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Antibiotic profiles of Microbial Infection in Diabetic Foot Ulcers

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ABSTRACT

Introduction and aim: Diabetic foot ulcers are a serious medical and economic worry. It is the most common reason for diabetic patients to be admitted to hospital. If not treated appropriately, the infection might lead to amputation of the infected foot. The current research aims to recognise the frequencies of isolated aerobic bacterial pathogens associated with diabetic foot ulcers and detect their antibiotic susceptibility and resistance patterns to reduce the risk of complications.

Methodology: This cohort study included 140 swab samples from diabetic foot ulcers from patients referred to the surgery department at Al-Azhar University Hospital in New Damietta, Egypt, in the period between January and December 2018. All samples were processed for the identification of aerobic bacterial strains were identified using routine bacteriological culture methods and antimicrobial susceptibility by the Kirby-Bauer disc diffusion method.

Results: Out of 140 pus and tissue samples obtained of diabetic foot ulcers, 120 (85.7%) exhibited aerobic organism growth, yielding a total of 132 isolates with an average of 1.1 isolates per specimen. Polymicrobial infection was 10%. Gram-negative isolates were prominent (62.88%), with Pseudomonas aeruginosa (26.5%) being the most common strain causing diabetic foot infections, followed by E. coli (20.5%). Staph aureus was the most common Gram-positive bacteria (8.9%). Out of 83 Gram-negative bacteria, 54 (65%) produced extended-spectrum lactamase (ESBL). The majority of Gram-negative isolates were resistant to levofloxacin, gentamicin, ampicillin-sulbactam, and gatifloxacin. All Gram-negative bacteria were sensitive to imipenem. Of the 25 Staph aureus, 15 (60%) were Methicillin Resistant Staphylococcus aureus (MRSA) and susceptible to vancomycin and linezolid.

Conclusion: *Pseudomonas sp.* was the most prevalent cause of infections. Most isolates were multidrug resistant. In our study, Gram-negative bacteria were the main cause of diabetic foot infections. A significant number of MDR isolates were also observed. The inappropriate use of broad-spectrum antibiotics may be the main cause of the increase in the frequency of MDR isolates.

Keywords: Diabetic Foot Ulcer; Antibiotic Susceptibility; Bacterial Isolates; Polymicrobial Infections; Multidrug-resistant Organisms.

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Abbreviations: Diabetic foot ulcer (DFU), Anti-Microbial Resistance (AMR), Clinical and Laboratory Standards Institute (CLSI), Trimethoprim-Sulphamethoxazole (TMP/SMX), Multi-Drug Resistance (MDR), Extended Spectrum Lactamase (ESBL), Methicillin Resistant Staphylococcus aureus (MRSA).

INTRODUCTION

Diabetes mellitus is a serious medical condition that affects around 382 million people across the globe. This number is anticipated to rise to 592 million by 2035 (1).

Uncontrolled diabetes may predispose patients to diabetic foot ulcers (DFUs) (2).

Over the course of their lives, around 25% of diabetics will develop a DFU (3). This ulcer may develop an infection that causes significant morbidity and financial consequences, and is the most common reason for lower limb amputation (4).

To increase the chances of surviving the limb, suitable antibiotic therapy should be initiated right way, without waiting for microbiological culture findings or antibiotic sensitivity tests. As a result, most diabetic foot lesions are treated empirically first. It would be prudent if empirical treatment were based on knowledge of commonly identified bacteria and the most prevalent antibiogram pattern of these bacteria (5).

Understanding DFUs microbiology is crucial for treatment with this clinical trick. DFU infections are frequently mixed infections that defy immune system clearance and cause antibiotic resistance (6).

DFU management is restricted to surgical wound care and the use of proper antibiotics to avoid amputation (7).

THE AIM OF THIS STUDY

This study aimed to recognise and isolate the bacterial pathogens isolated from diabetic foot ulcers and to evaluate their antibiotic susceptibility patterns in order to assist physicians in effectively selecting optimum antibiotics to reduce the risk of complications.

MATERIALS AND METHODS

Study design:

From January 2018 to December 2019, a total of 140 swab samples were randomly taken from diabetic foot ulcers. Participants were recruited randomly from the outpatient clinic and inpatient surgery departments at Al-Azhar University Hospitals, Egypt.

The study was approved by the Damietta Faculty of Medicine, Al-Azhar University. All participants gave their informed consent to participate in the study.

Collection of samples:

Pus specimens were obtained during the initial hospitalisation (given that no antibiotics had been used within the previous two days). Using a sterile disposable swab, 140 pus and tissue samples were obtained from participants who had diabetic foot infections.

The specimen was handled with care to avoid contamination. The swab was immersed in Amies transport media and then delivered to the Microbiology lab. The samples were handled in accordance with standard guidelines.

Microbial Isolation and Identification:

Collected swabs were subjected to Gram stain to screen for the presence of bacterial pathogens. Samples were cultured on various selective and differentiating media (such as nutrient agar, MacConkey agar, blood agar, and mannitol salt agar); plates were incubated aerobically at 37°C for 24–48 h. Subculturing of mixed growth cultures was done by transferring isolated colonies to various media to ensure pure cultures.

Pathogens identification:

Observation of cultural:

All the incubated plates were observed for colony morphology, swarming, and hemolytic action on blood agar.

Microscopic examination:

Gram staining was performed on different colonies and examined for Gram reaction.

Biochemical examination:

Isolates were identified and confirmed by biochemical reactions such as carbohydrate utilisation tests, triple sugar iron test, catalase test, coagulase, oxidase test, indole production test, methyl red test, Voges-Proskauer test, urease test, citrate utilisation test, bile aesculin test, and motility). The obtained data were collected, analysed and interpreted in accordance with the Clinical Laboratory Standards Institute recommendations (CLSI) (8).

Antibiotic Sensitivity Testing

Gram-positive isolates were tested for ampicillin, amoxicillin/ clavulanic acid, cefoxitin, erythromycin, clindamycin, doxycycline, levofloxacin, rifampicin, gentamicin, trimethoprim/sulfamethoxazole, teicoplanin, vancomycin, and linezolid. Methicillin resistance staphylococcal spp. (MRSA) and vancomycin resistance (VRSA) can be diagnosed according to the CLSI standards (8). Gram-negative isolates were tested for piperacillin/tazobactam, cefaclor, cefotaxime, ceftazidime, cefepime, levofloxacin, trimethoprim/ sulfamethoxazole, amikacin, meropenem, aztreonam, imipenem, and tigecycline. After incubating the plates at 37°C for 24 hours, the diameter of the zone of inhibition of antibiotics was measured and interpreted according to the CLSI guidelines.

Data analysis:

Data was collected in Excel format, then validated, inputted, and analysed with SPSS for data processing and statistics. Numbers and percentages were used to convey categorical data. The Kolmogorov Smirnoff test was used to determine the normality of quantitative data. For normal distribution, quantitative data were reported as mean and standard deviation.

RESULTS

Study Subjects:

A total of 140 swab samples were randomly taken from diabetic foot ulcers. The study population included 84 (60%) males and 56 (40%) females. The patients' ages varied from 48 to 73, with an average (standard deviation) of 59.98 ± 4.98 years. The most common prevalence was in type 2 diabetes mellitus at about 91 (65%) and 49 (35%) were type 1 (Table 1).

About 94 (67.1%) participants were treated with insulin, while 46 (32.9%) were treated with anti-diabetic oral treatments. Most patients also suffer from other associated medical conditions as hypertension (80) bronchial asthma (30) congestive heart disease (70) and other diseases (Table 1).

The most common location of the ulcer was the toe 75 (53.57%), followed by the plantar surface 40 (28.57%) and dorsal portion 25 (17.9%) (Table 1).

The bacteriological profile of DFU infection

Excluding fungal and non-significant growth samples, 120 (85.7%) of the 140 collected showed positive culture growth, with 10% (12/120) being polymicrobial pathogens and 90% (108/120) being monomicrobial infections. There were 132 positive findings for aerobic bacterial growth, indicating 1.1 isolates per specimen (Table 2).

Gram-negative bacteria constituted 83 (62.9%) of the isolates, whereas Gram-positive bacteria made up 49/132 (37.1%). *Pseudomonas aeruginosa* 26.5% (35/132) were the most common isolates causing diabetic foot infections followed by *E. coli* 20.5% (27/132), *S. aureus* 18.9% (25/132), S. *epidermidis* 9.8% (13/132), *Klebsiella sp.*, 9% (12/132), *S. saprophyticus* 6% (8/138), *Proteus sp.* 4.5% (6/132) and *Enterococci*, 2.7% (3/132), *Acinetobacter sp.* 2.3% (3/132) (Table 2).

Table (3) showed the antibiogram pattern of Gram-negative bacteria. Gram-negative bacteria were fully sensitive (100%) to Piperacillin/tazobactam, Amikacin, meropenem, imipenem, and tigecycline. Most Gram-negative isolates have a high rate of resistance to the majority of the antibiotics examined, including cephalosporins, including the third-generation cephalosporins.

Table (4) showed the antibiogram pattern of Gram-positive bacteria. Gram-positive bacteria were more sensitive to linezolid, teicoplanin, vancomycin, and clindamycin. They have a high rate of resistance to various classes of antibiotics like ampicillin, amoxicillin/clavulanic acid, cefoxitin, erythromycin, clindamycin, doxycycline, levofloxacin, rifampicin, gentamycin, and trimethoprim/sulfamethoxazole. Out of 25 Staph aureus, 15 (60%) were resistant to cefoxitin and were recorded as Methicillin Resistant *Staphylococcus aureus* (MRSA). Vancomycin resistance (VRSA) was found in 2 (8%).

Distribution of ESBL and MDR organisms: Based on disc diffusion methods, ESBL production was suspected in all isolated Gram-negative bacilli. Out of 83 Gram-negative isolates, 51 (61.4%) were extended-spectrum β-lactamase (ESBL) producers. The highest ESBL production was among Pseudomonas aeruginosa 28/35 (80%), followed by E. coli 15/27 (55.5%), *Klebsiella sp.* 6/12 (50%) and *Proteus sp.* (2/6, 33.3%) (**Table 5**).

MDR pathogens are those that are unsusceptible to at least one antibiotic in three or more of the antibiotic categories. The overall prevalence of MDR organisms among the studied isolates was 81/132 (61.4%). These MDR isolates included 5 MRSA and 76 GNB. All *acinetobacter sp.* were completely resistant to all the common antibiotics.

Table (1): Demographic data of Patients with diabetic foot ulcers.

Variables		Statistics
Total patients (n,%)		140 (100%)
Sex (n,%)	Male	84 (60%)
	Female	56 (40%)
Age (years)	Range (Min. – Max.)	48-73
	Mean ± SD	59.98 ± 4.98
Type of diabetes (n, %)	Type 1	49 (35%)
	Type 2	91 (65%)
Type of treatment (n, %)	Insulin injection	94 (67.1%)
	Oral treatments	46 (32.9 %)
Other associated disease (n, %)	Hypertension	80 (57.1%)
	Bronchial asthma	30 (21.4%)
	CHD	70 (50.0%)
	others	50 (35.7%)
Location of foot ulceration (n,%)	Toe	75 (53.57%)
	Planter surface	40 (28.57%)
	Dorsal portion	Dorsal portion 25 (17.9%)

Table (2): Spectrum of Pathogens Isolated from the diabetic foot ulcers.

	Organisms	Frequency of isolate n (%)
Total Collected samples (140)	Positive aerobic bacterial	120/140 (85.7%)
	NO Bacterial growth	20/140 (14.3%)
	Aerobic bacterial isolates	132/120 (110%)
	Poly-microbial infection was	12/120 (10%)
	Mono-microbial infection	108/120 (90%)
Gram negative bacteria **83/132 (62.9%)**	Pseudomonas aeruginosa	35 (26.5%)
	Escherichia coli	27 (20.5%)
	Klebsiella. sp	12 (9%)
	Proteus sp	6 (4.5%)
	Acinetobacter sp.	3 (2.7%)
Gram positive bacteria **49/132 (37.1%)**	Staphylococcus aureus	25 (18.9%)
	S. epidermidis	13 (9.8%)
	S. saprophyticus	8(6%)
	Enterococcus faecalis	3 (2.7%)

Table (3): Analysis of Antibiotic Resistance of Gram-negative bacilli isolated from diabetic foot ulcers

Antibiotic	Gram-negative bacilli, n (%) 83 (62.88%)				
	P. aeruginosa (n=35)	**E. coli (n=27)**	**K. sp (n=12)**	**Proteus sp (n=6)**	**Acinetobacter sp. (n=3)**
Piperacillin/tazobactam	0	0	0	0	0
Cefaclor	35 (100)	27 (100)	10 (100)	4 (66.6)	3(100)
Cefotaxime	35 (100)	15 (55.5)	10(100)	2 (33.3)	3(100)
Ceftazidime	30 (85.7)	14 (51.8)	8 (80)	3 (50)	3(100)
Cefepime	10 (50)	12 (44)	10 (100)	4 (66.6)	3(100)
Levofloxacin	35 (100)	27 (100)	9 (90)	1(46.6)	3(100)
Trimethoprim/sulfamethoxazole	28 (80)	0	10 (100)	3(50)	1(33.3)
Amikacin	0	0	0	0	0
Meropenem	0	0	0	0	0
Aztreonam	18 (51.4)	27 (100)	10 (100)	4(66.6)	3(100)
imipenem	0	0	0	0	0
Tigecycline	0	0	0	0	0

Table (4): Analysis of Antibiotic Resistance of Gram-positive cocci isolated from diabetic foot ulcers

Antibiotic	**Gram-positive cocci, n (%)**		**49 (37.2%)**	
	S. aureus (n=25)	S. epidermidis (n=13)	S. sapr (n=8)	E fecalis (n=3)
Ampicillin	25 (100)	13 (100)	8 (100)	3 (100)
Amoxicillin/clavulanic acid	25 (100)	12 (92)	7 (87.5)	3 (100)
Cefoxitin	15 (60)	10 (76.9)	6 (75)	1 (33.3)
Erythromycin	22 (88)	11 (84.6)	7 (87.5)	1 (33.3)
Clindamycin	7 (28)	6 (46.1)	3 (37.5)	1 (33.3)
Doxycycline	20 (80)	7 (53.8)	4 (50)	1 (33.3)
Levofloxacin	18 (72)	8 (61.5)	6 (75)	1 (33.3)
Rifampicin	22 (88)	13 (100)	8 (100)	3 (100)
Gentamicin	11 (44)	4 (33.3)	3 (37.5)	2 (66.6)
Trimethoprim/sulfamethoxazole	19 (76)	13 (30.8)	8 (100)	2 (66.6)
Vancomycin	2 (8)	0	1 (12.5)	0
Teicoplanin	1 (4)	0	0	0
Linezolid	0	0	0	0

Table (5): Distribution of ESBL in isolated Gram–negative

Organisms	Frequency of isolate n (%) 51/83 (61.4%)
Pseudomonas aeruginosa	28/35 (80%)
Escherichia coli	15/27 (55.5%)
Klebsiella. sp	6/12 (50%)
Proteus sp	2/6 (33.3%)
Acinetobacter sp.	0/3 (0%)

DISCUSSION

The newly revised International Working Group for Diabetic Foot (IWGDF) recommendations on foot infection propose using the Infectious Disease Society of America (IDSA) classification system to offer targeted therapy to determine the clinical severity of any diabetic foot infection (5).

A consensus suggestion among these and other recommendations is to collect wound samples prior to the administration of empirical antibiotics in order to identify the causal organisms (9). However, there are still gaps in our knowledge, notably about when and how specialists give antibiotics. About 33% of hospital patients were given empirical treatments that were perhaps compatible with local guidelines. However, after culture results, antibiotic medication was modified, resulting in a considerable improvement at the desired prescription stage (10).

This cohort investigates the microbiology of DFUs. The aim was to recognize the antibiotic sensitivity and resistance pattern of isolated aerobic bacterial pathogens associated with diabetic foot ulcers to lessen the risk of complications. The majority of DFU patients were older, with an average age of 60.2±10.1 years, which may be due to sensory neuropathy (5, 11).

In the current research, males accounted for 60% of DFU cases. Many prior investigations (9, 12-14) confirm the current findings. This conclusion can be explained by increased outside work, poor foot care compliance, and gender-related lifestyle variations. As in previous studies, the majority of DFUs patients (91, or 65%) had Type-2 diabetes mellitus (12).

The number of samples with no significant growth 20/140 (14.3%) has been recorded in some previous study (9). The majority of the sample results were monomicrobial, which is consistent with the findings of Anvarinejad *et al.* (11) and Yoga *et al.* (15). Most of the studies presented bacterial polymicrobial ecology (14). Clinically mild and superficial subcutaneous infections might explain the low frequency of polymicrobial infection in the current investigation.

Although bacterial diversity and the prevalence of certain bacteria vary greatly between studies, despite the fact that Gram-positive cocci are the most prevalent aetiological organisms in Europe and the United States (5,14), Gram-negative bacteria constituted around 83/132 (62.88%) in our study. These findings corroborate previous research by Murali *et al.* (2) and Miyan *et al.* (13).

P. aeruginosa is the most predominant isolate 35 (26.5%) in our investigations, followed by *E. coli* 27 (20.5%), *S. aureus* 25 (18.9%). This finding is consistent with earlier research, notably among patients from developing countries and warm climates in Asia and Africa (9,15,16).

P. aeruginosa poses a significant therapeutic challenge in terms of treatment and cost of care. It's unclear if this is related to environmental variables, personal hygiene, footwear, antimicrobial pretreatment, or anything else. The prevalence rate of other detected Gram-negative bacilli in DFUs was variable in different studies. Hefni *et al.* found that Klebsiella spp. was the most prevalent in DFUs (17).

Regarding Gram-positive isolates, *Staphylococcus aureus* reported among 18.9% (25/132) and remains the most frequent Gram-positive cocci, as found in a previous study (13).

Concerning the antibiogram pattern, most Gram-negative isolates have a high rate of resistance to the majority of the antibiotics examined, including cephalosporins and third-generation cephalosporins. This is consistent with other studies (18). In contrast, other antibiotics such as Piperacillin/tazobactam, Amikacin, meropenem, imipenem, and tigecycline were reported to be the most sensitive antibiotics (100%) (19, 20).

In regards of the antibiotic sensitivity profile of Gram-positive bacteria, a large proportion of the isolated bacteria were multidrug-resistant. Gram-positive bacteria have a high rate of resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, erythromycin, clindamycin, doxycycline, levofloxacin, rifampicin, gentamycin, and trimethoprim/sulfamethoxazole; similar results were reported in some studies (2,11). The frequency of MRSA and VRSA is increasing worldwide, for which appropriate and aggressive therapy is required to control them. We found a high frequency of MRSA among S aureus isolates 15/25 (60%), which was also seen in a study on the microbiological aspect of diabetic foot (13). Contrary to our findings, a very low frequency of MRSA was found in another study by Hefni, *et al.* VRSA was found in 2 (8%). Gram-positive bacteria were more sensitive to linezolid, teicoplanin, vancomycin, and Clindamycin (2, 11).

Based on disc diffusion methods, ESBL production was suspected in all isolated Gram-negative bacilli. Out of 83 Gram-negative isolates, 51 (61.4%) were extended-spectrum β lactamase (ESBL) producers. The highest ESBL production was among Pseudomonas aeruginosa 28/35 (80%), followed by E. coli 15/27 (55.5%), Klebsiella sp 6/12 (50%) and Proteus sp 2/6 (33.3%). This result was found in a previous study (21).

Antimicrobial resistance is a major public health concern which increases morbidity and mortality as well as the cost of health care. During the last decade, an increase in the rates of antimicrobial resistance worldwide and an increased frequency of MDR isolates in the clinical setting have also been demonstrated. We also found a high frequency of MDR isolates, the overall prevalence of MDR organisms among the studied isolates was 81/132 (61.4%). There was a predominance of Gram-negative MDR isolates 76/81(93.8%) (2, 13, 15, 19). Acinetobacter sp was completely resistant to all the common antibiotics tested, not only in our study but also in another study on the antimicrobial susceptibility patterns in diabetic foot ulcers (13).

Conclusions:

Gram-negative aerobes predominated in diabetic foot infections. MDR isolates were observed in diabetic foot ulcers. Delayed referral and incorrect use of broad-spectrum antibiotics may be the primary causes of the rise in MDR isolates. More large-scale research, particularly in primary and secondary care settings, is required to verify our findings.

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