Aerobic bacteria isolated from diabetic foot ulcers of Egyptian patients: types, antibiotic susceptibility pattern and risk factors associated with multidrug-resistant organisms

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Abstract

Introduction **Diabetic foot infection (DFI) is one of the common diabetic complications. Pathogens causing DFI and their antibiotic susceptibility vary with location. Therefore, empirical antibiotic therapy should be based on the pathogens that are most likely to be present. Aim: To identify the frequent aerobic bacteria causing DFI with detection of their antibiotic susceptibility to help clinicians in our community choose the best empirical antibiotic for DFI.**

Methods **Swabs were collected from 104 diabetic foot ulcers (DFUs). Aerobic bacterial cultures were done followed by bacterial identification and antibiotic susceptibility testing on VITEK® 2 system. Extended-spectrum beta-lacatamase (ESBL) detection was performed phenotypically and confirmed by multiplex-PCR for *bla*$_{CTX-M}$, *bla*$_{TEM,}$ and *bla*$_{SHV}$ genes.**

Results **Aerobic bacterial infection was detected in 82/104 (78.8%) of the DFUs. Gram-negative bacilli (GNB) were isolated more frequently (56.1%) than Gram-positive cocci (GPC) (43.9%). The most common single-isolated bacteria were *K. pneumoniae* (26.8%), *S. aureus* and coagulase negative staphylococci (22% for each). The only significant independent predictors of DFI with GNB or GPC were long DM duration and frequent hospitalizations, respectively. The most active antibiotics were amikacin, tigecycline and meropenem for GNB, and linezolid and vancomycin for staphylococci. Multidrug-resistance prevalence was 95.1%. ESBL was detected in 52.6% of Enterobacteriaceae; the *bla*$_{CTX-M}$ gene was the most common (90%), followed by *bla*$_{TEM}$ (65%) and *bla*$_{SHV}$ (35%). Peripheral neuropathy was the single independent predictor for DFI with ESBL producers (adjusted OR=15.5).**

Conclusions **There is a notable local pattern of DFI bacteriology in our community. Our findings could be valuable in developing the future empirical treatment guidelines for DFIs.**

Keywords **Diabetic foot infection, multidrug resistance, bacteriology, ESBL, MRSA, Egypt.**

Introduction

Diabetes mellitus (DM) is considered a major public health concern across the world, and its prevalence is rising, especially in developing countries.[1,2] Globally, adults with diabetes accounted for 463 million in 2019, and this figure is anticipated to rise to 642 million by 2040.[3] According to the International Diabetes Federation, Egypt is one of the ten countries with the greatest prevalence of diabetes. The number of diabetic patients in Egypt is likely to rise from 9 million in 2019 to 13.1 million by 2035.[3]

Diabetic foot ulcers (DFUs) represent one of the most frequent complications of DM, particularly in developing countries, and can cause disability and morbidity. Approximately 25% of diabetics will develop foot ulcers throughout their lifetime.[4] DFUs are found in

Received: 08 May 2021; revised: 22 November 2021; accepted: 28 November 2021.

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Published December 2021

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

7.2% of Africans, 5.5% of Asian and 3% of Europeans.[5] In Egypt, the frequency of DFUs is significant, ranging from 6.1% to 29.3%.[6] About half of the DFUs will be infected across their lifetime.[4] This infection starts superficially, but if the therapy is delayed and immunity is compromised, it can spread to the deeper tissues and cause gangrene and amputations.[7]

To enhance the possibility of saving the limb, appropriate antibiotic therapy should be started immediately without waiting for microbiological results of culture and antibiotic susceptibility testing.[8] Therefore, most diabetic foot lesions are initially treated empirically. It would be wise if the empirical therapy was based on knowledge of the common isolated bacteria and the most prevalent pattern of antibiogram of these bacteria.[9]

Many variables, such as age, sex, geographic location, ulcer severity, and ulcer duration could influence the types of bacteria implicated in diabetic foot infections (DFIs) and their patterns of antibiotic susceptibility.[10,11] As the number of DFUs infected with multidrug-resistant (MDR) bacteria is increasing, doctors are faced with a more difficult challenge when treating DFIs due to a limited number of antibiotic choices.

Infection of DFUs with MDR organisms might be caused by a variety of variables, including repeated hospitalizations for the same ulcer, inappropriate use of antibiotics, certain ulcer features, and the presence of additional diabetes complications.[12-14] There is limited data in our area on the causative organisms of the DFIs and their antibiotic susceptibility profiles. Therefore, we designed this study to identify the most common aerobic bacteria causing DFIs, as well as in vitro detection of their antibiotic susceptibility pattern and detection of the prevalence rate of MDR organisms and their associated risk factors in order to guide doctors in our community hospitals in selecting the best empirical antibiotic therapy for DFIs.

Methods

Study design

This prospective study was conducted on 104 type 2 diabetes mellitus patients with DFUs admitted at the surgery department, Mansoura University Hospital, Egypt in the duration between May 2016 and April 2017. A foot ulcer was defined as a lesion located below the malleoli and penetrating all layers of the skin. Clinical suspicion of infection was based on the existence of two or more of these criteria: induration or edema of the ulcer area, redness (>0.5 cm) surrounding the ulcer, local tenderness and/or pain, local rise in temperature and discharge of pus.[15]

Diabetic patients having foot ulcers not etiologically caused by their diabetic status or its complications, such as vasculitis, varicose ulcers, or those with wounds due to specific infections or tumors, were excluded. Also, this study did not include DFIs in patients who had received antibiotics for a period of more than 24 hours within 72 hours of sample collection. From all patients included in this study, we collected the following data; age, gender, diabetes duration, DFU duration, past history of hospital admissions for the same ulcer, and previous use of antibiotics in the preceding 6-12 months. Ulcers were examined for size and location. Patients were examined for sensory peripheral neuropathy.

Sample collection

For aerobic bacterial culture, samples were obtained aseptically from each DFU by the use of sterilized swabs. To avoid isolation of colonizing bacterial flora, samples were obtained from the deepest part of the ulcer after the wound area was rinsed with saline and the wound was debrided. Thereafter, samples were immediately transported to the microbiology laboratory in the clinical pathology department at Mansoura University Hospital for immediate handling.

Isolation of aerobic bacteria

All collected specimens were cultured on blood, mannitol salt, and MacConkey agar plates (Oxoid Ltd., UK). The inoculated plates were then incubated aerobically for 24 hours at 37°C. The revealed growth was identified according to the morphology of the resulting colonies and results of Gram-staining. Furthermore, identification of all isolates to the species level

was performed using the VITEK® 2 compact system (bioMérieux, France) following the instructions of the manufacturer.

Antibiotic susceptibility testing

Susceptibility testing of the isolated bacteria to antibiotics was performed using the Kirby-Bauer disc diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines were used for interpretation of the results.[16] The antibiotic discs were bought from Hi-Media Labs, India.

The antibiotics panel tested for staphylococci included ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), doxycycline (30 µg), levofloxacin (5 µg), rifampicin (5 µg), gentamicin (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), linezolid (30 µg) and vancomycin (30 µg). Detection of methicillin-resistance among staphylococcal spp. was done using a cefoxitin disk (30 µg) according to the criteria of the CLSI.[10] The diameter of the inhibition zone around the cefoxitin disc should be ≤21 mm and 24 mm to diagnose methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MR-CoNS), respectively. Vancomycin resistance was detected using the broth microdilution method with determination of the minimal inhibitory concentration (MIC).[16] The isolate was defined as vancomycin resistant *S. aureus* (VRSA) if the vancomycin MIC was ≥16 µg/mL.

The antibiotics panel tested for Gram-negative bacilli (GNB) were: cefotaxime (30 µg), piperacillin/tazobactam (100/10 µg), cefaclor (30 µg), ceftazidime (30 µg), cefepime (30 µg), levofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), amikacin (30 µg), meropenem (10 µg), aztreonam (30 µg) and tigecycline (15 µg). MDR pathogens are those that were unsusceptible to at least one antibiotic in three or more of the antibiotic categories.[17]

Detection of extended-spectrum beta-lactamase (ESBL) production

ESBL production among isolated Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*) was screened according to CLSI guidelines, 2017. The isolate was considered as a potential ESBL producer if inhibition zones of either ceftazidime, cefotaxime, ceftriaxone, or aztreonam antibiotic discs were ≤22 mm, ≤27 mm, ≤25 mm or ≤27 mm, respectively.[16]

Phenotypic confirmation of ESBL production was done by combination disc method: ceftazidime (30 µg) and cefotaxime (30 µg) alone and combined with clavulanic acid (10 µg) (BioRad, France) were used. The tested isolate was considered an ESBL producer if there was an increase equal to ≥5 mm in the diameter of the inhibitory zone around any of the combined ceftazidime/clavulanic acid or cefotaxime/clavulanic acid discs in comparison to the inhibition zone diameter around discs containing ceftazidime or cefotaxime alone, respectively.[16]

Genotypic confirmation of the ESBL phenotype was done by multiplex PCR for determination of the most prevalent ESBL-

Table 1. Primer sequence of ESBL genes and their amplicon size

Gene	Primer	Sequence	Amplicon size (bp)
bla$_{CTX-M}$	CTX-M-F	5'CGATGTGCAGTACCAGTAA3'	585
	CTX-M-R	5'TTAGTGACCAGAATCAGCGG3'	
bla$_{TEM}$	TEM-F	5'GCGGAACCCCTATTTG3'	1080
	TEM-R	5'ACCAATGCTTAATCAGTGAG3'	
bla$_{SHV}$	SHV-F	5'TTATCTCCCTGTTAGCCACC3'	795
	SHV-R	5'GATTTGCTGATTTCGCTCGG3'	

bp – base pair**; F** – forward; **R** – reverse.

encoding genes, including; bla_{TEM}, bla_{SHV}, bla_{CTX-M}. Extraction of the bacterial DNA was done by DNA QIAmp mini kits (Qiagen, USA) following the protocol provided by the manufacturer. Amplification of genes that encode ESBLs was achieved with the gene-specific primers presented in Table 1.

Briefly, the PCR reaction volume was 25 µL and contained 12.5 µL of hot start Taq master mix DNA polymerase (2X) (Qiagen), 0.2 µL of each gene-specific primer (10 picomole), DNA template (2 µL), and nuclease-free water (9.2 µL). The PCR was carried out in thermal cycler (MJ Research PTC-100) as follows: initial denaturation (one cycle at 98°C for 10 minutes), then 32 cycles, including: denaturation (at 94°C for 30 seconds), annealing (at 62°C for 30 seconds) and lastly extension (at 72°C for 1 minute), followed by an extension cycle at 72°C for 10 minutes. The resulting amplicons were examined by gel electrophoresis (2% agarose) under an UV lamp and bands were identified by comparing their sizes to a 100 bp DNA ladder.[18]

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 22; USA) was used to analyze the obtained data. The variables were expressed as mean ± standard deviation (SD) and range if continuous and as numbers and percentages if categorical. A Chi-square test was used for comparing categorical variables. The binary logistic regression test was used to identify risk factors for DFIs and acquisitions of ESBL-producing organisms. Multivariate logistic regression was used to identify predictive associations of factors that showed statistical significance by univariate analysis. Statistical significance was considered at a p value <0.05.

Ethical approval

This study was designed and conducted in accordance with the Helsinki Declaration. Ethical approval was obtained from the Ethical Committee of Mansoura Faculty of Medicine-Institutional Research Board with a code number of R.20.09.1006. All participants gave their informed consent.

Results

Demographic characteristics of the studied patients

The present study included 104 type 2 diabetic patients who presented with foot ulcers. There were 63 males and 41 females. Their ages ranged from 42 to 66 years. The duration of diabetes was ≥10 years in 57 (54.8%) and <10 years in 47 (45.2%) of the investigated patients. Ulcers were located on the plantar aspect in 86/104 (82.7%) and on the dorsal aspect in 18/104 (17.3%) of the studied diabetic feet. The size of ulcers was ≤4 cm^2 in 72.1% and >4 cm^2 in 27.9% of the participants. The duration of DFUs was <3 months in 89.4% and >3 months in 10.6% of the enrolled patients.

Based on the bacteriological analysis of the swabs obtained from 104 DFUs, these ulcers were 82 (78.8%) infected and 22 (21.2%) non-infected. Factors that were significantly associated with infection of DFUs included: history of previous hospitalization for the same ulcer in the previous month (OR=13.4), peripheral neuropathy (OR=10.978), severe ulcers (OR=5.143), ulcers larger than 4 cm^2 (OR=4.90) and those with DM duration of more than 10 years (OR=3.348). However, the multivariate logistic regression analysis showed that the only significant independent risk factors for infection of DFUs were previous hospital admission (adjusted OR=12.2), peripheral neuropathy (adjusted OR=9.5) and DM of more than 10 years (adjusted OR=6.718) – Table 2.

Distribution of organisms isolated from DFUs

Aerobic bacterial cultures of the infected DFUs (N=82) yielded one type of the organisms for each DFU. GNB were more frequent than GPC (56.1% vs. 43.9%). The most frequently isolated species were *K. pneumoniae* (22, 26.8%), *S. aureus* and CoNS (18, 22% for each), and *P. mirabilis* (14, 17.1%), followed by *P. aeruginosa* (6, 7.3%). On the other hand, each of the *E. coli* and *Raoultella ornithinolytica* was isolated from only 2.4% of the DFU cultures. *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. simulans* accounted

for 38.9%, 27.8%, 22.2%, and 11.1% of the isolated CoNS, respectively (Table 3).

Although the long duration of DM (≥10 years), location of ulcer on the plantar aspect of the foot and previous hospitalizations were all significantly associated more with GPC infection, the strongest independent risk factor for infection GPC was hospital admission in the previous month for treating of the same DFU (adjusted OR=8.15; 95%CI=1.46-45.54). On the other hand, the only significant independent risk factor for infection with GNB was long duration of DM (≥10 years) (adjusted OR=12.04; 95%CI=2.278-63.682).

Antibiotic susceptibility profiles

Antibiogram pattern of staphylococcal spp. is presented in Table 4. All staphylococcal spp. (18 *S. aureus* and 18 CoNS) were resistant to cefoxitin and therefore identified as MRSA and MR-CoNS, respectively. These methicillin-resistant staphylococci showed the highest frequency of resistance to ampicillin (100%), amoxicillin/clavulanic acid (97.2%), rifampicin (94.4%), and erythromycin, trimethoprim/sulfamethoxazole (88.9% for each) followed by levofloxacin (77.8%), doxycycline (69.4%), clindamycin (41.7%), and gentamicin (38.9%). On the other hand, these staphylococcal spp. were more sensitive to linezolid (91.7%) and vancomycin (88.9%). Vancomycin resistance was detected in 3 (16.7%) of MRSA and in 1 (5.6%) of MR-CoNS. Susceptibility to vancomycin was validated by the microbroth dilution method, which identified 2 (11.1%) of MRSA and none of MR-CoNS as vancomycin resistant.

The antibiotic resistance patterns of GNB species are shown in Table 5. They showed a high percentage of resistance to the majority of the tested antibiotics, including cefaclor (97.8%), cefotaxime (93.5%), trimethoprim/ sulfamethoxazole (91.3%), aztreonam (84.8%), and ceftazidime (82.6%), followed by levofloxacin (76.1%) and cefepime (73.9%). However, they were most sensitive to piperacillin/tazobactam, amikacin and tigecycline (95.7% for each) and meropenem (89.1%).

K. pneumoniae was resistant to many of the tested antibiotics but was fully susceptible (100%) to amikacin. The next most active antibiotics against *K. pneumoniae* were piperacillin/tazobactam, meropenem, and tigecycline with resistance detected in only 2 (9.1%), 4 (18.2%), and 2 (9.1%), respectively. Similarly, all tested isolates of *P. mirabilis*, *E. coli* and *P. aeruginosa* exhibited high resistance to many of the antibiotics but full susceptibility to piperacillin/tazobactam, meropenem, amikacin and tigecycline. Cefepime was active against 50% of these isolates. All tested *E. coli*, 66.7% of *P. aeruginosa* and 71.4% of *P. mirabilis* were resistant to aztreonam.

Distribution of MDR organisms and risk factor analysis

The overall prevalence of MDR organisms (showing resistance to at least one antibiotic member in at least three antibiotic classes) among the studied isolates was 78/82 (95.1%). These MDR isolates included 18 MRSA, 18 MR-CoNS, and 42 GNB. Based on disc diffusion testing, ESBL production was suspected in all isolated Enterobacteriaceae. However, the combined disc method confirmed ESBL production in 20/38 (52.6%) of the Enterobacteriaceae. The highest ESBL production was among *K. pneumoniae* (12/22, 54.5%), followed by *P. mirabilis* (7/14, 50%) and *E. coli* (1/2, 50%).

Multiplex PCR analysis of ESBL genes revealed that all phenotypically confirmed ESBL producers contained genes coding for ESBLs. Overall, 38 ESBL genes were detected in 20 isolates. $bla_{CTX\text{-}M}$ (18/20, 90%) was the most frequently detected gene for ESBL, followed by bla_{TEM} (13/20, 65%) and bla_{SHV} (7/20, 35%). Nine isolates (45%) had a single ESBL gene, whereas 11 (55%) isolates had multiple ESBL genes. The $bla_{CTX\text{-}M}$ and bla_{TEM} genes were detected as a single ESBL gene in 7 (35%) and in 2 (10%) of the investigated isolates, respectively. On the other hand, four isolates (20%) contained both $bla_{CTX\text{-}M}$ and bla_{TEM} genes, whereas 7 (35%) isolates contained $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV}. There was no statistically significant difference in the distribution of ESBL genes among different types of the studied Enterobacteriaceae isolates (p=0.8) – Table 6 and Figure 1.

Table 2. Demographic data of the studied patients and clinical characters of their DFUs with infection risk analysis

Variables	DFUs No (%)		Univariate analysis Crude OR (95%CI)	Multivariate analysis Adjusted OR (95%CI)
	Infected (No=82)	Non-infected (No=22)		
Age				
<50 years	34 (42.5)	7 (31.8)	0.659 (0.243-1.789)	
≥50 years	48 (58.5)	15 (68.2)		
Sex				
Male	51 (62.2)	12 (54.5)	1.371 (0.530-3.547)	
Female	31 (37.8)	10 (45.5)		
Duration of DM				
<10 years	32 (39)	15 (68.2)	3.348 (1.231-9.109)*	6.718 (1.3-34.3)*
≥10 years	50 (61)	7 (31.8)		
Duration of DFU				
<3 months	74 (90.2)	19 (86.4)	0.685 (0.166-2.831)	
≥3 months	8 (9.8)	3 (13.6)		
Severity of DFU				
Severe	54 (65.9)	6 (27.3)	5.143 (1.812-14.6)*	1.845 (0.43-7.8)
Non-severe	28 (34.1)	16 (72.7)		
Size of DFU				
<4 cm	55 (67.1)	20 (90.9)	4.90 (1.07-22.55)*	4.26 (0.67-27.23)
≥4 cm	27 (32.9)	2		
Site of ulcer				
Dorsal	12 (14.6)	6 (27.3)	2.188 (0.713-6.707)	
Plantar	70 (85.4)	16 (72.7)		
Previous hospitalization				
Yes	47 (57.3)	2 (9.1)	13.4 (2.9-61.27)*	12.2 (1.97-75.61)*
No	35 (42.7)	20 (10090.9)		
Previous antibiotics intake				
Yes	50 (61)	5 (22.7)	5.31 (1.78-15.82)*	1.56 (0.336-7.186)
No	32 (39)	17 (77.3)		
Peripheral neuropathy				
Yes	52 (63.4)	3 (13.6)	10.978 (2.998-40.197)*	9.5 (1.9- 48.14)*
No	30 (36.6)	19 (86.4)		

*Statistical significance by logistic regression analysis at p value <0.05.
CI – confidence interval; **DFU** – diabetic foot ulcer; **DM** – diabetes mellitus; **OR** – odds ratio.

Table 3. Pathogens isolated from infected diabetic foot ulcers

Gram stain (No, %)	Organism	Number	Frequency (%)
Gram-positive cocci (36, 43.9%)	*S. aureus*	18	22
	S. epidermidis	7	8.5
	S. haemolyticus	5	6.1
	S. hominis	4	4.8
	S. simulans	2	2.4
Gram-negative bacilli (46, 56.1%)	*K. pneumoniae*	22	26.8
	P. mirabilis	14	17.1
	P. aeruginosa	6	7.3
	E. coli	2	2.4
	R. ornithinolytica	2	2.4
Total		**82**	**100**

Table 4. Antibiotic resistance among Gram-positive cocci isolated from diabetic foot ulcers

Antibiotic	Gram-positive cocci, n (%)		
	S. aureus (n=18)	CoNS (n=18)	Total (n=36)
Ampicillin	18 (100)	18 (100)	36 (100)
Amoxicillin/clavulanic acid	18 (100)	17 (94.4)	35 (97.2)
Cefoxitin	18 (100)	18 (100)	36 (100)
Erythromycin	16 (88.9)	16 (88.9)	32 (88.9)
Clindamycin	8 (44.4)	7 (39)	15 (41.7)
Doxycycline	15 (83.3)	10 (55.6)	25 (69.4)
Levofloxacin	14 (77.8)	14 (77.8)	28 (77.8)
Rifampicin	16 (88.9)	18 (100)	34 (94.4)
Gentamicin	8 (44.4)	6 (33.3)	14 (38.9)
Trimethoprim/sulfamethoxazole	14 (77.7)	18 (100)	32 (88.9)
Vancomycin	3 (16.7)	1 (5.6)	4 (11.1)
Linezolid	2 (11.1)	1 (5.6)	3 (8.3)

CoNS – coagulase-negative staphylococci.

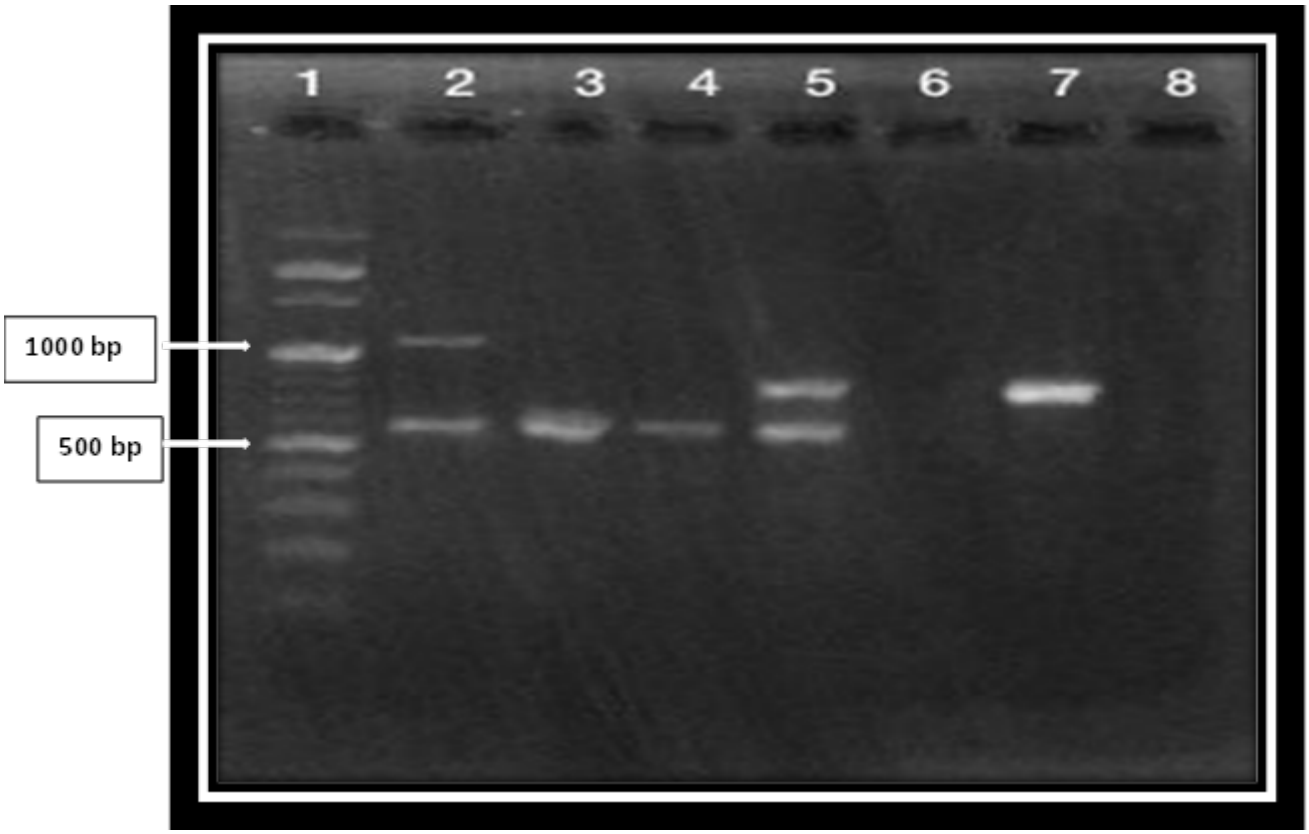
Table 5. Antibiotic resistance among Gram-negative bacilli isolated from diabetic foot ulcers

Antibiotic	Gram-negative bacilli, n (%)					
	K. pneumoniae (n=22)	*P. mirabilis* (n=14)	*P. aeruginosa* (n=6)	*E. coli* (n=2)	*R. ornithinolytica* (n=2)	Total (n=46)
Piperacillin/tazobactam	2 (9.1)	0	0	0	0	2 (4.3)
Cefaclor	22 (100)	13 (92.9)	6 (100)	2 (100)	2 (100)	45 (97.8)
Cefotaxime	22 (100)	13 (92.9)	6 (100)	1 (50)	1 (50)	43 (93.5)
Ceftazidime	18 (81.8)	12 (85.7)	5 (83.3)	1 (50)	2 (100)	38 (82.6)
Cefepime	22 (100)	7 (50)	3 (50)	1 (50)	1 (50)	34 (73.9)
Levofloxacin	20 (90.9)	6 (42.9)	6 (100)	2 (100)	1 (50)	35 (76.1)
Trimethoprim/sulfamethoxazole	22 (100)	14 (100)	5 (83.3)	0	1 (50)	42 (91.3)
Amikacin	0	0	0	0	2 (100)	2 (4.3)
Meropenem	4 (18.2)	0	0	0	1 (50)	5 (10.9)
Aztreonam	22 (100)	10 (71.4)	4 (66.7)	2 (100)	1 (50)	39 (84.8)
Tigecycline	2 (9.1)	0	0	0	0	2 (4.3)

Table 6. Distribution and frequency of ESBL genes among isolated Enterobacteriaceae

ESBL gene	Type of Enterobacteriaceae, n (%)				P value
	K. pneumoniae	*P. mirabilis*	*E. coli*	Total	
bla_{CTX-M}	4 (33.3)	3 (42.9)	0	7 (35%)	
bla_{TEM}	1 (8.3)	1 (14.3)	0	2 (10)	
bla_{CTX-M}, bla_{TEM}	3 (25)	1 (14.3)	0	4 (20)	0.8
bla_{CTX-M}, bla_{TEM}, bla_{SHV}	4 (33.3)	2 (28.6)	1 (100)	7 (35%)	
Total	12	7	1	20	

ESBL – extended-spectrum beta-lactamase.



Lane 1: 100 bp DNA ladder, Lane 2: *bla*$_{CTX-M}$ (585 bp) and *bla*$_{TEM}$ (1080 bp), Lane 3 and 4: *bla*$_{CTX-M}$ (585 bp), Lane 5: *bla*$_{CTX-M}$ (585 bp) and *bla*$_{SHV}$ (795 bp) and, Lane 6 and 8: no amplified product, Lane 7: *bla*$_{SHV}$ (795 bp)

Figure 1. Agarose gel electrophoresis of the amplified product of extended-spectrum beta-lactamase genes by multiplex-PCR

Table 7. Risk factor analysis for infection of DFUs with ESBL-producing Enterobacteriaceae

Variables	Univariate analysis			Multivariate analysis		
	P value	Crude OR	95% CI	P value	Adjusted OR	95% CI
Age	0.281	0.375	0.063-2.23			
Sex	0.804	1.182	0.316-4.424			
Duration of DM	0.911	1.125	0.142-8.937			
Duration of DFU	0.049	0.105	0.011-0.986	0.538	0.442	0.033-5.93
Severity of DFU	0.105	3.606	0.765-17.00			
Size of DFU	0.054	4.533	0.972-21.14			
Site of DFU	0.112	0.343	0.092-1.28			
Previous hospitalization	0.005	9.286	1.98-43.44	0.756	1.426	0.152-13.427
Previous antibiotic intake	0.025	7.2	1.27-40.67	0.207	5.003	0.410-61.09
Peripheral neuropathy	<0.001	20	3.8-104.6	0.007	15.5	2.104-114.28

CI – confidence interval; DFU – diabetic foot ulcer; DM – diabetes mellitus; ESBL – extended-spectrum beta-lactamase; OR – odds ratio.

Further analysis was performed to identify risk factors for infection of DFUs by MDR ESBL-producing organisms – Table 7. Peripheral neuropathy, hospital admissions within the previous month, previous antibiotic intake, and DM duration of ≥10 years were all found to be significantly associated with infection by ESBL-producing organisms. Peripheral neuropathy, on

the other hand, was the only identified significant independent risk factor for DFUs being infected with ESBL producers (adjusted OR=15.5, 95%CI=2.104-114.28).

Discussion

In the present study, DFUs patients were mostly males (61%). This agrees with another previous study that has shown that males are more susceptible to foot infection than females.[1] This might be due to factors such as variations in their lifestyles and work activities that force the feet to withstand greater pressure.[19] All patients were over the age of 40 years old. This might be because foot lesions in diabetic patients are more common in those with sensory neuropathy, who are usually elderly.[20]

Out of 104 DFUs with clinical suspicion of being infected, 78.8% revealed growth on aerobic bacterial culture. This is consistent with Markakis et al. who found that 60% of their patients with DFUs were infected at the time of presentation.[21]

In contrast to previous studies, all DFUs in the current study showed monobacterial infection with a single pathogen.[22,23] However, our finding of the predominance of monomicrobial DFI is in line with other studies.[19,24] These discrepancies could be related to variations in the duration of DFUs included in different studies where polymicrobial infection is usually associated with chronic DFUs.[25] In this study, 89.4% of the investigated patients had DFUs of less than three months, whereas only 10.6% had DFUs of three months or more. Additionally, here we did not investigate the presence of anaerobic bacteria, so monobacterial infections were found to be the most prevalent.

This present study showed that GNB were more frequently isolated from DFUs than GPC, representing 56.1% and 43.9%, respectively. Similarly, Dwedar et al., Gadepalli et al., and Bansal et al. reported GNB as the most predominant pathogens in DFIs (56%, 76%, and 51.4%, respectively).[26-28] In Egypt, Kamel et al. and Hefni et al. also demonstrated a higher prevalence of gram-negative bacteria than gram-positive bacteria in DFIs (65.5% vs. 34.5% and 67% vs. 30%, respectively).[29,30] The high frequency of DFIs with gram-negative bacteria in developing countries could be because most of the patients seek healthcare in a late advanced stage of the disease.[31] This was evident in our study, where more than half of the studied patients had severe DFUs on presentation. Additionally, the warm and humid climates in Egypt could be the reason behind the predominance of gram-negative bacteria in DFIs.[32]

However, in accordance to previous studies by Xie et al. and Spichler et al., aerobic gram-positive bacteria were the most common pathogens infecting DFUs.[33,34] Thus, there are wide variations in the causative pathogens causing infection of diabetic feet in the different regions. This disparity could be due to variety of factors such as variations in age, sex, ulcer severity, duration of ulcer, duration of DM, previous hospitalization, etc.[11] In this study, history of previous hospital admissions for the same DFU was the strongest independent risk factor for acquisition of GPC (8.15 fold). This suggests that stringent hygienic and isolation measures should be strictly enforced when admitting diabetic foot patients to hospitals in order to prevent infection cross-transmission.

K. pneumoniae was the most prevalent GNB in this study (47.8%), followed by *P. mirabilis* (30.4%) and *P. aeruginosa* (13%), while *E. coli* and *R. ornithinolytica* (2.4% for each) were the least common. The prevalence rate of single GNB in DFIs was variable in different studies. Ramani et al., Gadepalli et al., and Hamid et al. found that *Proteus* spp. was the most prevalent GNB in DFIs.[27,35,36] On the contrary, Chincholikar and Pal showed that *Pseudomonas* spp. was the most common.[37] In another study, the implication of *E. coli* and *Klebsiella* spp. as the principal causal pathogens in pyogenic DFIs was emphasized.[38] Likewise, previous studies conducted in our country have revealed different frequencies of GNB spp. than ours. Kamel et al. reported that the predominant GNB in DFIs were *Proteus* spp. (24.4%), followed by *E. coli* (11.6%), *Pseudomonas* spp. (9.2%) and *Klebsiella* spp. (8.2%) and the least common isolated organisms were *Serratia* spp., *Enterobacter* spp. and *Providencia* spp., each

accounting for 2% of the studied isolates.[29] Another study conducted in our country revealed different frequencies of GNB types, where *P. aeruginosa* was the most prevalent (19.4%), followed by *K. pneumoniae* (15.3%), and *Acinetobacter* spp. (10.2%).[30]

Regarding GPC, unlike other studies that recovered a variety of GPCs from DFIs, including staphylococci, streptococci, enterococci, and micrococci, we isolated only staphylococci (50% were *S. aureus* and the other 50% were CoNS).[9,29] Thus, it seems that DFIs in our community have a distinctive pattern of the causative organisms that differs from those in other countries, as well as within our own country. Interestingly, in clinical practice, understanding the microbiological profile of DFIs in a given area is critical for selecting the proper antibiotic and adopting the most effective infection control strategies based on the frequency of causal organisms.

Regarding the antibiotic susceptibility profile of the studied organisms, a high proportion of the isolated bacteria were multidrug-resistant. This finding is consistent with other studies elsewhere in Africa and Asia reporting high rates of MDR in *S. aureus*, Enterobacteriaceae, and *P. aeruginosa*.[26,39]

Gram-negative isolates in this study showed a high percentage of resistance to most of the tested antibiotics and this is consistent with other studies.[9,23] On the other hand, piperacillin/tazobactam, amikacin, tigecycline and meropenem were the most effective antibiotics against GNB. This is also similar to the findings of other previous studies.[9,40]

For GPC, all isolates of staphylococcal spp. were resistant to methicillin. This is similar to Shahi and Kumar, 2016, who have shown that almost 100% of *S. aureus* isolates from DFIs are MRSA.[11] However, Anvarinejad et al. and Murali et al. detected MRSA in 78% and 67% of their staphylococci isolated from diabetic wounds.[19,41] Other earlier studies have identified MRSA in lower proportions such as 15-30%.[24] The reason of such increase in prevalence of MRSA isolated from DFIs may be attributed to the absence of strict regulations for the use of antibiotics, non-adherence to measures of infection control in hospitals and an increased spread of MRSA in the community.[42]

Moreover, we found that both MRSA and MR-CoNS showed some degree of resistance to linezolid (8.3%) and to vancomycin (11.1%). This is in contrast to the previous studies, that reported a full susceptibility of the methicillin-resistant staphylococci to vancomycin and linezolid.[9,42] Therefore, the concept of using vancomycin and linezolid as empirical treatments for DFIs in our region should be revised to avoid treatment failures in such cases.

ESBL production is an important mechanism of plasmid mediated MDR among GNB. This study found a high percentage of ESBL producers (52.6%) among Enterobacteriaceae isolated from DFIs, which is higher than the prevalence rate of ESBL producers (14% and 6%) detected among isolates of DFIs in other studies performed in our locality.[29,43]

In our study, ESBL was highest among *K. pneumoniae*, followed by *P. mirabilis* and *E. coli*. The bla_{CTX-M} gene was the most frequent detected ESBL gene (90%), followed by the bla_{TEM} gene (65%) and the bla_{SHV} gene (35%). This is consistent with the previous findings of Kamel et al. who found that bla_{CTX-M} was the most prevalent ESBL gene (100%), followed by the bla_{TEM} gene (50%) and bla_{SHV} (37.5%).[29] Similarly, Zubair et al. in India found that bla_{CTX-M} was the most frequently detected ESBL gene among DFUs (82%), followed by bla_{TEM} (50%) and bla_{SHV} (46.9%).[31]

Microbial resistance to antibiotics is a threat among patients with DFIs because it leads to treatment failure with a bad prognosis.[44] We found a high frequency of MDR bacteria (95.1%) infecting DFUs, which is substantially higher than that reported by Gadepalli et al., Amini et al. and Adeyemi et al. (72%, 75%, and 80%, respectively).[27,39] In developing countries, the rising rate of MDR organisms in DFIs could be due to many factors, such as the unwise use of broad-spectrum antibiotics, unrestricted access to antibiotics, the chronicity of the wound and frequent times of admissions to hospitals.[23]

Antibiotic resistance mechanisms in routine bacteriology laboratory take a long time (48 h or more). However, to improve the prognosis of patients with DFIs, they should receive the most appropriate antibiotics as soon as they are admitted to the hospital. Therefore, knowing the risk factors for MDR infection is important for prompt, accurate antibiotic treatment and a favorable prognosis.

In this study, peripheral neuropathy was the only significant independent predictor for infection with ESBL-producing bacteria. Impaired peripheral sensation in diabetics makes them more likely to develop foot ulcers and could make these ulcers more widespread as a result of continual painless damage. In developing countries, such patients with painless DFU could depend on the use of self-prescribed antibiotics for a long time rather than attending medical centers for proper treatment. This would lead to the selective pressure of MDR organisms.[45]

Similar to our findings, Adeyemi et al. also reported peripheral neuropathy as an independent risk factor for infection with MDR organisms, with an adjusted odds ratio of 4.05 and 95%CI of 1.08-15.13.[46] On the other hand, other different risk factors were reported by other studies, such as large ulcer size by Noor et al., long duration of DFU by both Adeyemi et al. and Hartemann-Heurtir et al., and prolonged hospital admissions by Kandemir.[12,46-48] The variation in risk factors observed in different studies could be attributed to geographical differences and the inclusion of patients with varying clinical characteristics.[44]

Conclusions

A specific pattern of aerobic bacteria causing DFIs has been found in our community. Furthermore, the pattern of antibiotic sensitivity of these bacteria differs from other communities. GNB were found more often than GPC. Amikacin, tigecycline, and meropenem were the best antibiotics for GNB, whereas linezolid and vancomycin were the best for staphylococci. These microbiological data should be available to practitioners in our area for planning the best empirical antibiotic treatment of DFIs for a good prognosis.

Authors' contributions statement: MM carried out of the laboratory work of the study and wrote the original draft of the manuscript. MAEK contributed to selection of patients and revision of the statistical analysis. MI contributed to collection of clinical samples and their relevant data. WK contributed to concept and design of the study, revision of the manuscript and approved the final version for publishing. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

Acknowledgements: The authors would like to thank the patients & staff of Mansoura University Hospital and to express their sincere gratitude to the laboratory technicians of their microbiology unit for their valuable efforts.

References

1. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. Int J Microbiol. 2011;2011:605195. https://doi.org/10.1155/2011/605195
2. Hadadi A, Omdeh Ghiasi H, Hajiabdolbaghi M, Zandekarimi M, Hamidian R. Diabetic foot: infections and outcomes in Iranian admitted patients. Jundishapur J Microbiol. 2014;7:e11680. https://doi.org/10.5812/jjm.11680
3. International Diabetes Federation. IDF Diabetes Atlas. 9th edition. IDF; 2019. Accessed on: 25 December 2019. Available at: https://www.diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-fial-web.pdf.
4. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. Clin Infect Dis 2007;44:562-5. https://doi.org/10.1086/511036
5. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Ann Med 2017;49:106-16. https://doi.org/10.1080/07853890.2016.1231932
6. Assaad-Khalil SH, Zaki A, Abdel Rehim A, et al. Prevalence of diabetic foot disorders and related risk factors among Egyptian subjects with diabetes. Prim Care Diabetes. 2015;9:297-303. https://doi.org/10.1016/j.pcd.2014.10.010
7. Lipsky BA, Senneville É, Abbas ZG, et al. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diabetes Metab Res Rev. 2020;36 Suppl 1:e3280. https://doi.org/10.1002/dmrr.3280
8. Gentry LO. Diagnosis and management of diabetic foot ulcer. J Antimicrob Chemother. 1993;32 Suppl A:77-89. https://doi.org/10.1093/jac/32.suppl_A.77
9. Banashankari GS, Rudresh HK, Harsha AH. Prevalence of gram negative bacteria in diabetic foot - a clinico-

microbiological study. Al Ameen J Med Sci 2012;5:224-32.
10. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer – a review on pathophysiology, classification and microbial etiology. Diabetes Metab Syndr. 2015;9:192-9. https://doi.org/10.1016/j.dsx.2015.04.007
11. Shahi SK, Kumar A. Isolation and genetic analysis of multidrug resistant bacteria from diabetic foot ulcers. Front Microbiol. 2016;6:1464. https://doi.org/10.3389/fmicb.2015.01464
12. Kandemir Ö, Akbay E, Şahin E, Milcan A, Gen R. Risk factors for infection of the diabetic foot with multiantibiotic resistant microorganisms. J Infect. 2007;54:439-45. https://doi.org/10.1016/j.jinf.2006.08.013
13. Shahi SK, Kumar A, Kumar S, Singh SK, Gupta SK, Singh TB. Prevalence of diabetic foot ulcer and associated risk factors in diabetic patients from north India. J Diab Foot Compl, 2012;4:83-91.
14. Dos Santos VP, da Silveira DR, Caffaro RA. Risk factors for primary major amputation in diabetic patients. Sao Paulo Med J. 2006;124:66-70. https://doi.org/10.1590/S1516-31802006000200004
15. Demetriou M, Papanas N, Panopoulou M, Papatheodorou K, Bounovas A, Maltezos E. Tissue and swab culture in diabetic foot infections: neuropathic versus neuroischemic ulcers. Int J Low Extrem Wounds. 2013;12:87-93. https://doi.org/10.1177/1534734613481975
16. Clinical and Laboratory Standards Institute. Performance standards of antibiotic susceptibility testing. 27th ed. Supplement M100. Wayne, PA: CLSI; 2017.
17. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18: 268-81. https://doi.org/10.1111/j.1469-0691.2011.03570.x
18. Roschanski N, Fischer J, Guerra B, Roesler U. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-Type AmpCs in Enterobacteriaceae. PLoS One. 2014;9:e100956. https://doi.org/10.1371/journal.pone.0100956
19. Anvarinejad M, Pouladfar G, Japoni A, et al. Isolation and antibiotic susceptibility of the microorganisms isolated from diabetic foot infections in Nemazee Hospital, Southern Iran. J Pathog. 2015;328796. https://doi.org/10.1155/2015/328796
20. Lipsky BA, Pecoraro RE, Ahroni JH. Foot ulceration and infections in elderly diabetics. Clin Geriatr Med. 1990;6:747-69. https://doi.org/10.1016/S0749-0690(18)30579-2
21. Markakis K, Bowling FL, Boulton AJ. The diabetic foot in 2015: an overview. Diabetes Metab Res Rev. 2016;32 Suppl 1:169-78. https://doi.org/10.1002/dmrr.2740
22. Papini M, Cicoletti M, Fabrizi V, Landucci P. Skin and nail mycoses in patients with diabetic foot. G Ital Dermatol Venereol. 2013;148:603-8.
23. Perim MC, Borges Jda C, Celeste SR, et al. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. Rev Soc Bras Med Trop. 2015;48:546-54. https://doi.org/10.1590/0037-8682-0146-2015
24. Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot infections in South India. Eur J Int Med. 2005;16:567-70. https://doi.org/10.1016/j.ejim.2005.06.016
25. Omar NS, El-Nahas MR, J. Gray J. Novel antibiotics for the management of diabetic foot infections. Int J Antimicrob Agents. 2008;31:411-9. https://doi.org/10.1016/j.ijantimicag.2007.10.016
26. Dwedar R, Ismail DK, Abdulbaky A. Diabetic foot infection: microbiological causes with special reference to their antibiotic resistance pattern. Egypt J Med Microbiol. 2015;24:95-102. https://doi.org/10.12816/0024935
27. Gadepalli R, Dhawan B, Kapil A. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care. 2006;29:1727-32. https://doi.org/10.2337/dc06-0116
28. Bansal E, Garg A, Bhatia S, Attri A, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol. 2008;51:204-8. https://doi.org/10.4103/0377-4929.41685
29. Kamel N, Abouelwafa M, Eltayeb W, Aboshanab K. Antibiotic resistance pattern of aerobic bacteria isolated from patients with diabetic foot ulcers in Egypt. Afr J Microbiol Res. 2014;8:2947-54. https://doi.org/10.5897/AJMR2014.6909
30. Hefni AA, Ibrahim AR, Attia KM, et al. Bacteriological study of diabetic foot infection in Egypt. J Arab Soc Med Res. 2013;8:26-32.
31. Zubair M. Prevalence and interrelationships of foot ulcer, risk factors and antibiotic resistance in foot ulcers in diabetic populations: a systematic review and meta-analysis. World J Diabetes 2020;11:78-89. https://doi.org/10.4239/wjd.v11.i3.78
32. Shakil S, Khan AU. Infected foot ulcers in male and female diabetic patients: a clinico-bioinformative study. Ann Clin Microbiol Antimicrob. 2010;9:2. https://doi.org/10.1186/1476-0711-9-2
33. Xie X, Bao Y, Ni L, et al. Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: focus on the differences among different Wagner's grades, IDSA/IWGDF grades, and ulcer types. Int J Endocrinol. 2017;2017:8694903. https://doi.org/10.1155/2017/8694903
34. Spichler A, Hurwitz BL, Armstrong DG, Lipsky BA. Microbiology of diabetic foot infections: from Louis Pasteur to 'crime scene investigation'. BMC Med. 2015;13:2. https://doi.org/10.1186/s12916-014-0232-0
35. Ramani A, Ramani R, Shivananda PG, Kundaje GN. Bacteriology of diabetic foot ulcers. Indian J Pathol Microbiol. 1991;34:81-7.

36. Hamid MH, Arbab AH, Yousef BA. Bacteriological profile and antibiotic susceptibility of diabetic foot infections at Ribat University hospital; a retrospective study from Sudan. J Diabetes Metab Disord. 2020;19:1397-406. https://doi.org/10.1007/s40200-020-00660-8
37. Chincholikar DA, Pal RB. Study of fungal and bacterial infections of the diabetic foot. Indian J Pathol Microbiol. 2002;45:15-22.
38. Dezfulian A, Salehian MT, Amini V, et al. Bacteriological study of diabetic foot infections in an Iranian hospital. Iran Red Crescent Med J. 2011;13:590-1.
39. Amini M, Davat A, Piri M. Determination of the resistance pattern of prevalent aerobic bacterial infections of diabetic foot ulcer. Iranian J Pathol. 2013;8:21-6.
40. Saseedharan S, Sahu M, Chaddha R, et al. Epidemiology of diabetic foot infections in a reference tertiary hospital in India. Braz J Microbiol. 2018;49:401-6. https://doi.org/10.1016/j.bjm.2017.09.003
41. Murali TS, Kavitha S, Spoorthi J, et al. Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. J Med Microbiol. 2014;63(Pt 10):1377-85. https://doi.org/10.1099/jmm.0.076034-0
42. Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health. 2012;5:1-8. https://doi.org/10.1016/j.jiph.2011.07.004
43. Motta RN, Oliveira MM, Magalhães PS, et al. Plasmid-mediated extended-spectrum beta lactamase-producing strains of Enterobacteriaceae isolated from diabetes foot infections in a Brazilian diabetic center. Braz J Infect Dis. 2003;7:129-34. https://doi.org/10.1590/S1413-86702003000200006
44. Richard JL, Sott A, Jourdan N, et al. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. Diabetes Metab. 2008;34(4 Pt 1):363-9. https://doi.org/10.1016/j.diabet.2008.02.005
45. Akash MSH, Rehman K, Fiayyaz F, Sabir S, Khurshid M. Diabetes-associated infections: development of antimicrobial resistance and possible treatment strategies. Arch Microbiol. 2020;202:953-65. https://doi.org/10.1007/s00203-020-01818-x
46. Adeyemo AT, Kolawole B, Rotimi VO, Aboderin AO. Multicentre study of the burden of multidrug-resistant bacteria in the aetiology of infected diabetic foot ulcers. Afr J Lab Med. 2021;10:1261. https://doi.org/10.4102/ajlm.v10i1.1261
47. Noor S, Borse AG, Ozair M, Raghav A, Parwez I, Ahmad J. Inflammatory markers as risk factors for infection with multidrug-resistant microbes in diabetic foot subjects. Foot (Edinb). 2017;32:44-8. https://doi.org/10.1016/j.foot.2017.05.001
48. Hartemann-Heurtir A, Robert J, Jacqueminet S, et al. Diabetic foot ulcer and multirug-resistant organisms: risk factors and impact. Diabet Med. 2004;21:710-5. https://doi.org/10.1111/j.1464-5491.2004.01237.x

Please cite this article as:
Mashaly M, Abo El kheir M, Ibrahim M, Khafagy W. Aerobic bacteria isolated from diabetic foot ulcers of Egyptian patients: types, antibiotic susceptibility pattern and risk factors associated with multidrug-resistant organisms. GERMS. 2021;11(4):570-582. doi: 10.18683/germs.2021.1292