

Original Research Article

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Bacteriological Profile of Diabetic Foot Infections and its Antibiotic Resistance Pattern in Alexandria Main University Hospital

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ABSTRACT

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Diabetic foot is one of the serious complications of DM and may be the initial presentation of undiagnosed diabetes. Foot problems are associated with significant morbidity and impairment in the diabetic patient's quality of life. This work aimed at studying the bacteriological profile of diabetic foot infections and its antibiotic resistance pattern. This study was carried out on 60 diabetic patients with foot lesions admitted at Diabetic Foot Unit, Alexandria Main University Hospital, during the period from March 2017 to October 2017. An interview questionnaire sheet was filled in, including all the relevant data. Swab samples were collected from each wound after the wound had been cleansed and debrided. A total of 85 microorganisms were isolated, majority of isolates were gram negative (94.1 %), *P. aeruginosa* isolates were the predominant (34.1%), followed by *K.pneumoniae* (29.4%), *P.mirabilis* (12.9%), *E.coli* (9.4%) then *P.vulgaris* (8.2%). *S.aureus* was the only isolated gram positive bacteria (3.51%) and *C.albicans* was the only isolated fungus (2.4%). Almost all isolated microorganisms were resistant to various antimicrobials. Gram negative organism's infection predominates in DFI. Monomicrobial infection was the most common followed by the polymicrobial infection. Deep wounds were more associated with polymicrobial infection.

Introduction

Diabetic patients have an increased propensity to develop a variety of infections, which are often more severe than in the general population. Foot infections are probably the commonest and most important of them, being responsible for more hospital days than any other complication of diabetes (Ramsey *et al.*, 1999; Reiber, 1996).

The most common cause of amputations in diabetic patients is ischaemia and infection: gangrene or non-healing foot ulcer is the cause of amputations in 50–70% and infection in 20–50% of patients with diabetes (Tentolouris *et al.*, 2004). In most cases, however, amputation had to be performed because of the combination of infection and ischaemia (Zargarzadeh *et al.*, 2018).

Diabetic foot infections pose a potentially serious acute medical problem, usually requiring immediate medical attention, appropriate diagnostic evaluations and various therapeutic modalities (Lipsky *et al.*, 2004). At other times they constitute a long-term medical problem, with increased morbidity (due to recurrences, bone involvement and the need for surgical resections or amputations) and even, though seldom, increased mortality, especially if not managed properly (Frykberg *et al.*, 2006).

The incidence of foot problems and amputations remains very high, accounting for up to 20% of diabetes-related hospital admissions.

This can be easily attributed to several practices prevalent in Egypt, such as barefoot walking, inadequate facilities for diabetes care, low socioeconomic status, and illiteracy (Shankar *et al.*, 2005). The burden of diabetic foot is set to increase further in the future as its contributory factors such as peripheral

neuropathy and peripheral vascular disease are present in more than 10% of cases at the time of diagnosis (Citron *et al.*, 2007).

Staphylococcus aureus is the most frequent and perhaps the most virulent pathogen in diabetic foot ulcers. β -haemolytic streptococci are also common and obligate anaerobes (*Bacteroides*, *Peptostreptococcus* species, etc.) infect deep wounds with accompanying gangrene or ischaemia.

Previous antibiotic therapy tends to alter the colonizing flora of the skin and wounds, favouring organisms resistant to the agent administered (Rao and Lipsky, 2007).

Recent lesions tend to have monomicrobial infections (Lipsky *et al.*, 1990), whereas chronic wounds tend to develop more complex infections, with aerobic gram-negative rods (*E. coli*, *P. mirabilis*, *P. aeruginosa*, etc.), anaerobes (gram-positive and gram negative) and enterococci, in addition to the gram positive aerobes (O'Meara *et al.*, 2001).

Fungi (*Candida* and *Tinea* species) are also found more frequently in diabetes, although their contribution to infection is questionable (Thomson, 1998).

In an Egyptian study (2015) of the bacterial profile from DFI, gram negative aerobic bacilli were isolated more frequently (56.08%) than gram positive cocci (27.7%).

The commonest isolates were *P. mirabilis* (16.8%) followed by *E. coli* (13.5%), Methicillin sensitive *S. aureus* (MSSA) (11.4%), *Pseudomonas* spp (10.8%), and Methicillin resistant *S. aureus* (MRSA) (10.1%) (Dwedat *et al.*, 2015).

This work aimed at studying the bacteriological profile of diabetic foot infections and its antibiotic resistance pattern.

Materials and Methods

Study setting

The present cross sectional study was conducted over nine-months period from March 2017 to October 2017. The study was carried out at the Vascular Surgery and Diabetic Foot Unit, Surgery Department, Alexandria Main University Hospital. After being approved by the Ethics Committee at the HIPH. Verbal and written consent were obtained from each patient.

Sample size

The sample size was calculated using Epi Info 7.2.0.1, 2016. Assuming a prevalence of diabetic foot infection 96.25% among diabetic foot lesions (Egypt, 2015) and a 5% confidence limit, the resulted sample size at 95% level of confidence was 55, and was increased to 60 suspected DFI patients (Dwedat *et al.*, 2015).

Study population

The study involved 60 diabetic foot patients (previously or newly diagnosed), whoever admitted for surgical intervention due to clinically suspected DFI lesion.

Clinical diagnosis of infection was defined by the presence of at least 2 of the following indicators: local swelling or induration, >0.5cm of erythema around the wound, local tenderness or pain, local warmth, and purulent discharge (Lipsky *et al.*, 2016; Lipsky *et al.*, 2012).

An interview questionnaire sheet for each patient was filled in, including all the relevant data.

Clinical severity of diabetic foot lesions was assessed by Wagner-Meggitt classification system (Lipsky *et al.*, 2006).

Specimen collection

Swab samples were collected from each wound after the wound had been cleansed (using 0.9% sterile saline and gauze) and debrided (removal of necrotic tissue, foreign material, calluses, and undermined wound edges) (Huang *et al.*, 2016). No antimicrobial agent or antiseptic was introduced into the wound before specimen collection.

Each wound was swabbed by rotation of a wound swab over a 1cm² area of the wound for 5 seconds, using sufficient pressure to extract fluid from the inner part of the wound (Rondas *et al.*, 2013).

The specimens were placed into sterile transport containers and sent to the Microbiology laboratory for aerobic culturing within 30 minutes. Anaerobic culturing was not performed in this study.

Sample processing

One swab was Gram-stained for direct examination of the lesion.

The other swab was cultured on each of blood and MacConkey's agar plates and incubated aerobically at 37°C. All plates were examined for growth at 24 and 48 hours after which were discarded as negative.(Tille)

Identification of bacterial isolates

All isolates were identified by conventional microbiological methods according to Tille.

Antimicrobial susceptibility testing

All bacterial isolates were subjected to antimicrobial susceptibility testing using the single disc diffusion method described by the Clinical and Laboratory Standard Institute (CLSI) on Muller Hinton's agar (Jorgensen and Turnidge, 2015)

Selected 20 panels of antimicrobial agents for gram-positive and gram-negative bacteria were used. After aerobic incubation period (18-24 hs) at 35°C for all organisms, inhibition zones were measured and susceptibility was recorded as susceptible, intermediate, and resistant according to CLSI tables (Jorgensen and Turnidge, 2015).

Multidrug resistance (MDR) was defined as the resistance to 3 \geq different antimicrobial classes, except *Staphylococcus* infections. (Saltoglu *et al.*, 2018)

Results and Discussion

Figure 1 shows the distribution of the study sample according to some risk factors for DFI. According to risk factors 53% of study population were smokers, 71.7% were subjected to trauma, hypertension was found in 76.7% and PAD was found in 53.3% of the patients.

According to Wagner-Meggitt Grade, 45% of the patients were in grade II followed by grade I (26.7%) then grade III (25%) and the least was for grade IV only 3.3%.

Most of the diabetic foot lesions were in the toe region (31.7%) followed by hallux (23.3%) the heel (18.3%), sole (15%) and finally infected stump (11.7%). About 52.0% of the diabetic foot lesions were in the right side and 48.0% were in the left side.

The culture results of the 60 diabetic foot lesions yielded sterile (no growth) from one sample (1.7%), monomicrobial bacterial growth in 32 samples (53.3%), polymicrobial bacterial growth (2-3 microorganisms) in 25 samples (41.7%) and *C.albicans* in 2 samples (3.3%) (Table 1).

Table 2 shows that a total of 85 microorganisms were isolated from the 59

infected diabetic foot lesions. Majority of isolates were gram negative (94.1 %), *P.aeruginosa* isolates were the predominant (34.1%), followed by *K.pneumoniae* (29.4%), *P.mirabilis* (12.9%), *E.coli* (9.4%) then *P.vulgaris* (8.2%). *S.aureus* was the only isolated gram positive bacteria (3.51%) and *C.albicans* was the only isolated fungus (2.4%).

Table (3) shows that most of the twenty nine *P.aeruginosa* isolates were resistant to AMK, and TOB, ATM and LEV 58.7%, 51.7%, 37.9 % and 34.5% respectively. Most of the twenty five *K.pneumoniae* isolates were resistant to FOX (80%), LEV (76%) and AMK (52%). *P.mirabilis* were resistant to ATM, FOX, C and KZ (72.7%), TZP, LEV (63.3%) and AMK (54.5%). Most of *E.coli* isolates were resistant to KZ, LEV (87.5%) followed by AMC, CN, AMK and FOX (75%) then for AMP and FEP (62.5%). *P. vulgaris* isolates were resistant to AMP, LEV, AMK and KZ (100% resistance) then to C, (85.7%) AMC and FOX (57.1%).

All of the three *S.aureus* isolates were resistant to TE, resistance pattern for AZM and E was 66.7%, and only one isolate was resistance to FOX (MRSA).

Major risk factors in the present study were smoking, PAD and hypertension which agrees with epidemiological data of large retrospective cohort study in Saudi Arabia (Al-Rubeaan *et al.*, 2015) which also showed a more prevalence of type two diabetes among total diabetic foot cases (94.27%) compared to 91.7% in the present study.

Foot infections in diabetic patients can be caused by a variety of bacterial species, both mono-microbial and poly-microbial including gram positive and gram negative aerobes and anaerobes (Spichler *et al.*, 2015). The present study showed that 41.7% of patients were

infected by 2 – 3 microorganism (polymicrobial) compared with 56.6% of patients who had a monomicrobial etiology including *C. albicans*.

The results are similar to those reported by Raja and Renina *et al.*, who reported that most of patients developed mixed growth (Raja, 2007; Renina *et al.*, 2001).

Pradeep *et al.*, (2017) (Pradeep *et al.*, 2017) reported that gram negative organisms were isolated more frequently (72.3%) than gram positive organisms (27.7%). *K.pneumoniae* (37.2%) and *P.aeruginosa* (25.6%) were the predominant gram negative bacilli.

In the present study, isolated gram-negative microbes were the predominant pathogens

(94.1%) and gram positive 3.5% this has also been observed by Bansal *et al.*, (2008), Shankar *et al.*, (2005), and by Gadepalli *et al.*, (2005), (76 vs. 24%, 57.6 vs. 42.3%, and 51.4 vs. 33.3%, respectively) Raja (2007), and Renina *et al.*, (2001) also documented more gram-negative bacteria than gram-positive bacteria (52 vs. 45% and 67 vs. 33%, respectively) (Hefni *et al.*, 2013).

The prevalence of gram negative was higher than the positive aerobes in a Chinese study which had the same warm and humid climate as Egyptian conditions (Xie *et al.*, 2017). Gram negative organisms' predominance was attributed to warm climates especially in Asia and Africa (Martínez-Gómez *et al.*, 2009; Shakil and Khan, 2010).

Table.1 Culture results of 60 diabetic foot lesions

Growth pattern in culture	No.	%
No growth	1	1.7
Bacterial growth	57	95.0
Monomicrobial	32	53.3
Polymicrobial microorganism (2 – 3)	25	41.7
Fungal growth		
<i>Candida albicans</i>	2	3.3
Total	60	100.0

Table.2 Frequency of microbial isolates from diabetic foot lesions

(n = 85)

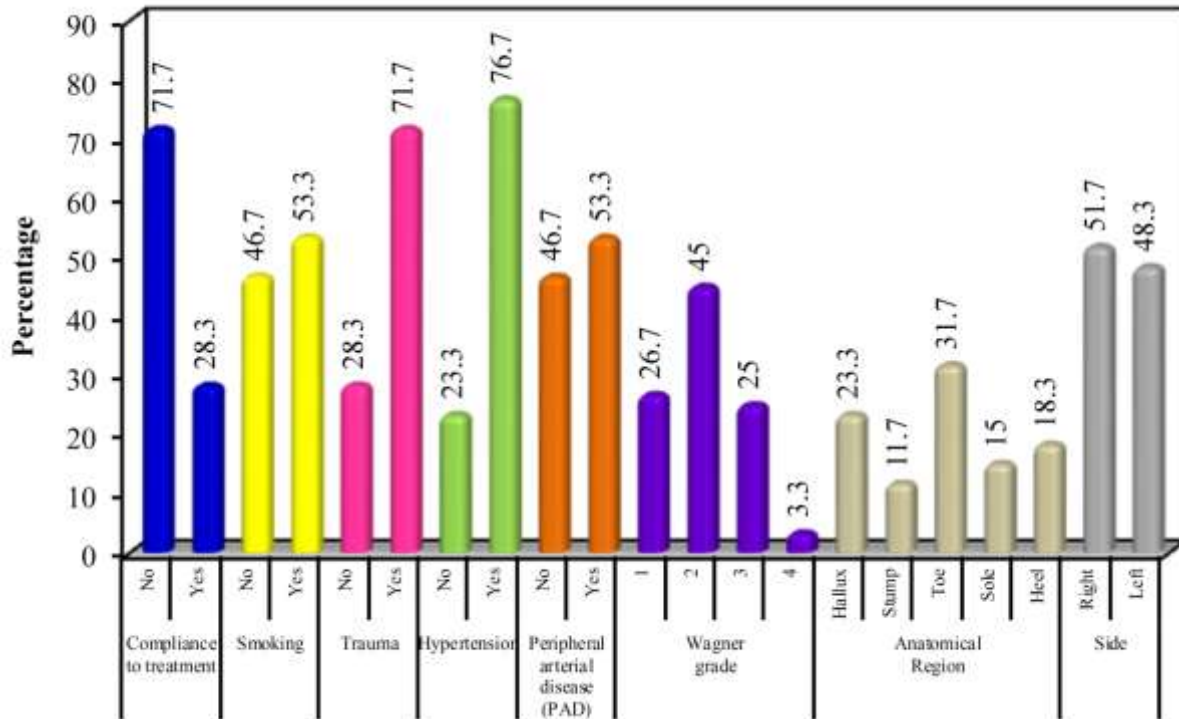
Gram reaction		No.	%
Gram negative	<i>P. aeruginosa</i>	29	34.1
	<i>K. pneumoniae</i>	25	29.4
	<i>P. mirabilis</i>	11	12.9
	<i>E. coli</i>	8	9.4
	<i>P. vulgaris</i>	7	8.2
Gram positive	<i>S.aureus</i>	3	3.5
Fungi	<i>C. albicans</i>	2	2.4
Total		85	100.0

Table.3 Antibiotic resistance pattern of 83 isolated bacteria from DFI

Name of microorganism	Total No. of isolates	Antibiotics % of Resistance																			
		AZM	E	ATM	MEM	IPM	FEP	CAZ	AMC	AMP	TMP/S MX	TZP	LEV	TOB	CN	AMK	FOX	KZ	LZD	C	TE
<i>P. aeruginosa</i>	29	–	–	11(37.9)	12(41.4)	–	4(13.8)	12(41.4)	2(6.9)	13(44.8)	–	4(13.8)	10(34.5)	15(51.7)	6(20.7)	17(58.6)	–	–	–	–	–
<i>K. pneumoniae</i>	25	–	–	4(16.0)	–	3(12.0)	7(28.0)	–	13(52.0)	–	–	6(24.0)	19(76.0)	–	–	4(16.0)	20(80.0)	12(48.0)	–	7(28.0)	–
<i>P. mirabilis</i>	11	–	–	8(72.7)	–	1(9.1)	0(0.0)	–	2(18.2)	7(63.6)	–	7(63.6)	7(63.6)	–	2(18.2)	6(54.5)	8(72.7)	8(72.7)	–	8(72.7)	–
<i>E. coli</i>	8	–	–	3(37.5)	–	2(25.0)	5(62.5)	–	6(75.0)	5(62.5)	–	2(25.0)	7(87.5)	–	6(75.0)	6(75.0)	7(87.5)	7(87.5)	–	1(12.5)	–
<i>P. vulgaris</i>	7	–	–	4(57.1)	–	0(0.0)	0(0.0)	–	4(57.1)	7(100)	–	2(28.6)	7(100)	–	0(0.0)	7(100)	4(57.1)	7(100)	–	6(85.7)	–
<i>S. aureus</i>	3	2(66.7)	2(66.7)	–	–	–	–	–	–	–	0(0.0)	–	1(33.3)	–	–	–	1(33.3)	–	0(0.0)	1(33.3)	3(100)

AZM: Azithromycin, E: Erythromycin, ATM: Aztreonam, CN: Gentamycin, MEM: Meropenem, FEP: Cefepime, CAZ: Ceftazidime, AMC: Amoxicillin-Clavulanate, TMP/SMX: Trimethoprim- Sulfamethoxazole, TE: Tetracycline, AMP: Ampicillin, TZP: Piperacillin –Tazobactam, LEV: Levofloxacin, TOB: Tobramycin, IPM: Imipenem, FOX: Cefoxitin, KZ: Cefazolin, AMK: Amikacin, C: Chloramphenicol (–) indicates not done

Fig.1 Risk factors including: compliance to treatment, Smoking, Trauma, Hypertension, Peripheral arterial disease (PAD), Wagner-Meggit grade, Anatomical Region and Side of 60 diabetic foot patients



The predominant causative microbiological organisms of DFI in Western population are gram-positive aerobes, especially *S. aureus*, and the infection rate of MRSA has increased dramatically over the past 15 years (Al Benwan *et al.*, 2012; Boulton *et al.*, 2005; Citron *et al.*, 2007; Dang *et al.*, 2003; Lipsky *et al.*, 2012; Ramakant *et al.*, 2011; Tentolouris *et al.*, 1999).

The difference observed in the prevalence of gram negative bacilli and gram positive in DFI between diabetic patients of Eastern and Western countries remains largely unknown (Samant *et al.*, 2018).

In the present study among the gram negative aerobes the most commonly encountered were *P.aeruginosa*, *K. pneumoniae* and *E.coli* in agreement with Kumar results (Kumar *et al.*,

2017). Joseph *et al.*, (Joseph *et al.*, 2017), reported that gram positive (*S.aureus*) isolates were most susceptible to Vancomycin and Linezolid these findings are similar to results of the present study where *S.aureus* was sensitive to Linezolid and Trimethoprim-sulfamethoxazole.

In this study *P.aeruginosa* showed high degree of resistant to Amikacin (58.6%), this is in agreement with Noor study (Noor *et al.*, 2017). *K.pneumoniae* showed high degree of resistance to Cefoxitin (80%) and Levofloxacin (76%), This is in agreement with Alexis results (67%), (79%) respectively (Alexis and Sakthivennila, 2018).

Protus mirabilis showed high degree of resistant to Amikacin, this is in agreement with the study done by Sugandhi and Prasanth,

2018 (Sugandhi and Prasanth, 2018). *E.coli* was resistant to Amoxicillin /Clavuanate (75%), this was in agreement with the study done by Bello, *et al.*, 2018 (Bello *et al.*, 2018). *Protus vulgaris* was 100% resistant to Amikacin, while in other study *P.vulgaris* showed the Cephalosporins was most resistant antibiotic (Thangamani *et al.*, 2017).

Antimicrobial resistance is now a major challenge to diabetic foot infection healthcare providers for treating patients (Basak *et al.*, 2016).

In the present study most of *K. pneumonia* isolates were MDR; resistance to Amoxicillin-clavulanate, Levofloxacin and Cefoxitin were 52%, 76% and 80% respectively, In addition, two *S.aureus* isolates were MDR; (resistant to Azithromycin, Erythromycin and Tetracycline with 66.7%, 66.7% and 100% respectively) while, one isolate was methicillin-resistant.

Majority of *P. mirabilis* isolates were XDR i.e. resistance to Aztreonam, Ampicillin, Piperacillin –Tazobactam, Levofloxacin, Amikacin, Cefoxitin, Cefazolin and Chloramphenicol were 72.7%, 63.6%, 63.6%, 63.6%, 54.5%, 72.7%, 72.7% and 72.7% respectively. Same for the *P. vulgari* (resistance to Aztreonam, Amoxicillin-Clavulanate, Ampicillin, Levofloxacin, Amikacin, Cefoxitin, Cefazolin, and Chloramphenicol 57.1%, 57.1%, 100%, 100%, 100%, 57.1%, 100% and 85.7%, respectively).

E.coli isolates resistance pattern was XDR mostly to Cefepime, Amoxicillin-Clavulanate, Ampicillin, Levofloxacin, Gentamycin, Amikacin, Cefoxitin and Cefazolin (62.5%, 75%, 62.5%, 87.5%, 75%, 75%, 75% and 87.5% respectively). It should be mentioned that none of *P. aeruginosa* isolates were MDR.

The current study was carried out in the Vascular Surgery Diabetic Foot Unit ward, Alexandria University, in which the empirical

regimen for treatment of DFI is following IDSA guidelines 2012 which include the use of Trimethoprim/ sulfamethoxazole, Levofloxacin and imipenem (Tienam®) respectively.

These guidelines are so far controlling most of isolated bacteria in this study; as MDR and XDR isolates were sensitive to Imipenem (Lipsky *et al.*, 2012). None of the isolated bacteria were resistant to Trimethoprim/sulfamethoxazole, while Levofloxacin showed high degree of resistance among the isolates this highlights the importance of bacteriological culture for precise choice of the accurate antibiotic and give importance of the continuous surveillance to determine the changes of the bacterial growth pattern.

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