

# Identification and antibiotic susceptibility of microorganisms isolated from diabetic foot ulcers: A pathological aspect

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**Abstract.** Diabetic foot ulcers infected with microorganisms increase the risk of amputation. The presence of drug-resistant bacteria in diabetic foot ulcers creates a big challenge during the treatment. The objective of the present study was to determine the bacterial prevalence and antibiotic resistance among bacteria isolated from Chinese patients with diabetic foot ulcers. The present study studied the microbial colonization of diabetic foot ulcers of patients from a single center in China. Wound swabs from 89 patients with diabetic foot ulcers were collected and the presence of microorganisms detected. The isolated microorganisms were subjected to antibiotic susceptibility testing by the disk diffusion method. Of 89 patients, 56 (62.9%) were male and 33 (37.1%) were female, the mean age of patients was 53.2±5.4 years, the mean duration of diabetes was 14.8±2.9 years, the mean random blood sugar was 301±87 mg/dl, mean HbA1c was 7.9±1.4%. Patients with Wanger ulcer grade III (36.0%; P=0.034) and patients within the weight range of 51-75 kg (59.6%; P=0.012) were significantly higher. The prevalence rate of diabetic foot ulcers was 11.3%. Among 153 microorganisms, gram-positive bacteria (52.3%) were more prevalent than gram-negative bacteria (44.4%). Most of the patients with polymicrobial infection were classified to have Wanger III ulcer grade diabetic foot ulcers. *Staphylococcus aureus* (38.2%) was the most predominant bacteria isolated followed by *Staphylococcus epidermidis* (29.2%) and *Escherichia coli* (28.1%). Most of the gram-positive and gram-negative bacteria were resistant to dicloxacillin (73.8%, P=0.021) and cefotaxime (50%), respectively and ~53.4% of the isolates were multi-drug resistance isolates, 61.8% of the *Staphylococcus aureus* were identified as methicillin-resistant *Staphylococcus aureus* and 61.8% of the

gram-negative bacteria were extended-spectrum  $\beta$ -lactamase producers. *Staphylococcus aureus* and *Escherichia coli* were the predominant gram-positive and gram-negative bacteria isolated, respectively. Penicillin resistance was significantly higher among the gram-negative bacteria (P=0.019). *Staphylococcus aureus* and *Escherichia coli* were the predominant gram-positive and gram-negative bacteria isolated and levofloxacin and nitrofurantoin were the most effective antibiotics among the gram-positive and gram-negative bacterial isolates, respectively.

## Introduction

Diabetes, a disease related to lifestyle, is characterized by chronic hyperglycemia and imposes serious problems and a heavy health burden on the Chinese population (1). Uncontrolled diabetes will lead to several complications; for example, retinopathy, neuropathy, cardiopathy, nephropathy and diabetic foot ulcers (2). A diabetic foot ulcer is an inframalleolar infection on the feet of patients with type 2 diabetes (3). It is caused due to several risk factors including trauma, peripheral neuropathy, peripheral vascular disease and the development of resistance to infectious bacteria (4). In 2017, the worldwide prevalence of diabetic foot ulcers was 6.3%; of which 13% was in North America, 7.2% in Africa, 5.5% in Asia and 5.1% in Europe. The countrywide prevalence was reported to be 13% in the United States of America, 11.6% in India and 4.1% in China (5). On presentation, 60% of diabetic foot ulcers can be infected with microorganisms and can increase the risk of amputation by 50% compared to patients with foot ulcers without infection (6,7).

Several microorganisms have been isolated from diabetic foot ulcers with aerobic gram-positive bacteria, especially *Staphylococcus aureus*, which is the predominantly responsible organisms (8). However, deep and chronic wounds have often yielded aerobic gram-negative or obligate anaerobic bacteria (9). Mild bacterial infections are mostly attributed to mono-bacterial infection while severe infection is attributed to polymicrobial infections (10). Ischemia and neuropathy at the infected site leads to the development of necrosis, which requires drastic measures including debridement and/or amputation of the lower limb (11). Antibiotic resistance is a serious challenge in the treatment of diabetic foot ulcers, especially multidrug resistance (MDR), extended-spectrum  $\beta$ -lactamase (ESBL)

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**Key words:** antibiotic resistance, diabetic foot ulcer, gram-positive bacteria, gram-negative bacteria, extended-spectrum  $\beta$ -lactamase, methicillin-resistant *Staphylococcus aureus*, polymicrobial infection

producing bacteria and methicillin-resistant *Staphylococcus aureus* (MSRA) (12,13). MDR bacteria, ESBL-producing strains and MRSA have been isolated from diabetic foot ulcers (14,15). The presence of these resistant strains restricts the present treatment possibilities and makes the treatment more demanding with a high threat to the life of patients (16). Diabetic infection can worsen aggressive diagnosis and proper selection of antibiotic(s), which is important to the successful management of diabetic foot ulcers, as overuse of antibiotics will develop resistance from gram-negative bacteria (16). The knowledge of the bacterial profile and antibiotic profile in a specific geographical condition can improve the treatment of diabetic foot ulcers. Overuse of antibiotics develops bacterial resistance making these drugs useless, for example, overuse of third-generation cephalosporin can lead to the development of resistance against it from penicillin-resistant *pneumococci*. Ceftriaxone overuse causes sleep disorders (16).

The object of the present study was the identification of microorganisms and the antibiotic susceptibility of bacteria among isolated strains retrieved from foot ulcers of patients with diabetes.

## Materials and methods

**Patients.** A total of 787 consecutive patients with diabetes mellitus who visited the Department of Infection Management at the Huaihua Cancer Hospital, Hunan, China between January 2018 and March 2021 were evaluated. Of these patients, 89 patients who had diabetic foot ulcers were included in this study. A total of 698 diabetic patients who did not have foot ulcers were excluded. Patient details such as age, sex, weight, blood sugar level, type of diabetes and ulcer type based on Wanger's classification (17) and amputation details were collected. Other clinical conditions such as retinopathy, neuropathy, nephropathy, vasculopathy and hypertension were also collected (Table I). The present study was approved under the ethical approval number PR-2017-21 by the Human Ethics Committee of the Huaihua Cancer Hospital. Informed consent was obtained from each patient or their legal guardian for the commencement of the present study.

**Isolation and identification of microorganisms.** Wound exudates were obtained from foot ulcers. The deep wound technique (taking sample from deep inside the wound) was followed, using a sterile scalpel. Wounds were debrided and rinsed with sterile saline before swabbing. Using sterile swabs, samples were collected from the depth of the wounds. The swabs were transferred to brain heart infusion broth (Neogen Culture Media; Neogen), transported immediately to the laboratory and processed by standard culture techniques. Then swabs were inoculated onto blood agar, MacConkey agar and mannitol salt agar and incubated at 37°C for 24 h. To identify fungi, the swabs were inoculated onto Sabouraud's dexterosus agar (SDA) media and incubated at 27°C for 5 days. The plates were observed for the presence of growth after the incubation period, bacterial strains were subjected to the Gram's staining, then the isolates were identified using conventional biochemical tests. Bacterial isolation was detected phenotypically and confirmed by multiplex-PCR genes (18).

Table I. Patient characteristics.

Characteristic	Number of isolates (%)
Sex	
Male	56 (62.9)
Female	33 (37.1)
Age (years)	53.2±5.4
Weight	
≤50 kg	8 (9)
51-75 kg	53 (59.6)
>75 kg	28 (31.5)
Body mass index (kg/m <sup>2</sup> )	26.7±2.5
Duration of diabetes (years)	14.7±3.4
Random blood sugar level (mg/dl)	256.7±58.9
%HbA1c	7.9±1.4
Wound size	
≤5 mm	32 (36.0)
>5 mm	57 (64.0)
Amputation	41 (46.1)
Wanger ulcer grade	
Grade 0	4 (4.5)
Grade I	12 (13.5)
Grade II	20 (22.5)
Grade III	32 (36)
Grade IV	21 (23.6)
Clinical complications	
Hypertension	52 (58.4)
Neuropathy	34 (38.2)
Retinopathy	27 (30.3)
Nephropathy	19 (21.3)
Vascular diseases	48 (53.9)

Continuous variables are presented as mean ± standard deviation. Categorical variables are presented as frequency (percentages).

**Antibiotic susceptibility testing.** Antibiotic susceptibility testing was performed by the disk diffusion method as per the established protocol provided in the Clinical Laboratory Standard Institute guidelines (19). The following routinely used antibiotics were tested: ampicillin (10 µg), amikacin (30 µg), cefotaxime (30 µg), cefepime (30 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), dicloxacillin (1 µg), levofloxacin (5 µg), vancomycin (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), penicillin (10 U), netilmicin (30 µg) and tetracycline (30 µg). All reagents and antibiotics were purchased from HiMedia Laboratories LLC.

**MRSA and ESBL detection.** Methicillin resistance in *Staphylococcus aureus* was detected using a 30 µg cefoxitin disk and oxacillin agar screen plate. Briefly, 10 µl of overnight culture adjusted to 0.5 McFarland's standard was swabbed onto Muller-Hinton Agar plates with 4% NaCl and 6 µl/ml of oxacillin. The plates were incubated at 37°C for 48 h and any presence of growth after the incubation period

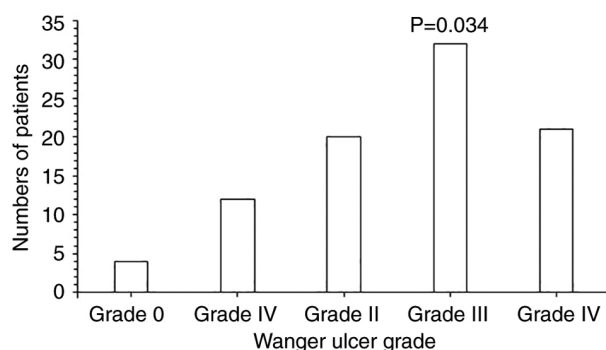


Figure 1. Distribution of Wanger ulcer grade among enrolled patients.

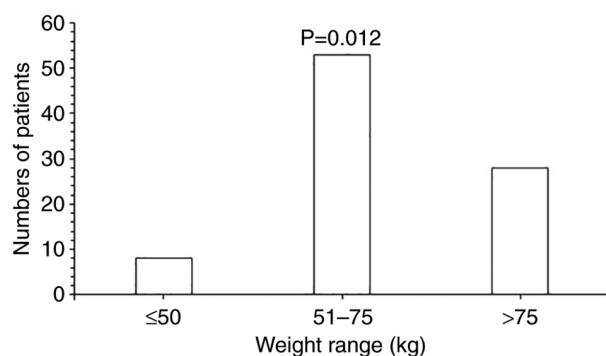


Figure 2. Distribution of the weight range among enrolled patients.

was determined to be oxacillin resistant. For gram-negative bacteria, ESBL production was determined by the combined disk method; cefotaxime (30 µg) and cefotaxime/clavulanic acid (30 µg/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30 µg/10 µg).

**Statistical analysis.** Continuous variables were given as mean, median (range) and percentages. Chi-Square test ( $\chi^2$ -test), unpaired Student's *t*-test and one-way analysis of variance (ANOVA) were performed using SPSS version 20 statistical software (IBM Corp.). The Dunnett multiple comparison test was used for post hoc analysis. Pearson correlation between resistance bacteria and patient characteristics parameters was utilized.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Patient data.** Of a total of 787 patients with diabetes, 89 (11.3%) patients had diabetic foot ulcers. Of these, 56 (62.9%) were male and 33 (37.1%) were female, the mean age of diabetic patients was  $53.2 \pm 5.4$  years, the mean duration of diabetes was  $14.8 \pm 2.9$  years, the mean random blood sugar was  $301 \pm 87$  mg/dl, mean HbA1c was  $7.9 \pm 1.4\%$ . Patients with Wanger ulcer grade III (36.0%;  $P = 0.034$ ,  $\chi^2$ -test, Fig. 1) and those within the weight range of 51-75 kg (59.6%;  $P = 0.012$ ,  $\chi^2$ -test, Fig. 2) were significantly higher among the enrolled patients (Table I).

**Identification of microorganisms.** Of the 89 samples, a total of 153 microorganisms were isolated. Of these, 80 (52.3%) were

Table II. Prevalence of microorganisms isolated from diabetic foot ulcer.

Microorganisms	Numbers of isolates (%)
<b>Gram-Positive</b>	
<i>Staphylococcus aureus</i>	34 (38.2)
<i>Staphylococcus epidermidis</i>	26 (29.2)
<i>Enterococcus</i> species	14 (15.7)
<i>Streptococcus</i> species	4 (4.5)
Other Gram-positive bacteria	2 (2.2)
<b>Gram-Negative</b>	
<i>Escherichia coli</i>	25 (28.1)
<i>Klebsiella</i> species	18 (20.2)
<i>Acinetobacter</i> species	12 (13.5)
<i>Pseudomonas aeruginosa</i>	9 (10.1)
<i>Protease</i> species	2 (2.2)
Other Gram-negative bacteria	2 (2.2)
<b>Fungus</b>	
<i>Candida</i> species	3 (3.4)
<i>Aspergillus niger</i>	2 (2.2)

Variables are presented as frequency (percentages).

gram-positive bacteria, 68 (44.4%) were gram-negative bacteria and five (3.3%) were fungal isolates. A summary diagram of the study is presented in Fig. 3. Overall, *Staphylococcus aureus* (34, 38.2%) was the predominant bacteria isolated followed by *Staphylococcus epidermidis* (26, 29.2%) and *Escherichia coli* (25, 28.1%). Of the five fungal species isolated, three (3.4%) were identified as *Candida* species and two (2.2%) as *Aspergillus niger* (Table II). No significant difference was found among the isolates ( $P > 0.05$ ). Among the 89 patient samples, 37 (41.6%) revealed a polymicrobial infection with more than two isolates from each sample. Of these 37 patients, 18 patients were classified to have Wanger III ulcer grade diabetic foot ulcer.

**Antibiotic susceptibility test.** A total of 80 gram-positive bacteria and 68 gram-negative bacteria were subjected to antibiotic susceptibility testing using different sets of antibiotics. Most of the gram-positive bacteria were resistant to dicloxacillin (50, 73.8%), followed by penicillin (47, 58.8%), tetracycline (40, 50%) and vancomycin (40, 50%). Of the 34 *Staphylococcus aureus* isolates strains, 28 (82.4%) isolates were resistant to penicillin and 20 (58.8%) were resistant to erythromycin. None of the *Streptococcal* isolates was resistant to penicillin and vancomycin (Table III). Dicloxacillin resistance was significantly higher among the gram-positive bacteria ( $P = 0.021$ ). The majority of gram-negative bacteria were resistant to cefotaxime (34, 50.0%), followed by vancomycin (33, 48.5%), ampicillin (32, 47.1%), cefepime (32, 47.1%) and ceftriaxone (31, 45.6%). Of the 25 *Escherichia coli* isolates, 14 (56%) were resistant to levofloxacin, 13 (52%) were resistant to ampicillin, 12 (48%) were resistant to cefotaxime and 11 (44%) were resistant to cefepime. None of the *Klebsiella* isolates was resistant to nitrofurantoin and netilmicin (Table IV). Penicillin

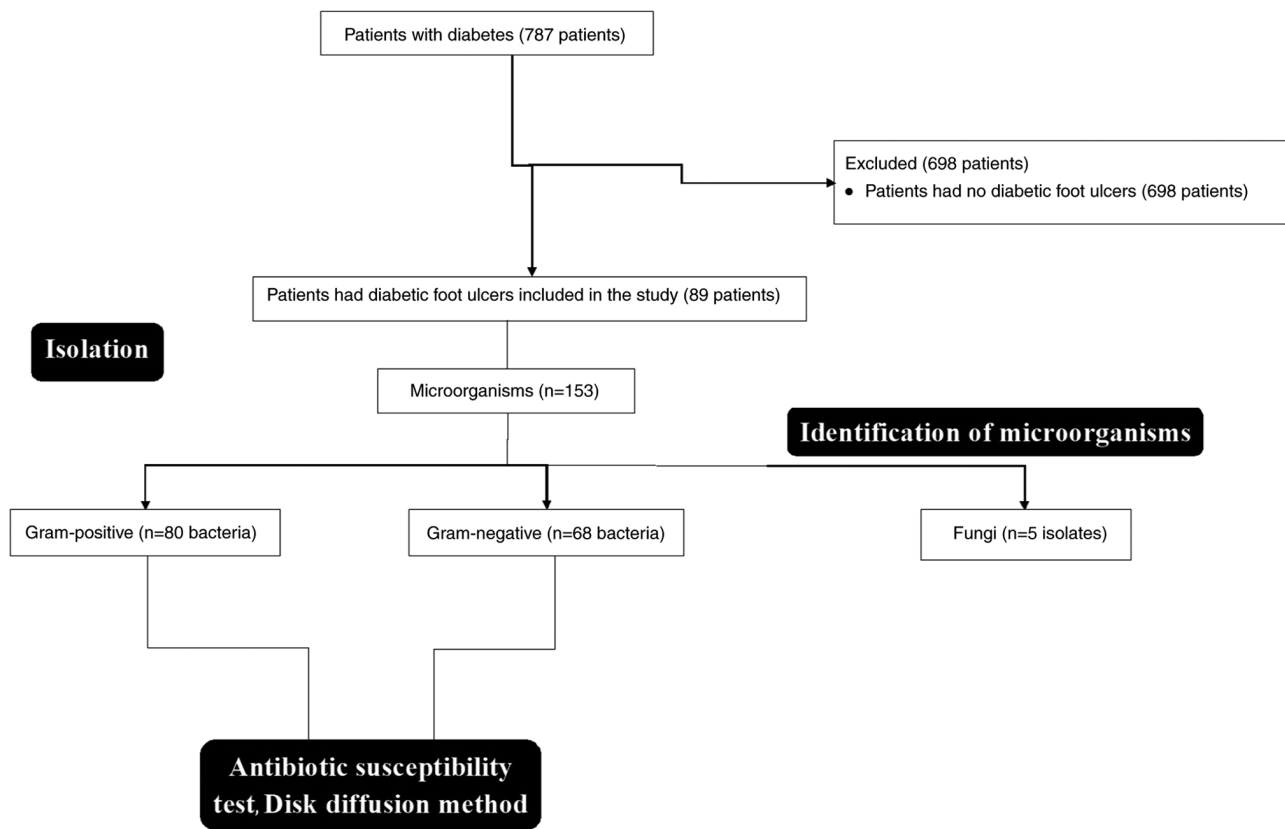


Figure 3. Summary diagram of the present study.

resistance was significantly higher among the gram-negative bacteria ( $P=0.019$ ).

Of the 148 bacterial isolates, 79 (53.4%) isolates were found to be MDR isolates. Among the 80 gram-positive bacteria, 38 (47.5%) isolates were found to be MDR and among the 68 gram-negative bacteria, 41 (60.3%) isolates were found to be MDR isolates. Of the 34 *Streptococcus aureus* isolates, 21 (61.8%) isolates were found to be MRSA. Among the 68 gram-negative bacteria, 42 (61.8%) isolates were ESBL producers. ESBL production was higher among *Escherichia coli* (17/25, 68%) and *Klebsiella* (12/18, 66.7%) isolates (Table V).

More than 25 kg kg/m<sup>2</sup> body mass index ( $P=0.0492$ ), >7 years duration of diabetes ( $P=0.0481$ ),  $\geq 6.5$  HbA1C ( $P=0.0481$ ), >5 mm wound size ( $P=0.0472$ ), history of amputation ( $P=0.0462$ ) and Wanger ulcer grade  $\geq$ III ( $P=0.0451$ ) were associated with resistant bacteria (Table VI).

## Discussion

A diabetic foot ulcer is a severe problem that is not confined to the superficial subcutaneous tissue (20). Uncontrolled diabetes or improper management of it leads to the development of diabetic foot ulcers (21,22). In the current study majority of the patients with diabetic foot ulcers were male (62.9%), these results are in line with that reported by other studies (23,24). In the current study, the overall prevalence of patients with diabetic foot ulcers was 11.3%, which is higher than that of the global prevalence of diabetic foot ulcers reported by Zhang *et al* (5). Zhang *et al* (5) used a systematic review

and meta-analysis to calculate the global epidemiology of diabetic foot ulcers. The study included 67 published papers and reported a prevalence rate from 1.5-16.6%. The lowest prevalence (1.5%) was reported in the Australian population and the highest prevalence (16.6%) was reported in the Belgium population. The study included 10 publications from China and reported a prevalence rate of 4.1% in the Chinese population which is lower than that reported (11.6%) in the current study. While the reported prevalence of diabetic foot ulcers in the Belgium population (16.6%), the Canadian population (14.8%) and the North American population (13%) were higher compared to the current study. The prevalence rate in the Indian population (11.6%) (5) was similar to that reported in the current study.

In the present study, the fraction of gram-positive bacteria was higher than gram-negative bacteria (52.3% vs. 44.4%). Similar to the current report, some studies have reported that gram-positive bacteria are the predominant bacteria isolated from diabetic foot ulcers compared with gram-negative bacteria (9,14). In contrast to the current study, other studies reported that the fraction of gram-negative bacteria was higher than gram-positive bacteria (3,8,20-23). Although gram-negative bacteria were predominantly isolated, *Staphylococcus aureus* (38.2%) was the predominant bacteria isolated in several studies (3,8,20-22). In the current study, among the gram-negative bacteria, *Escherichia coli* (28.1%) was the predominant bacteria isolated, which is similar to that reported by Xie *et al* (8). While the other studies report *Proteus* species and/or *Pseudomonas aeruginosa* as the predominant isolates after *Staphylococcus*

Table III. Antibiotic susceptibility among gram-positive bacteria.

Gram-positive (n=80; R(%) / S(%))	Ampicillin	Cefotaxim	Cefuroxime	Dicloxacillin	Levofloxacin	Tetracycline	Gentamicin	Vancomycin	Erythromycin	Penicillin
<i>Staphylococcus aureus</i> (n=34)	17 (50.0)/ 11 (32.4)	13 (38.2)/ 16 (47.1)	19 (55.9)/ 8 (23.5)	27 (79.4)/ 6 (17.6)	13 (38.2)/ 18 (52.9)	15 (44.1)/ 13 (38.2)	15 (44.1)/ 16 (47.1)	18 (52.9)/ 16 (47.1)	20 (58.8)/ 8 (23.5)	28 (82.4)/ 3 (8.8)
<i>Staphylococcus epidermidis</i> (n=26)	12 (46.2)/ 7 (26.9)	9 (34.6)/ 13 (50.0)	13 (50.0)/ 7 (26.9)	19 (73.1)/ 4 (15.4)	8 (30.8)/ 14 (53.8)	16 (61.5)/ 6 (23.1)	18 (69.2)/ 5 (19.2)	14 (53.8)/ 12 (46.2)	8 (30.8)/ 15 (57.7)	10 (38.5)/ 6 (23.1)
<i>Enterococcus</i> species (n=14)	5 (35.0)/ 5 (35.0)	7 (50.0)/ 4 (35.7)	3 (21.4)/ 8 (57.1)	10 (71.4)/ 2 (14.3)	4 (28.6)/ 6 (42.9)	5 (35.7)/ 3 (21.4)	4 (28.6)/ 5 (35.7)	8 (57.1)/ 4 (28.6)	4 (28.6)/ 4 (28.6)	7 (50.0)/ 5 (35.7)
<i>Streptococcus</i> species (n=4)	3 (75.0)/ 1 (25.0)	2 (50.0)/ 2 (50.0)	2 (50.0)/ 2 (50.0)	3 (75.0)/ 1 (25.0)	2 (50.0)/ 1 (25.0)	3 (75.0)/ 1 (25.0)	3 (75.0)/ 1 (25.0)	0 (0)/ 4 (100)	1 (25.0)/ 3 (75.0)	0 (0)/ 4 (100)
Other Gram-positive bacteria (n=2)	2 (100)/ 0 (0)	1 (50)/ 1 (50)	1 (50)/ 1 (50)	0 (0)/ 2 (100)	2 (100)/ 0 (0)	1 (50)/ 1 (50)	2 (100)/ 0 (0%)	0 (0)/ 2 (100)	2 (100)/ 0 (0)	2 (100)/ 0 (0)
Total	39 (43.8)/ 24 (30.0)	32 (40.0)/ 36 (45.0)	38 (47.5)/ 26 (32.5)	59 (73.8)/ 15 (18.8)	29 (36.3)/ 39 (48.8)	40 (50.0)/ 24 (30.0)	42 (52.5)/ 27 (33.8)	40 (50.0)/ 38 (47.5)	35 (43.8)/ 30 (47.5)	47 (58.8)/ 18 (22.5)

Variables are presented as frequency (percentages). R, Resistance; S, Susceptible.

Table IV. Antibiotic susceptibility among gram-negative bacteria.

Gram-Negative R(%) / S (%)	Ampicillin	Chloramphenicol	Ceftriaxone	Cefotaxime	Cefepime	Gentamicin	Amikacin	Levofloxacin	Netilmicin	Nitrofurantoin
<i>Escherichia coli</i> (n=25)	13 (52)/ 8 (32)	8 (32.0)/ 12 (48)	6 (24)/ 15 (60)	12 (48)/ 13 (52)	11 (44)/ 14 (56)	9 (36)/ 14 (56)	8 (32)/ 15 (60)	14 (56)/ 8 (32)	6 (24)/ 18 (72)	8 (32)/ 17 (68)
<i>Klebsiella</i> species (n=18)	9 (50.0)/ 7 (38.9)	10 (55.6)/ 7 (38.9)	7 (38.9)/ 5 (27.8)	9 (50)/ 8 (44.4)	5 (27.8)/ 12 (66.7)	9 (50)/ 8 (44.4)	7 (38.9)/ 5 (27.8)	5 (27.8)/ 12 (66.7)	0 (0)/ 18 (100)	0 (0)/ 18 (100)
<i>Acinetobacter</i> species (n=12)	4 (33.3)/ 8 (66.7)	6 (50)/ 6 (50)	10 (83.3)/ 2 (16.7)	7 (58.3)/ 5 (41.7)	5 (41.7)/ 8 (66.7)	4 (33.3)/ 8 (66.7)	6 (50)/ 5 (41.7)	7 (58.3)/ 5 (41.7)	4 (33.3)/ 8 (66.7)	2 (16.7)/ 8 (66.7)
<i>Pseudomonas</i> <i>aeruginosa</i> (n=9)	3 (33.3)/ 6 (66.7)	4 (44.4)/ 4 (44.4)	5 (55.6)/ 3 (33.3)	6 (66.7)/ 3 (33.3)	8 (88.9)/ 1 (11.1)	4 (44.4)/ 5 (55.6)	4 (44.4)/ 4 (44.4)	6 (66.7)/ 2 (22.2)	8 (88.9)/ 1 (10.1)	7 (77.8)/ 2 (22.2)
<i>Protease</i> species (n=2)	2 (100)/ 0 (0)	1 (50)/ 1 (50)	1 (50)/ 1 (50)	0 (0)/ 2 (100)	2 (100)/ 0 (0)	1 (50)/ 1 (50)	0 (0)/ 2 (100)	1 (50)/ 1 (50)	2 (100)/ 0 (0)	1 (50)/ 1 (50)
Other Gram- negative bacteria (n=2)	1 (50)/ 1 (50)	0 (0)/ 2 (100)	2 (100)/ 0 (0%)	0 (0)/ 2 (100)	1 (50)/ 1 (50)	0 (0)/ 2 (100)	2 (100)/ 0 (0)	0 (0)/ 2 (100)	1 (50)/ 1 (50)	0 (0)/ 2 (100)
Total	32 (47.1)/ 30 (44.1)	29 (42.6)/ 32 (47.1)	31 (45.6)/ 26 (38.2)	34 (50.0)/ 33 (48.5)	32 (47.1)/ 36 (52.9)	27 (39.7)/ 38 (55.9)	27 (39.7)/ 31 (45.6)	33 (48.5)/ 30 (44.1)	21 (30.9)/ 46 (67.6)	18 (26.5)/ 48 (70.6)

Variables are presented as frequency (percentages). R, Resistance; S, Susceptible.

Table V. Extended-spectrum  $\beta$ -lactamase producing gram-negative bacteria.

Gram-negative	Numbers of isolates (%)
<i>Escherichia coli</i> (n=25)	17 (68.0)
<i>Klebsiella</i> species (n=18)	12 (66.7)
<i>Acinetobacter</i> species (n=12)	7 (58.3)
<i>Pseudomonas aeruginosa</i> (n=9)	5 (55.6)
<i>Protease</i> species (n=2)	1 (50.0)
Total	21 (61.8)

Variables are presented as frequency (percentages).

Table VI. Correlation between resistance bacteria and patient characteristics parameters.

Characteristics	Odd ratio	95% confidence interval	P-value
Sex (female vs. male)	0.8521	0.7521-0.8624	0.0631
Age (>50 years vs. $\leq$ 50 years)	0.8922	0.7123-0.9214	0.0592
Weight (>50 kg vs. $\leq$ 50 kg)	0.9521	0.8214-0.9952	0.0512
Body mass index (>25 kg kg/m <sup>2a</sup> vs. $\leq$ 25 kg kg/m <sup>2</sup> )	1.0211	0.8522-1.1211	0.0492
Duration of diabetes (>7 years <sup>a</sup> vs. $\leq$ 7 years)	1.0222	0.8422-1.2311	0.0481
Random blood sugar level (abnormal* vs. tolerated and normal)	1.1232	0.8524-1.2455	0.0472
%HbA1c ( $\geq$ 6.5 <sup>a</sup> vs. <6.5)	1.2244	0.8852-1.3221	0.0481
Wound size (>5 mm <sup>a</sup> vs. $\leq$ 5 mm)	1.3241	0.8951-1.3354	0.0472
Amputation (yes <sup>a</sup> vs. no)	1.2541	0.9924-1.2514	0.0462
Wanger ulcer grade ( $\geq$ III <sup>a</sup> vs. <III)	1.2544	0.8542-1.4211	0.0451
Clinical complications (presence vs. absent)	0.8522	0.7842-0.9122	0.0612

Odd ratio >1 and P<0.05 were considered significant. <sup>a</sup>Significant parameter associated with resistance bacteria.

*aureus* (3,23). In the present study, *Staphylococcus epidermidis* was the second most predominant organism isolated among the gram-positive bacteria followed by *Enterococcus* species. Similar to the study result of Anvarinejad *et al* (14), *Staphylococcus* species (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were the most prevalent organisms isolated followed by *Enterococcus* species. The frequent detection of *Enterococcus* species in patients compromised with such as diabetes and foot ulcers raises a serious health problem. However, its role in the infection is not established (25). In the current study, 41.6% of patient samples revealed a polymicrobial infection, while Xie *et al* (8) reported that 59.8% of their samples revealed polymicrobial infection, higher than that reported in the current study. Akhi *et al* (9) reported that mild infection was usually associated with mono-bacterial while severe infections were reported with polymicrobial infections, which is in line with the current study result that the majority of the patients who had polymicrobial infection were classified to have Wanger III ulcer grade diabetic foot ulcers (9).

Antibiotic selection for early treatment is empirical, and most clinicians will prescribe an antibiotic based on experience and/or observations. Appropriate background of antibiotic resistance before treatment will help in the successful management of the disease(s) (16). The current

study results will provide suggestions to physicians and surgeons regarding the potential antibiotics to be used for the treatment of diabetic foot ulcers. In the current study, among the gram-positive bacteria, dicloxacillin resistance was higher (73.8%) followed by penicillin resistance (58.8%). The current study findings corroborated with those reported by Sánchez-Sánchez *et al* (3), which reported the highest resistance to penicillin (93%) and dicloxacillin (88%) among the gram-positive bacteria. Among the different species identified in the current study *Staphylococcus aureus* was showed the highest resistance to penicillin (82.4%). A study from Pakistan reported that 100% of their *Staphylococcus aureus* isolates were resistant to penicillin (24). In the current study, 59.2% of the *Staphylococcus aureus* were resistant to vancomycin which was consistent with that reported by Sánchez-Sánchez *et al* (3). By contrast, another study reported that none of their *Staphylococcus aureus* isolates was resistant to vancomycin (14). Levofloxacin (36.3%) was the most effective antibiotic among our gram-positive bacteria, which corroborates the report by Sánchez-Sánchez *et al* (3). A higher number of gram-negative bacteria isolated in the current study showed resistance to cefotaxime (50%), ampicillin (47.1%) and cefepime (47.1%). While Sánchez-Sánchez *et al* (3) reported a much higher number of their gram-negative bacteria were resistant to ampicillin (92%), cefotaxime (60%) and



ceftriaxone (54%). Previous studies report that amikacin is the most effective antibiotic followed by netilmicin and levofloxacin (3,26). However, in the current study, nitrofurantoin (26.5%) was the most effective antibiotic among gram-negative bacteria followed by netilmicin (30.9%) and amikacin (39.7%). Although the study sample size was not large, the results suggested the need for a change in the empirical strategies to control the spread of antibiotic resistance.

Among the *Staphylococcus aureus* isolates, 61.8% found MRSA. Results of MRSA isolates were similar to that reported by Pontes *et al* (63.1% MRSA) (23); lower (78%) than that reported by Anvarinejad *et al* (14) and higher (10.1-55%) than that reported by the other studies (3,8,27). In the current study, 61.8% of the gram-negative bacteria were ESBL producers which is higher than that reported by other studies (9,14,28). About 53.4% of the isolates in the current study were MDR, while another study reported that 91% of their isolates were MDR, which is higher than the current study (14). The very high rate of resistant bacteria might be due to several reasons including unsound use of antibiotics, prolonged hospitalization, history of surgeries and recent use of broad-spectrum study antibiotics (14).

The current study limitations include the lack of single anaerobic culturing and the small sample size. The high rate of MRSA and ESBL strains posed an alarming situation since infection due to these isolates are difficult to treat as these isolates may not respond to commonly used antibiotics. The present study neglected microbiological data according to the best empirical antibiotic treatment for diabetic foot ulcers for a good prognosis. The possible justification for this is that the present study has presented data and recommended antibiotic(s) for diabetic foot ulcers. However, the current study results were useful irrespective of the fact that it did not specify best empirical antibiotic treatment for diabetic foot ulcers; the results were too preliminary.

The present study investigated the prevalence and antibiotic resistance of bacteria isolated from diabetic foot ulcers. The prevalence rate of diabetic foot ulcers among patients with diabetes was 11.3%. *Staphylococcus aureus* and *Escherichia coli* were the predominant gram-positive and gram-negative bacteria isolated, respectively. Levofloxacin and nitrofurantoin were the most effective antibiotics among the gram-positive and gram-negative bacterial isolates, respectively. The high rate of MRSA and extended-spectrum  $\beta$ -lactamase-producing strains reiterates the need for the judicious use of antibiotics for the appropriate management of diabetic foot ulcers. However, further studies with a large patient population are required to validate the results.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

MS was the project administrator and contributed to supervision, conception, visualization, validation, interpretation of data, resources and the literature review of the present study. XQ contributed to conceptualization, resources, methodology, validation and the literature review of the present study. LT contributed to resources, investigation, methodology and the literature review of the present study. HZ contributed to resources, formal analysis, data curation and the literature review of the present study. AY contributed to resources, acquisition of data, analysis and interpretation of data, and the literature review of the present study, and was involved in drafting the manuscript, and revising it critically for important intellectual content. All authors agreed to be accountable for all aspects of work ensuring integrity and accuracy. MS and AY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was performed in line with the principles of the V2008, Declaration of Helsinki and the laws of China. The present study was approved under the ethical approval number PR-2017-21 by the Human Ethics Committee of the Huaihua Cancer Hospital. Informed consent was obtained from each patient or their legal guardian for the commencement of the present study.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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