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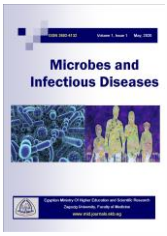

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Original article

Microbiological profile of diabetic foot infections

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ABSTRACT

Background: About 15% to 25% of people with diabetes will develop a foot ulcer. These wounds are often resistant to healing; therefore, people with diabetes are 20 times more likely to experience lower limb amputations than non-diabetic individuals. **Aim of the study*:*** To identify the causative organisms causing diabetic foot ulcers (DFUs) and to determine their antibiotic susceptibility. **Methods:** This is an observational cross-sectional study that included 100 different diabetic foot wound specimens collected from the patients attending at the vascular outpatient clinics of Cairo University Hospitals over the period from April 2022 to October 2022. Antibiotic susceptibility was identified by disc diffusion method and MIC. **Results:** The prevalence of Gram-negative isolates (75.4%) (89/118) was more than the Gram-positive (22%) (26/118). The most common isolated organisms were *Klebsiella* spp. (24%) (28/118 isolates), *Proteus* spp. (17.8%) (21/118 isolates), *Pseudomonas* spp. (16%) (19/118 isolates) and *Staphylococcus aureus* (13.5%) (16/118 isolates). Three *Candida albicans* isolates were recovered from the 118 isolates (2.6%). Multidrug-resistance (MDR) was detected in 67% (79/118 isolates), extensive drug resistant (XDR) was found in 24.5% (29/118 isolates). Extended-spectrum β-lactamase (ESBL) was found in 45% of the Gram-negative isolates (40/89 isolates), 22.5% of the Gram-negative isolates were carbapenem resistant *Enterobacteriaceae* (CRE) (20/89 isolates) and 46% of the Gram-positive isolates were methicillin-resistant *Staph*ylococcus *aureus* (MRSA) (12/26 isolates). Colistin resistance was found in 6% (4/66) of the Gram-negative isolates by broth microdilution method. **Conclusions:** DFUs are mostly monomicrobial, more in type 2 DM. As per Wagner's classification, the prevalence of grade 3 ulcers is the highest.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. As the prevalence of diabetes has increased, foot complications also increased along with infections [1].

Diabetic foot ulcers (DFUs) are microvascular complications of DM associated with a marked increase in morbidity and mortality. They are common with potentially devastating complications. Infection thrives in more than half of foot ulcers and is the main factor that DFUs most often lead to lower extremity amputation [2].

To prevent the exacerbation of the condition and eliminate the potential for amputation, on-time forceful management of DFUs is needed.

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This could be done by employing multidisciplinary management approaches focusing on prevention, learning, regular foot assessment, aggressive intervention and optimal use of therapeutic footwear [3].

Managing infections require proper diagnosis, accurate classification of lesions, obtaining appropriate specimens for culture and selecting empirical then definitive antimicrobial therapy. The quick determination of when surgical intervention is needed and providing all the necessary types of wound care is also crucial. Certain factors such as illiteracy, poor socioeconomic status, barefoot walking, ignorance about diabetic foot care and delayed presentation of patients to clinics worsen the situation and increase the prevalence of DFUs [4].

Methodology

Study settings: This study included 100 different diabetic foot wound specimens collected from the patients attending at the vascular outpatient clinics of Cairo University Hospitals over the period from April 2022 to October 2022. The samples were submitted to the laboratory of the Medical Microbiology and Immunology department within 1-2 hours and cultivated immediately.

Study design: This is an observational cross-sectional study.

Sample size calculation: The sample size was calculated using "statistics and sample size pro"; considering the following data: *Staphylococcus aureus* is isolated from 38.4% of cases (18) with alpha error 0.05 and the power of the study is 90%. So, the sample size should include 91 patients.

Study subjects' inclusion criteria: Infected diabetic foot wounds grade 1-5 according to Wagner's classification system for DFU **(Table 1)**.

Study subjects' exclusion criteria: Infected wounds in non-diabetic patients.

- Note: Grade 1-3 ulcers are termed non-gangrenous ulcers and Grade 4 and 5 ulcers are termed gangrenous ulcers.

Specimens collection, transport and storage [6]:

Swabs were collected from the deeper portion of the ulcers with a sterile swab under aseptic conditions. Two swabs were collected from each patient and one was soaked in thioglycolate broth for anaerobic cultivation. The samples were submitted to the laboratory of the Medical Microbiology and Immunology department within 1-2 hours and cultivated immediately.

Cultivation of the specimens [6]:

Clinical specimens were cultivated on blood agar, MacConkey's agar and sabouraud dextrose agar (SDA) plates (Himedia, India). The plates were incubated aerobically at 37^{o}C for 24 hours.

Swabs impeded in thioglycolate broth were cultured on blood agar and incubated anaerobically at 37 ^{o}C for 3-5 days.

Identification of the isolates [6]:

Identification of the isolates was done according to colony morphology and the conventional microbiological standard tests (Gram stain, oxidase test, Triple Sugar Iron test (TSI), citrate test, urease test, Lysine Decarboxylase test (LDC) and Motility Indole Ornithine (MIO) test).

Candida albicans were identified by forming creamy white colonies on SDA, confirmed by morphological features under the microscope and germ tube test.

Germ tube test was done for presumptive discrimination of *Candida albicans* from other *Candida* species [6].

Antibiotic susceptibility testing [7]:

The antibiotic susceptibility testing was done for all the bacterial isolates using Kirby-Bauer modified disc diffusion technique [7].

All Gram-negative bacterial isolates (except *Proteus* spp., *Providencia* spp. and *Morganella* spp.) were examined by the broth microdilution method and agar dilution method for colistin minimum inhibitory concentration (MIC) determination while for Gram-positive *Staphylococcus aureus* isolates, vancomycin MIC determination was done by agar dilution method [7].

Determination of colistin MIC using broth microdilution method for Gram-negative bacteria [7]:

Colistin sulphate powder (6 million I.U. /gm.) (AMOUN Pharmaceuticals Co., Egypt) was used for the determination of MIC as recommended by CLSI, 2022 [7].

MICs were determined as the highest dilution of the antibiotic that visually inhibited the growth of the tested organism as demonstrated by

turbidity and reading the turbidity using microplate reader.

Determination of colistin MIC using agar dilution method for Gram-negative bacteria [7]:

The MICs of colistin were determined by agar dilution method using Mueller Hinton agar (Oxoid, UK) according to the CLSI 2022 [7].

Determination of MICs of vancomycin by agar dilution method for *Staphylococcus aureus* isolates [7]:

The MICs of vancomycin were determined by agar dilution method using Mueller Hinton agar (Oxoid, UK) according to the CLSI 2022 [7].

Phenotypic tests for the detection of ESBL, MRSA and inducible clindamycin resistance:

When using the CLSI 2022 [7] breakpoints, routine ESBL testing is no longer necessary before reporting results. However, ESBL testing may still be useful for epidemiological or infection prevention purposes. ESBL production was suspected when reduced susceptibility to two or more indicator antibiotics (cefotaxime 30µg, ceftriaxone 30µg, ceftazidime 30µg, aztreonam 30µg) was observed, which was confirmed by detecting an increase in the zone of inhibition towards a disk containing amoxicillin-clavulanate 20/10µg placed 15mm from the cephalosporins disks (ceftriaxone, cefotaxime or ceftazidime) [8,9].

The phenotypic test for the detection of MRSA was done by using a cefoxitin 30µg disc. A zone of inhibition which was equal to or more than 22 mm was considered susceptible to cefoxitin and the organism was reported as methicillin-sensitive *Staphylococcus aureus* (MSSA). Those isolates which produced a zone of inhibition less than or equal to 21 mm were considered MRSA [7].

Inducible clindamycin resistance was tested by placing 15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart. Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) was considered as ICR [7].

Determination of multi-drug resistant (MDR) phenotype and extensively drug resistance (XDR) phenotype [10]:

Multi-drug resistant (MDR) phenotype is defined as bacteria which is resistant to more than one antimicrobial agent in three or more antimicrobial categories. Extensively drug resistance (XDR) phenotype is defined as bacteria which is resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less [10].

Statistical methods:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum and maximum for quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. For comparing categorical data, the Chi square ($\chi 2$) test was performed. An exact test was used instead when the expected frequency is less than 5 [11]. Standard diagnostic indices including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic efficacy were calculated as described by Galen [12]. P-values less than 0.05 were considered statistically significant.

Results

Demographic distribution of patients

In the present study, a total of 118 spp. was isolated from 81 different specimens collected from patients visiting the vascular surgery department clinics at Cairo University Hospitals during the period from April 2022 to October 2022. 70% of the patients were males (57 male patients) and 30% were females (24 female patients). Their ages ranged from 30 to 84 with a mean of 60.67 and a standard deviation of 11.76.

Number of organisms in each specimen

In the present study, growth of one organism after 24h incubation was found in 56% (45 specimens), two organisms in 43% (35 specimens) and 3 organisms in one percent (one specimen) **(Figure 1)**.

Presence of co-morbidities with diabetes

In the present study, 53% of the patients had co-morbidities (43 patients) in the form of hypertension and cardiovascular diseases while 47% did not suffer from any co-morbidities (38 patients).

Type of DM

In the present study, 16% (13 patients) had type 1 diabetes mellitus (T1DM) and 84% (68 patients) had type 2 diabetes mellitus (T2DM).

Duration of DM

In the current study, 44% of the patients had DM for up to 10 years (36 patients) while 56% suffered from it for more than 10 years (45 patients).

Grading of the DFU according to Wagner's classification

In the present study, 20% had grade 1 ulcers (16 patients), 26% had grade 2 ulcers (21 patients), 32% had grade 3 ulcers (26 patients) and 22% had grade 4 ulcers (18 patients) according to Wagner's classification **(Table 1, Figure 2)**.

Types of organisms isolated from the studied specimens

In the current study, 118 isolates were recovered, 75.4% were Gram-negative (89 isolates), 22% were Gram-positive (26 isolates) while 2.6% were *Candida albicans* (3 isolates). Out of the 118 isolates, 12% were *E. coli.* (14 isolates), 24% were *Klebsiella* spp. (28 isolates), 9.3% were *Proteus vulgaris* (11 isolates), 8.5% were other *Proteus* spp. (10 isolates), 0.8% was *Enterobacter* spp. (one isolate), 0.8% was *Providencia* spp. (one isolate), 0.8% was *Morganella* spp. (one isolate), 16% were *Pseudomonas* spp. (19 isolates), 3.3% were *Acinetobacter* spp. (four isolates), 13.5% were *Staphylococcus aureus* (16 isolates), 5% were *Enterococcus* spp. (6 isolates), 3.4% were *Streptococcus* spp. (four isolates) and 2.6% were *Candida albicans* (3 isolates).

Antibiotic susceptibility tests by disc diffusion method

A. Gram negative isolates

- Results of antibiotic susceptibility tests by disc diffusion method performed on *E. coli*, *Klebseilla* spp., *Proteus vulgaris*, Other *Proteus* spp., *Pseudomonas* spp., and *Acinetobacter* spp.isolates are presented in (**Table 2)**.
- Results of antibiotic susceptibility tests by disc diffusion method performed on *Enterobacter* spp., *Providencia* spp., and *Morganella* spp. isolates were as follows:
  - *Enterobacter* spp. isolates were resistant to AMP, AMC, A/S, PIT, CZ, CX, CTX, CAZ, CPM, TE, DO, CIP, LE and COT.
  - *Enterobacter* spp. isolates were susceptible to AT, GEN, ETP, IPM, MRP and AK.
  - *Providencia* spp. isolates were resistant to AMP, AMC, A/S, PIT, CZ, CX, CTX, CAZ, CPM, AT, GEN, AK, CIP, LE and COT.
  - *Providencia* spp. isolates were susceptible to ETP, IPM and MRP.
  - *Morganella* spp. isolates were resistant to AMP, AMC, A/S, CZ, CAZ, CIP and COT.
  - *Morganella* spp. isolates were susceptible to PIT, CX, CTX, CPM, AT, GEN, ETP, IPM, MRP, AK and LE.

Prevalence of antibiotic resistance in the isolated bacteria

In the current study, out of the 118 isolates, 67% were multidrug-resistant (MDR) (79 isolates), 24.5% were extensive drug resistant (XDR) (29 isolates).

Extended spectrum β-lactamase (ESBL) was found in 45% out of the 89 Gram-negative isolates (40 isolates), 22.5% of the 89 Gram-negative isolates were carbapenem-resistant *Enterobacteriaceae* (CRE) (20 isolates) and 46% of the 26 Gram-positive isolates were methicillin-resistant *Staph*ylococcus *aureus* (MRSA) (12 isolates).

Colistin MIC (broth microdilution method vs agar dilution method for Gram-negative isolates)

In the present study, 6% were resistant to colistin by the broth microdilution method (four Gram-negative isolates) while 94% were colistin susceptible by the same method (62 Gram-negative isolates). Out of the four colistin-resistant Gram-negative isolates; 25.0% were *E. coli* (one isolate) and 75% were *Klebsiella* spp. (three isolates).

Prevalence of vancomycin resistance among Gram-positive isolates

In the present study, all Gram-positive isolates were sensitive to vancomycin.

Table 1. Wagner's classification system for DFU [5].

Ulcer grading	Description
Grade 0	No ulcer but high-risk foot
Grade 1	Superficial ulcer
Grade 2	Deep ulcer, no bony involvement or abscess
Grade 3	Abscess with bony involvement (as shown by X-ray)
Grade 4	Localized gangrene e.g., Toe, heel etc.
Grade 5	Extensive gangrene involving the whole foot

Table 2. Antibiotic susceptibility profile of Gram-negative isolates.

Gram negative		***E. coli*** (n=14)		***Klebseilla* spp.** (n=28)		***P. vulgaris*** (n=11)		**Other *Proteus* spp.** (n=10)		***Pseudomonas* spp.** (n=19)		***Acinetobacter* spp.** (n=4)	
		R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
AMP	**N**	14	0	0	0	9	2	8	2	0	0	0	0
	%	100.0%	0.0%	0.0%	0.0%	81.8%	18.2%	80%	20%	0.0%	0.0%	0.0%	0.0%
A/S	**N**	13	1	20	8	8	3	6	4	0	0	2	2
	%	92.9%	7.1%	71.4%	28.6%	72.7%	27.3%	60%	40%	0.0%	0.0%	50.0%	50.0%
AMC	**N**	14	0	19	9	8	3	6	4	0	0	0	0
	%	100.0%	0.0%	67.8%	32.2%	72.7%	27.3%	60%	40%	0.0%	0.0%	0.0%	0.0%
PIT	**N**	12	2	20	8	5	6	4	6	5	14	2	2
	%	85.7%	14.3%	71.4%	28.6%	45.4%	54.6%	40%	60%	26.4%	73.6%	50.0%	50.0%
CZ	**N**	13	1	24	4	7	4	8	2	0	0	0	0
	%	92.9%	7.1%	85.7%	14.3%	63.6%	36.4%	80%	20%	0.0%	0.0%	0.0%	0.0%
CPM	**N**	12	2	22	6	6	5	6	4	15	4	3	1
	%	85.7%	14.3%	78.5%	21.5%	54.6%	45.4%	60%	40%	78.9%	21.1%	75.0%	25.0%
CTX	**N**	8	6	20	8	7	4	6	4	0	0	3	1
	%	57.1%	42.9%	71.4%	28.6%	63.6%	36.4%	60%	40%	0.0%	0.0%	75.0%	25.0%
CX	**N**	10	4	22	6	8	3	7	3	0	0	0	0
	%	71.4%	28.6%	78.5%	22.2%	72.7%	27.3%	70%	30%	0.0%	0.0%	0.0%	0.0%
CAZ	**N**	13	1	24	4	9	2	7	3	16	3	3	1
	%	92.9%	7.1%	85.7%	14.3%	81.8%	18.2%	70%	30%	84.2%	15.8%	75.0%	25.0%
ETP	**N**	4	10	11	17	2	9	2	8	0	0	0	0
	%	28.6%	71.4%	39.2%	60.8%	18.2%	81.8%	20%	80%	0.0%	0.0%	0.0%	0.0%
IPM	**N**	2	12	8	20	2	9	1	9	1	18	2	2
	%	14.3%	85.7%	28.6%	71.4%	18.2%	81.8%	10%	90%	5.3%	94.7%	50.0%	50.0%
MRP	**N**	2	12	10	18	3	8	2	8	2	17	1	3
	%	14.3%	85.7%	35.7%	64.3%	27.3%	72.7%	20%	80%	10.5%	89.5%	25.0%	75.0%
AT	**N**	9	5	14	14	4	7	4	6	6	13	0	0
	%	64.3%	35.7%	50%	50%	36.4%	63.6%	40%	60%	31.6%	68.4%	0.0%	0.0%
GEN	**N**	9	5	11	17	5	6	4	6	10	9	3	1
	%	64.3%	35.7%	39.2%	60.8%	45.4%	54.6%	40%	60%	52.6%	47.4%	75.0%	25.0%
AK	**N**	7	7	11	17	3	8	3	7	6	13	1	3
	%	50.0%	50.0%	39.2%	60.8%	27.3%	72.7%	30%	70%	31.6%	68.4%	25.0%	75.0%
TE	**N**	12	2	18	10	0	0	0	0	0	0	1	3
	%	85.7%	14.3%	64.2%	35.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	25.0%	75.0%
DO	**N**	11	3	16	12	0	0	0	0	0	0	2	2
	%	78.6%	21.4%	57%	43%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	50.0%	50.0%
LE	**N**	12	2	17	11	3	8	3	7	11	8	3	1
	%	85.7%	14.3%	60.8%	39.2%	27.3%	72.7%	30%	70%	57.9%	42.1%	75.0%	25.0%
CIP	**N**	13	1	17	11	3	8	4	6	12	7	3	1
	%	92.9%	7.1%	60.8%	39.2%	27.3%	72.7%	40%	60%	63.2%	36.8%	75.0%	25.0%
COT	**N**	12	2	23	5	9	2	9	1	0	0	3	1
	%	85.7%	14.3%	82%	18%	81.8%	18.2%	90%	10%	0.0%	0.0%	75.0%	25.0%

Ampicillin (AMP), amoxicillin-clavulanate (AMC), ampicillin-sulbactam (A/S), piperacillin-tazobactam (PIT), cefazolin (CZ), cefoxitin (CX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), aztreonam (AT), gentamycin (GEN), ertapenem (ETP), imipenem (IPM), meropenem (MRP), amikacin (AK), tetracycline (TE), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT)).

According to CLSI 2024 [13] gentamycin is no more used with *Pseudomonas* spp. and amikacin is only used with urine samples.

Table 3. Antibiotic susceptibility profile of Gram-positive isolates.

Gram positive	*Enterococcus* spp. (n=6)				*Streptococcus* spp. (n=4)				*S. aureus* (n=16)			
	Resistant		Sensitive		Resistant		Sensitive		Resistant		Sensitive	
	N	%	N	%	N	%	N	%	N	%	N	%
AMP	0	0.0%	6	100.0%	2	50.0%	2	50.0%	0	0.0%	0	0.0%
CTX	0	0.0%	0	0.0%	2	50.0%	2	50.0%	0	0.0%	0	0.0%
CX	0	0.0%	0	0.0%	0	0.0%	0	0.0%	12	75.0%	4	25.0%
ETP	0	0.0%	0	0.0%	0	0.0%	4	100.0%	0	0.0%	0	0.0%
MRP	0	0.0%	0	0.0%	0	0.0%	4	100.0%	0	0.0%	0	0.0%
GEN	0	0.0%	0	0.0%	0	0.0%	0	0.0%	9	56.3%	7	43.8%
TE	5	83.3%	1	16.7%	3	75.0%	1	25.0%	6	37.5%	10	62.5%
DO	2	33.3%	4	66.7%	0	0.0%	0	0.0%	4	25.0%	12	75.0%
LE	0	0.0%	6	100.0%	3	75.0%	1	25.0%	7	43.8%	9	56.3%
CIP	5	83.3%	1	16.7%	0	0.0%	0	0.0%	7	43.8%	9	56.3%
COT	0	0.0%	0	0.0%	0	0.0%	0	0.0%	7	43.8%	9	56.3%
AZM	0	0.0%	0	0.0%	4	100.0%	0	0.0%	0	0.0%	0	0.0%
E	5	83.3%	1	16.7%	4	100.0%	0	0.0%	11	68.8%	5	31.3%
CD	0	0.0%	0	0.0%	4	100.0%	0	0.0%	11	68.8%	5	31.3%
LZ	0	0.0%	6	100.0%	0	0.0%	4	100.0%	0	0.0%	16	100.0%
P	0	0.0%	6	100.0%	2	50.0%	2	50.0%	16	100.0%	0	0.0%
VA	0	0.0%	6	100.0%	0	0.0%	0	100.0%	4	0.0%	16	100.0%
FC	0	0.0%	0	0.0%	0	0.0%	0	0.0%	12	75.0%	4	25.0%

Ampicillin (AMP), cefotaxime (CTX), ertapenem (ETP), meropenem (MRP), penicillin (P), gentamycin (GEN), erythromycin (E), clindamycin (CD), tetracycline (TE), doxycycline (DO), ciprofloxacin (CIP), levofloxacin (LE) and trimethoprim-sulfamethoxazole (COT), linezolid (LZ) and fusidic acid (FC), vancomycin (VA), cefoxitin (CX).

Figure 1. Number of organisms in each specimen.

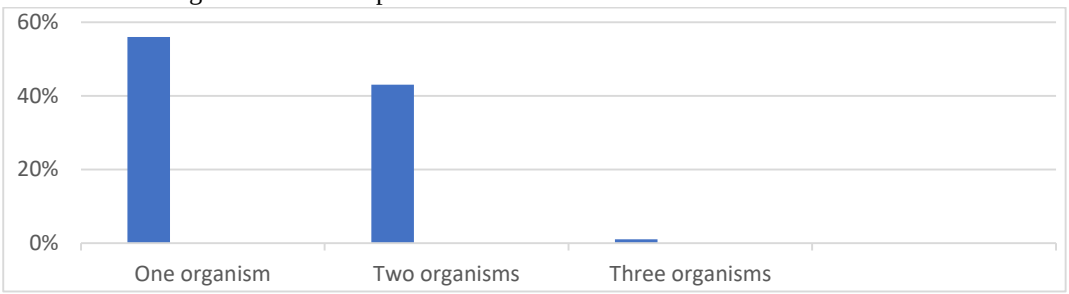
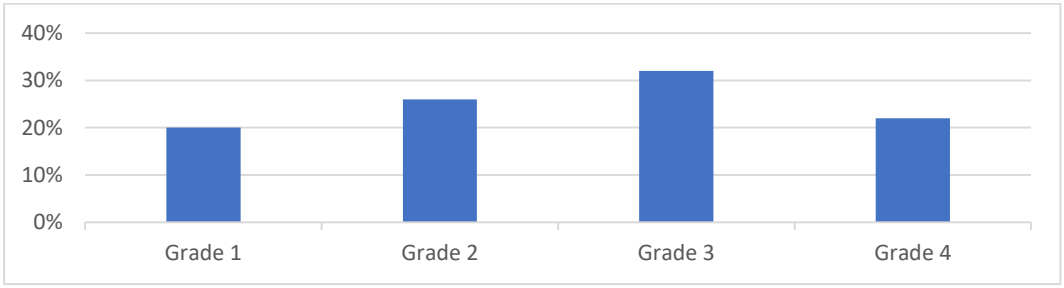


Figure 2. Grading of the DFU according to Wagner's classification.



Discussion

Diabetic foot infections (DFIs) are common devastating complications in DM patients. The pattern of bacterial profile and their antibiotic susceptibility changes from one region to another within the country and between different countries [14].

In the present study, we attempted to study the microbiological profile of DFUs and their susceptibility to different antibiotics by using disc

diffusion method. Broth microdilution method, agar dilution method for determination of colistin MIC and agar dilution method for determination of vancomycin MIC as outlined in CLSI, 2022 [7].

In our study, 70% were males (57 patients) and 30% were females (24 patients). An explanation for this gender difference might be the involvement of males in increased physical work, in association with diabetic neuropathy, making them more prone to injury. Also, it may be attributed to smoking habits that are more predominant in males.

This result was in line with a study conducted by Megallaa et al [15] who stated that out of 180 patients, DFUs were more common in males (75.6%) than females (24.4%). Another study conducted in southwest China by Wu et al [16] stated that 63.8% were males (273 patients/ 428) showing a distinct male predominance.

In disagreement with our study, a study conducted by Vibha et al [17] showed female predominance with a percentage of 57.4% (356/620 patients) while male patients only represented 42.6% (264/620 patients).

The difference in demographic distribution between the studies may be attributed to the difference in the countries where the studies were conducted in.

In our study, 118 isolates were recovered from 81 specimens. Growth of one organism after 24h incubation was found in 56% (45 specimens), two organisms in 43% (35 specimens) and three organisms in 1% (one sample). The explanation for the lower prevalence of polymicrobial infections in our study may be attributed to the proper sampling and the higher prevalence of mild and superficial ulcers.

This result was in line with a study conducted by Shareef et al [18] who stated that 53.52% of the patients had monomicrobial infections (38/71 patients) and 46.47% had polymicrobial infections (33/71 patients). Another study conducted in India by Sugandhi and Prasanth [19] stated that 66% of specimens were monomicrobial (34/51 specimens) while 18% were polymicrobial (9/51 specimens) and 16% had no growth of any microorganism after 48h incubation (8/51 specimens).

In disagreement with our study, a study conducted in Oman by Sannathimmappa et al [20] stated that 56% were polymicrobial (75 out of 133 specimens) while 44% were monomicrobial (58 out of 133 specimens). Another observational study was conducted at the Baqai Institute of Diabetology and Endocrinology (BIDE) by Miyan et al [21] stated that 78.6% were polymicrobial (269/342 specimens) while 21.4% were monomicrobial (73/342 specimens). An explanation for the higher prevalence of polymicrobial infections in the other studies opposing us may be attributed to the low immunity protectiveness against microbes in diabetic patients.

In this study, 53% of patients had co-morbidities in the form of hypertension and cardiovascular diseases (43 patients) and 47% didn't suffer from any co-morbidities (38 patients). The increased incidence of other co-morbidities with DM could be due to the vascular complications of DM, predisposing to hypertension and coronary heart diseases.

This result was in accordance with a study conducted in southeastern Brazil by Verrone et al [22] that stated that 72% suffered from co-morbidities (72/100 patients) while 28% didn't have any co-morbidities (28/100 patients). In contrast to our study, a study conducted in Egypt by Galal et al [23] stated that 80% of patients didn't suffer from co-morbidities (127/159 patients) while 20% had co-morbidities (32/159 patients). Another study conducted in the capital of Sudan, Khartoum by Almobarak et al [24] stated that 55.4% didn't suffer from co-morbidities (31 patients) while 44.6% had co-morbidities (25 patients). An explanation for the lower prevalence of co-morbidities in the other studies might be explained by the lower age group among the population included.

In the present study, 16% of the patients had T1DM (13 patients) and 84% had T2DM (68 patients). An explanation for this could be the higher prevalence of T2DM than T1DM in our population.

This result was in agreement with a study conducted by Kapila et al [25] that stated that 4.1% had T1DM (8 patients) while 95.9% had T2DM (185 patients). Jouhar et al [26] also stated that 98% had T2DM (340/348 patients) while only 2% had T1DM (8 patients).

Rather than our study, a study conducted in India by Sannathimmappa et al [20] stated that 50.1% had T1DM (26/50 patients) while 45.9% had T2DM (24/50 patients).

In our study, 44% had DM for up to 10 years (36 patients) while 56% suffered from it for more than 10 years (45 patients).

An explanation of this is that the longer the duration of DM, the higher the severity of vascular complications, hence DFUs occurrence.

This result was matching with a study conducted by Megallaa et al [15] that stated that 35.6% had DM for up to 10 years (64/180 patients) while 64.4% had DM for more than 10 years (116/180 patients). In conflict with our study Nongmaithem et al [27] stated that 82% had DM for up to 10 years (41/50 patients) while 18% had DM for more than 10 years (9/50 patients).

According to Wagner's classification, the majority of ulcers in our study were grade 3.Twenty percent had grade 1 ulcers (16 patients), 26% had grade 2 ulcers (21 patients), 32% had grade 3 ulcers (26 patients) and 22% had grade 4 ulcers (18 patients).

The presentation of DFUs with higher-grade ulcers could be explained by the poor educational level of the population, self-medication and delay in seeking medical advice.

A study conducted in India by Sannathimmappa et al [20] showed similar results, whereas per Wagner's classification, 6% of ulcers were Grade 1 (3 patients), 32% were Grade 2 (16 patients), 44% were Grade 3 (22 patients) and 10% were Grade 4 (5 patients). Another study conducted in Egypt by Ismail et al [28] stated that grade 2 was found in 25% (30/120 patients), grade 3 in 50% (60/120 patients) and grade 4 in 25% (30/120 patients). Another study conducted by Erdoğan et al [29] stated that out of 130 DFUs, the majority of ulcers according to the Wagner classification belonged to grade 3 ulcers where 14.6% of the patients had grade 1 ulcers (19 patients), 40.8% had grade 2 ulcers (53 patients), 36.1% had grade 3 ulcers (47 patients) and 8.5% had grade 4 foot ulcers (11 patients).

In disagreement with our study, a study conducted by Otta et al [30] stated that out of 148 patients when the ulcers were graded as per Wagner's system, Grade 2 ulcers were the most predominant (31.08%). Another study conducted in India by Sadriwala et al [31] stated that 22 ulcers out of 25 had grade 2 ulcers.

According to our study, 118 isolates were recovered, 75.4% were Gram-negative (89 isolates), 22% were Gram-positive (26 isolates) while 2.6% were *Candida albicans* (3 isolates). An explanation of this could be the high resistance to antibiotics shown by Gram-negative bacteria compared to Gram-positive isolates, and therefore their persistence in infected wounds.

In concurrence with our study, a study conducted by Shareef et al [18] stated that out of the total 122 isolates, 64.75% isolates were found to be Gram-negative (79 isolates) and 35.24% were Gram-positive (43 isolates). Another study conducted in India by Sannathimmappa et al [20] stated that out of 135 isolates, Gram-negative bacteria comprised the major group of 54.1% (73 isolates) followed by Gram-positive bacteria 40% (54 isolates) and 5.9% Fungus (eight isolates) were observed on culture. In contrast to our study, a study conducted in Korea by Son et al [32] stated that 57.5% were Gram-positive (478/745 isolates) and 40.0% were Gram-negative (333/745 isolates). An explanation for the predominance of Gram-positive organisms in these studies might be due to the intake of antibiotics against the Gram-negative organisms by the patients before sampling.

After identification of the isolated organisms, we recovered in the present study 118 isolates, 75.4% were Gram-negative (89 isolates), 22% were Gram-positive (26 isolates) while 2.6% were *Candida albicans* (3 isolates). Out of the 118 isolates, 12% were *E. coli* (14 isolates), 24% were *Klebsiella* spp. (28 isolates), 9.3% were *Proteus vulgaris* (11 isolates), 8.5% were other *Proteus* spp. (10 isolates), 0.8% was *Enterobacter* spp. (one isolate), 0.8% was *Providencia* spp. (one isolate), 0.8% was *Morganella* spp. (one isolate), 16% were *Pseudomonas* spp. (19 isolates), 3.3% were *Acinetobacter* spp. (four isolates), 13.5% were *Staphylococcus aureus* (16 isolates), 5% were *Enterococcus* spp. (6 isolates), 3.4% were *Streptococcus* spp. (four isolates) and 2.6% were *Candida albicans* (3 isolates).

Similarly, a study conducted by Shareef et al [18] stated that *Pseudomonas aeruginosa* was isolated in 18.03% (22/122 patients) followed by *Klebsiella pneumonia* 14.75% (18/122 patients). Another study by Hatipoglu et al [33] found that the most commonly isolated organisms were *Escherichia coli* (15%) (58/387 isolates), *Pseudomonas aeruginosa* (12.4%) (48/387 isolates), *Proteus* spp. (9.6%) (37/387 isolates) while *Staphylococcus aureus* was only 11.4% of all the isolates.

In contrast to our study, a study conducted in Korea by Son et al [32] stated that out of 745 isolates, MRSA was identified as the most

commonly isolated organism (13.7%) followed by *Enterococcus faecalis* (12.6%), methicillin-sensitive *Staphylococcus aureus* (MSSA) (12.5%), *Streptococcus agalactiae* (6.5%) and Methicillin-resistant *Staphylococcus epidermidis* (MRSE) (3.5%). With reference to the Gram-negative organisms, *Pseudomonas aeruginosa* was identified as the most commonly isolated organism (9.4%) followed by *Escherichia coli (*7.2%), *Klebsiella pneumonia* (3.2%), *Enterobacter cloacae* (3.0%) and *Serratia marcescens* (2.4%). Another study conducted by Lebowitz et al [34] at Geneva University Hospital stated that the five most frequently isolated microorganisms out of 1018 isolates were *Staphylococcus aureus* (32%) (325 isolates), coagulase-negative *staphylococci* (3.4%) (35 isolates), *Enterococcus faecalis* (4%) (40 isolates), *Streptococcus agalactiae* (2.5%) (26 isolates), and *Pseudomonas aeruginosa* (6%) (61 isolates).

In the current study, out of the 118 isolates, 67% were multidrug-resistant (MDR) (79 isolates), 24.5% were extensive drug resistant (XDR) (29 isolates).

Other studies reported similar results as the study that was conducted at Alexandria University Hospital, Egypt by Ismail et al [28] which stated that 55.1% were MDR (54/98 isolates) and34.7% were XDR (44/98 isolates). Another study conducted in South India by Kathirvel et al [35] stated that out of 150 isolates, 66% of the isolates were MDR and/or XDR (99 isolates).

A study conducted in Oman by Sannathimmappa et al [20] wasn't in line with our study where it stated that 36% of the isolates only were found to be MDR Gram-negative pathogens (63/175 isolates).

In the present study, 45% were extended-spectrum β-lactamase (ESBL) (40 isolates) and 22.5% were carbapenem-resistant (20 isolates).

In agreement with our study, a study conducted in Salem, Tamil nadu, India by Sugandhi and Prasanth [19] stated that out of 51 patients, the presence of ESBL was found in 45% (23 isolates). Another study conducted at Alexandria University Hospital, Egypt by Ismail et al [28] stated that out of 98 isolates, 49% were ESBL and 40.8% were carbapenemase producers. Otta et al [30] stated in their study that out of 148 isolates ESBL production was noted in 42.1% of isolates.

In our study, 46% of the 26 Gram-positive isolates were methicillin-resistant *Staphylococcus aureus* (MRSA) (12 isolates).

Consistent with our study, a study conducted by Otta et al [30] stated that 35% (77.8% of *Staphylococcus aureus* strains) were MRSA (52 isolates). Another study conducted in Italy by Boschetti et al [36] stated that MRSA was isolated in 27.1% (27% of *Staphylococcus aureus* strains) (52 cases).

Contrary to our study, a study conducted at the Baqai Institute of Diabetology and Endocrinology (BIDE) by Miyan et al [21] stated that; MRSA was found in 11% (26.7% of *Staphylococcus aureus* strains) (38/342 isolates). Another study conducted in southwest China by Wu et al [16] stated that 4.8% were MRSA (20% of *Staphylococcus aureus* strains) (17/354 isolates).

Conclusion

In the present study, we found that DFUs were mostly monomicrobial. There is high prevalence of co-morbidities such as hypertension and cardiovascular diseases in diabetic patients suffering from DFUs. Type 2 DM (T2DM) was more prevalent than type 1 DM (T1DM) among diabetic patients suffering from DFUs. The incidence of developing DFUs increases as the duration of DM increases.

As per Wagner's classification, the prevalence of grade 3 ulcers is the highest. In DFUs, the prevalence of Gram-negative organisms was higher than Gram-positive. The most common isolated organisms were *Klebsiella* spp. (24%) (28/118 isolates), *Proteus* spp. (17.8%) (21/118 isolates), *Pseudomonas* spp. (16%) (19/118 isolates) and *Staphylococcus aureus* (13.5%) (16/118 isolates) .The prevalence of multi-drug resistance (MDR) was (67%) (79/118 isolates) and extensive drug resistance (XDR) was (24.5%) (29/118 isolates), while the prevalence of extended spectrum β-lactamase (ESBL) among Gram-negative isolates was (45%) (40/89 isolates) and carbapenem-resistant Enterobacteriaceae (CRE) was (22.5%) (20/89 isolates).The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among Gram-positive isolates was (46%) (12/26 isolates).

Recommendation

A further multicenter surveillance study using a larger sample size is needed to identify the prevalence of different bacterial species causing

DFUs among tertiary care hospitals as well as the possible antimicrobial agents to which they can be susceptible. Increasing the awareness of clinicians to the importance of performing antibiotic susceptibility testing before the start of empirical therapy. Increasing the awareness of patients to the importance of completing the antibiotic course fully.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

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