (78)	-	2 (22)
<i>Fusobacterium spp</i> NO. (%)	3 (75)	-	1 (25)	2 (50)	-	2 (50)	4 (100)	-	-	4 (100)	-	-
Total NO. (%)	70 (89.6)	2 (2.5)	6 (7.9)	45 (57.7)		33 (42.3)	74 (94.9)		4 (5.1)	73 (93.6)		5 (6.4)

TABLE 4: MICS LEVELS OF DIFFERENT ANTIBIOTICS TESTED ON ANAEROBIC ISOLATES

Organism/ Antibiotics	Amoxicillin clavulonic acid MIC range (MIC ₅₀ / MIC ₉₀) µg/ml	Clindamycin MIC range (MIC ₅₀ / MIC ₉₀) µg/ml	Metronidazol MIC range (MIC ₅₀ / MIC ₉₀) µg/ml	Imipenem MIC range (MIC ₅₀ / MIC ₉₀) µg/ml
<i>Bacteroides spp</i> <i>Bacteroid fragilis</i>	<0.06 - >256 (0.5/ 32)	<0.06 - >256 (>256/>256)	0.25 - 16 (0.5/1)	0.125- 16 (0.125/2)
<i>Non-Bacteroid Fragilis</i>	0.25- 2 (<0.06/0.5)	0.06- > 256 (>256/>256)	0. 5- 16 (<0.06/1)	0.125- 16 (0.5/2)
<i>Peptostreptococcus spp.</i>	<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)
<i>Porphyromonas spp.</i>	<0.06 - 4 (0.125/1)	0.06- 8 (1/8)	0.06- 4 (0.125/2)	0.06- 2 (0.125/0.5)
<i>Prevotella spp</i>	<0.06 -2 (<0.06/1)	0.25- >256 (1/ >256)	<0.06- 2 (.125/0.5)	<0.06->256 (0.25/>32)
<i>E.Nucleatum spp</i>	<0.06->32 (0.125/>32)	0.5- >256 (1/>256)	<0.06->2 (0.5/1)	<0.06- 2 (0.06/0.5)

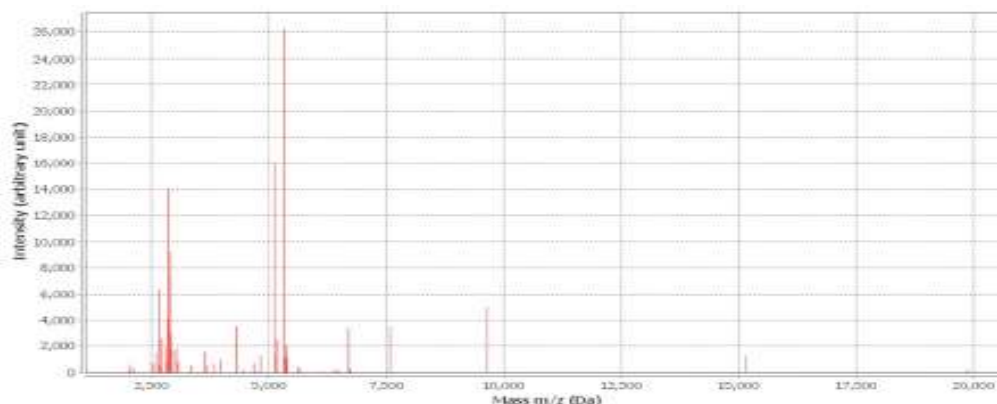


FIG1: MALDI-TOF CURVE OF BACTERIOD FRAGILIS

DISCUSSION

Anaerobic bacteria is part of the human flora; however, it can cause variety of life threatening infections. Culture and identification of anaerobes in the microbiology laboratory are difficult and require strict conditions, long time, and laboratory skills in isolation. Also, traditional methods do not always capable to differentiate 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 fast 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 methods. Most importantly, reducing the period required to identify an organism from days to few minutes that will improve the patient clinical outcome (14). Also, It has a great significance in the identification of biochemically inert, fastidious and slow growing anaerobic cocci (15).

The results of our work demonstrated that infections caused by anaerobic bacteria constituted 21.7% of different 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 a 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 the study of Jamal et al., 2013 (17) who identified fourteen species within five genera of anaerobic clinical isolates. In this study, *Bacteroides spp.* were the most frequent species (41%) isolated from the different surgical infection. This result was also demonstrated by the studies performed by Knoester and his colleagues, 2012 (18) and Jamal et al., 2013 (17) which revealed that *Bacteroides* species constituted more than one-third of the isolates that were identified by MALDI-TOF MS.

The second prevalent genus isolated in this work was *peptostreptococcus spp.* (27%). followed by *Peptostreptococcus spp.* then came *Prevotella spp* and lastly *Fusobacterium spp.* Frequency of different anaerobic species are widely variable between different studies, Knoester and his colleagues ,2012 (18) demonstrated that *Propionibacterium* (15%), *Prevotella* (13%) were the second frequently isolated genera. In contrast, the study of Scola et al., 2011 demonstrated that the most common anaerobes were *Propionibacterium spp.* (12%), followed by *Fusobacterium spp.* (6%) and *Bacteroides spp.*(12). This difference may be due to differences in site of infection 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 anaerobic pathogens were recovered from 87% of diabetic foot infections. This different finding 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 spp* were the predominant isolates. However, El-Tahawy, 2000 (20) found that *Bacteroides fragilis* were responsible 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 discrepant 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 site infections are the third frequent nosocomial infections reported and responsible for a quarter of all nosocomial infections (24).

In the present study, 25% of cultures from SSIs revealed positive culture for anaerobes, which is 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 in nature.

The predominant anaerobic bacteria isolated from SSI and in line with the study done by Reddy , 2012 (25) was *Bacteroides spp.* While Rao et al., 2013 (24) results revealed that *Peptostreptococcus* species (2%) were the most frequently isolated species. However, the predominance of anaerobes bacilli contradicts previous reports that aerobic cocci were the primary contributor to SSI (26). Also, The importance of anaerobes such as *Peptostreptococcus spp.*, *Prevotella spp.*, *Fingoldia* and *Peptoniphilus* has been reported (27). This discrepant result may be 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 abscess cases were due to anaerobic infection. In association

with the results obtained by study of Solomkin et al., 2010, the major pathogen isolated by our work from appendicular abscess cases was *Bacteroides spp.* (70%), followed by *porphorymonas spp.* (35%).

In our study, anaerobic infection was demonstrated in 38.4% of the patients with psoas abscess and *Bacteroides spp.* were the most frequently isolated pathogen as it is responsible for 62.5% of these infections, followed by *peptostreptococcus spp.* (37.5%). However, Adelekan et al., 2004 (29) found that *clostridium difficile* was the most common anaerobic pathogen isolated from psoas abscess cases. This means that bacterial flora are responsible for these two types of infection in this study.

In agreement with results obtained by Knoester et al., 2012 (18), Jamal et al., 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 et al., 2014 (31) study revealed that the MALDI-TOF MS system provided the correct identification for 92% isolates to species level and 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 20 A in identification varies with different anaerobic genera or species. In this study, both tests achieved 100% agreement (Kappa; 1.0) for identification of *Porphorymonas spp.* and *Fusobacterium spp.* In addition, the comparison between both tools in identification of *Bacteroides spp.* and *Peptostreptococcus spp.* demonstrated very good agreement (kappa; 0.98). However, the least degree of agreement between both techniques was in identification of *Prevotella spp.* (kappa; 0.79). 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 et al., 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.

Clindamycin was considered the gold standard for anaerobic infection treatment since 1960, However, resistance to clindamycin has steadily increased among anaerobes in the last 15 years (39). According to our result, about one third of all the isolates were resistant to clindamycin. *Bacteroid spp.* strains showed the highest rate of resistance (69%) especially *Bacteroid fragilis*. While, one third of *Prevotella spp.* in this study were resistant to clindamycin, other studies showed that *Prevotella spp.* resistance to clindamycin ranges between (31%-70%) (40), (41). In this study, 25% of *Porphorymonas spp.* were resistant to clindamycin, compared to 1% in Belgium (40),(41), (42),(43).

Half of *Fusobacterium spp.* isolated by our work were resistant to clindamycin. However, resistance of *Fusobacterium spp.* 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 different hospitals, (44), (45). *Peptostreptococcus spp.* species resistance to clindamycin in our study was 14%, near to the resistance of 11% detected in a study in Taiwan Hospital (3).

Our results represented that *Peptostreptococcus* spp., *Porphoryomonas* spp., and *Fusibacterium* spp. had excellent sensitivity to imipenem with 100% sensitivity among the isolated strains. These results matches the results of Al-Jebouri and Al-Hadeethy 2014 (46) in Iraq. About 10% of *Bacteroid* spp. strains were resistant to Imipenem. Resistance of *Bacteroids* spp. to imipenem had also been also in earlier works done by (Hecht, 2004) (39) and (liu et al 2008)(3). Resistance of *provetella* spp. raised to 25% in another study performed by l-Jebouri and Al-Hadeethy 2014 (46).

Metronidazole has an excellent antimicrobial activity among most of anaerobes, this was supported by the study of Liu et al., 2008 (3). However, resistance of *Bacteroid fragilis* had been reported in several countries (47), (48), (4).

Our results showed that all *Fusobacterium*, *Porphoyromonas* spp., *Peptostreptococcus* spp. and *Prevotella* spp. isolates were sensitive to Amoxicillin clavulonic acid. However, only 78% of *Bacteroids* spp. were sensitive. In a study done by Jamal et al., 2015 (49) showed that the drug gave excellent activity against *Fusibacterium* spp., *Porphoyromonas* spp. and *Peptostreptococcus* spp.

Bacteroid fragilis MIC₅₀/MIC₉₀ in this study for amoxicillin clavulonic acid were (0.5/ 32) clindamycin (>256/>256) and Imipenem (0.125/2) were higher than those detected in Kuwait (0.75- 8), (4 >256) and (0.125-1) respectively(44), and these values were much higher than MIC₅₀/MIC₉₀ for amoxicillin clavulonic acid (0.016- 0.5) and clindamycin (0.016- >256) in Netherland (49). 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 *Bacteroid Non-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).

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Peptostreptococcus spp. showed the best sensitivity profile for all drugs as their MIC₅₀/MIC₉₀ has been reserved within acceptable ranges in relation to other studies (49).

Prevotella spp. in our study showed high level of resistance to clindamycin as MIC₅₀/MIC₉₀ were (1/ >256) and imipenem MIC₅₀/MIC₉₀ were (0.25/>32). This high resistant level to clindamycin has been detected before in previous studies (45), (44), (49) while, *provetella* spp. in this study were all sensitive to amoxicillin clavulonic acid and metronidazole.

F. Nucleatum spp. MIC₅₀/MIC₉₀ were (0.125/>32) for amoxicillin clavulonic acid, (1/ >256) for clindamycin, (0.5/1) for metronidazole and (0.06/0.5)for imipenem. Our results for *Porphoryomonas* spp. MIC₅₀/MIC₉₀ were (0.125/1) for amoxicillin clavulonic acid, (1/8) for clindamycin, (0.125/2) for metronidazole and (0.125/0.5) for imipenem. Values for *F.Nucleatum* spp and *Porphoryomonas* spp. were higher than previous studies (44), (49).

Analysis of MIC₅₀/MIC₉₀ 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 could 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) Misuse of antibiotic is still a problem.

We conclude that anaerobes are common causes of infection in surgical unit, In addition, MALDI-TOF is an accurate rapid test for diagnosis. Unfortunately there is increasing tendency toward developing resistance in many species, thus routine testing for antibiotic sensitivity is a must to treat affected patients. We also recommend continuous monitoring of patterns of resistance in our hospitals and elsewhere.

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