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Predominance of multidrug-resistant bacteria with high resistance to empiric antibiotics in diabetic foot ulcers: a cross-sectional study

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Diabetic foot ulcers (DFUs) pose a serious clinical challenge due to their chronic nature and high risk of infection. DFUs present polymicrobial infections frequently involving antibiotic-resistant bacteria, complicating treatment and increasing the risk of amputations. We conducted a cross-sectional study of 153 DFU patients to analyze the bacterial profile of DFUs and prevailing patterns of antibiotic resistance. The pathogenic bacteria isolated from DFUs were predominantly Gram-positive (62%), with *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) being the most frequent isolates along with Gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Over 60% of Gram-positive bacteria and 95% of Gram-negative bacteria were multidrug resistant with a median resistance to 9–11 antibiotics, most of which are in the prescribed regimen for diabetic foot infections. Multidrug resistance (MDR) was observed in approximately 95% of Gram-negative and 60–87% of Gram-positive isolates. We categorized the antibiotics following the AWaRe (Access (A), Watch (W), Reserve (R)) classification and have identified the antibiotics that can be prioritized for treatment of DFU leaving out the reserve category only for treating MDR bacteria. The findings underscore the need for prudent use of antibiotics, microbiological profiling of DFU bacteria and periodic updates to the empirical treatment regimen to improve patient outcomes.

Keywords Diabetic foot ulcer, Antibiotic resistance, Multidrug resistance, AWaRe classification

Abbreviations

AWaRe	Access (A), Watch (W), Reserve (R)
CoNS	Coagulase-negative Staphylococci
DFU	Diabetic foot ulcer
DFI	Diabetic foot infection
GLASS	Global antimicrobial resistance and use surveillance system
MAR	Multiple antibiotic resistance
MDR	Multidrug resistant
MRSA	Methicillin-resistant *S. aureus*

Diabetes mellitus (DM) is a chronic metabolic disorder that leads to complications such as hypertension, obesity, neuropathy and nephropathy. Globally, the prevalence of diabetes has been increasing steadily and is projected

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to affect approximately 578 million people by 2030[1]. The National Health and Morbidity Survey 2023 reported that one in six adults in Malaysia are living with diabetes[2]. A common complication of longstanding and poorly managed diabetes is diabetic foot ulcers (DFUs) that arise from neuropathic, ischemic or mixed neuro-ischemic causes[3]. Globally, about one-third of diabetic patients in the world are at risk of developing DFUs, and about 50% of these ulcers are likely to get infected, leading to the development of diabetic foot infections (DFI)[4].

DFIs are frequently polymicrobial and often involve diverse bacterial populations. *Staphylococcus aureus*, including Methicillin-resistant *S. aureus* (MRSA) is commonly isolated. Other prevalent bacteria are *Streptococcus spp.* and *Enterococcus spp.* Among Gram-positive bacteria, and *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among Gram-negative bacteria[5–7]. In chronic polymicrobial DFIs, Staphylococcus spp. and Streptococcus spp. typically predominate, while Gram-negative bacilli often serve as co-pathogens[8]. The microbial composition of DFIs can vary significantly across patients and geographical locations. Studies from Portugal and Germany report a predominance of Gram-positive bacteria in DFU, whereas reports from China and Bangladesh highlight a high prevalence of Gram-negative bacteria[9–13].

Biofilms, with their matrix of extracellular polymeric substances, provide a protected environment for symbiotic coaggregation of different bacterial species, and with their complex pathophysiology, contribute significantly to antibiotic resistance. In DFIs, biofilms are frequently observed playing a central role in the chronicity of the ulcers and the development of multidrug resistance, thereby impeding wound healing and necessitating prolonged antibiotic therapy[14]. The presence of biofilms substantially complicates treatment as the antibiotic concentrations required to eradicate biofilm-associated bacteria can be 10–1000 times higher than those needed for planktonic forms[15].

In China, Gram-positive bacteria isolated from DFUs showed resistance to multiple antibiotic classes, including beta-lactams (penicillin group), aminoglycosides (gentamycin), macrolides (erythromycin and clindamycin), fluoroquinolones (ciprofloxacin and levofloxacin), and tetracyclines. Gram-negative bacteria in the same settings demonstrated resistance to cephalosporins[9,16]. Similar patterns of antibiotic resistance have been reported in studies from Peru and Brazil, where isolates were resistant to one or more of these commonly used antibiotics[17,18]. The increasing prevalence of antibiotic-resistant organisms not only complicates treatment, contributes to prolonged hospitalization, higher healthcare costs and an elevated risk of treatment failure. Ultimately, the presence of antibiotic-resistant bacteria significantly increases morbidity and mortality among DFU patients.

The emergence of multidrug resistant (MDR) bacteria in DFUs is a significant risk of lower limb amputations. A meta-analysis reported that MDR bacteria are prevalent in approximately 51% of DFUs, with *S. aureus* and *Enterococcus spp.* being the most common Gram-positive bacteria, and *E. coli*, *P. aeruginosa* predominant among the Gram-negative isolates[19]. Prior exposure to broad-spectrum antibiotics has been identified as a major risk factor for subsequent infection with MDR bacteria[8].

This cross-sectional study was designed to investigate the bacterial profile of DFU patients in Malaysia and assesses their antibiotic resistance patterns, to provide insight into emerging resistance patterns in this geographical region to help re-evaluate the treatment plan for DFU.

Results

Patient demography

A total of 153 patients with active diabetic foot ulcers were recruited for this study. The majority were Malays (64%), followed by Indian (24%) and Chinese (approximately 12%) reflecting the major ethnic groups in Malaysia (Table 1). About 68% of the patients were male. In terms of age distribution, 21% were between 46 and 55 years; 38% were aged 56–65 years and around 23% were over 65 years of age. Most patients (~ 62%) had been living with diabetes for more than 5 years (Table 1). According to Wagner's grading system ([20]), the majority presented Grade 2 ulcers (61%), while 23% had Grade 3 ulcers. Most patients (51%) had an ulcer duration of 1–4 weeks whereas around 12% of the patients had ulcers persisting for more than 20 weeks (Table 1). There was no significant correlation between diabetic foot ulcer duration and Wagner's grade or the other parameters. Most of the patients (97%) had diabetic neuropathy.

Bacterial characteristics

A total of 383 bacterial isolates were cultured from 153 patients. Among these, 61% of the isolates were Gram-positive. Monomicrobial infections were observed in 19% of patients, while 25% had polymicrobial infections involving two bacterial species and 12% had three different bacterial isolates from their wounds (Table 1). Gram-positive bacteria were predominant across different Wagner's Grade ulcers.

The bacterial species commonly isolated were *Staphylococcus aureus* (47%), Coagulase-negative Staphylococci (CoNS) (39.3%), *Streptococcus pyogenes* (11.8%), *Streptococcus pneumoniae* (5.9%), *Pseudomonas aeruginosa* (20.3%), *Klebsiella pneumoniae* (19.6%), *Escherichia coli* (10.5%) and *Proteus spp.* (4.6%) (Table 2). The aerobic cocci, *S. aureus* and CoNS were the most isolated Gram-positive bacteria prevalent in 86% of the patients followed by *S. pyogenes* (12%). Among Gram-negative bacteria, *P. aeruginosa* and *K. pneumoniae* (~ 20% each) were the most prevalent followed by *E. coli* (10.5%).

Biofilm formation

Both crystal violet staining of biofilms and Congo red agar method were used to evaluate biofilm forming capacity of bacteria. Biofilm forming bacteria were present in 60% of the patients (Table 1). Across the different Wagner's ulcer grades, the number of biofilm-forming bacterial isolates was slightly higher than that of non-biofilm formers. Moderate to high biofilm formers were present mostly in ulcers with grades 3 and 4 (Table 3). In patients with grade 2 ulcers, 13 had mild biofilm forming bacteria while 26 patients had moderate and 17 patients had high (17) biofilm formers. Majority of the patients examined presented with grade 2 ulcers (~ 61%),

Variables	Number (%)	Variables	Number (%)
Gender		**Duration of diabetes**	
Male	105 (68.63%)	< 1 year	5 (3.27%)
Female	48 (31.37%)	1–5 years	25 (16.34%)
Age group (years)		6–10 years	40 (26.14%)
25–35	7 (4.58%)	11–20 years	34 (22.22%)
36–45	18 (11.76%)	21–30 years	17 (11.11%)
46–55	33 (21.57%)	31–40 years	4 (2.61%)
56–65	58 (37.91%)	Unknown	28 (18.3%)
66–75	27 (17.65%)	***Wagner's Grade***	
75–85	8 (5.23%)	Grade 1	15 (9.8%)
Unknown	2 (1.31%)	Grade 2	93 (60.8%)
Race		Grade 3	35 (22.9%)
Malay	98 (64.05%)	Grade 4	8 (5.2%)
Indian	37 (24.18%)	Unknown	2 (1.3%)
Chinese	18 (11.76%)	**Number of bacteria per patient**	
Ulcer duration		None	22 (14.4%)
1–4 weeks	78 (51%)	1	29 (19%)
5–8 weeks	25 (16.3%)	2	39 (25.5%)
9–12 weeks	12 (7.8%)	3	19 (12.4%)
13–20 weeks	10 (6.5%)	4	15 (9.8%)
> 20 weeks	18 (11.8%)	5	18 (11.8%)
Unknown	10 (6.5%)	6	7 (4.6%)
Biofilm formation		> 6	4 (2.6%)
Biofilm-positive	91 (59.5%)		
Biofilm-negative	37 (24.2%)		
n/a	25 (16.3%)		

Table 1. Demographic characteristics of DFU patients.

Bacteria	No. of patients	Percentage	No. of isolates
Gram-positive			
S. aureus	72	47%	113
CoNS	60	39.2%	87
S. pyogenes	18	11.8%	19
S. pneumoniae	9	5.9%	10
Bacillus spp.	4	2.6%	4
E. faecalis	3	2%	3
Others	1	0.65%	1
Gram-negative			
P. aeruginosa	31	20.3%	64
K. pneumoniae	30	19.6%	39
E. coli	16	10.5%	19
Proteus spp.	7	4.6%	7
P. vulgaris	5	3.3%	6
Others	9	5%	11

Table 2. Bacterial distribution in DFU patients.

thereby contributing to high numbers in all groups. The most common biofilm formers were *S. aureus* and CoNS found in 46% of the patients (Table 4). Other biofilm formers were Gram-positive *S. pyogenes*, *S. pneumoniae* and Gram-negative *P. aeruginosa*, *K. pneumoniae* and *E. coli*.

Antibiotic sensitivity

The bacteria were tested against 21 antibiotics, belonging to 10 classes and their sensitivity/resistance pattern was identified. The Multiple antibiotic resistance (MAR) index analysis showed that among Gram-positive

Wagner's Grade	No. of Patients with Biofilms		
	Mild	Moderate	High
1	1	2	2
2	13	26	17
3	2	11	11
4	1	2	3

Table 3. Correlation between biofilm forming bacteria and Wagner's grade.

Bacteria	No. of patients	Percentage
S. aureus	41	26.8%
CoNS	29	19%
S. pyogenes	16	10.5%
K. pneumoniae	15	9.8%
P. aeruginosa	8	5.2%
E. coli	7	4.6%
S. pneumoniae	6	3.9%
Proteus spp.	4	2.6%
E. faecalis	2	1.3%
P. vulgaris	1	0.7%
Bacillus spp.	1	0.7%
Others	3	2%

Table 4. Distribution of biofilm-forming bacteria in patients.

Bacteria	MAR index	No. of MDR isolates/ Total no. of isolates	Percentage of MDR isolates
Gram-positive			
S. aureus	0.41	68/113	60.2%
CoNS	0.54	76/87	87.4%
S. pyogenes	0.41	15/19	78.9%
Gram-negative			
P. aeruginosa	0.57	62/64	97%
K. pneumoniae	0.53	37/39	95%
E. coli	0.51	18/19	94.7%

Table 5. MAR index and multidrug resistant isolates of the most prevalent bacteria.

bacteria, *S. aureus* had a MAR index of 0.41 with 68/113 isolates (60%) exhibiting multidrug resistance (MDR). In contrast, CoNS had a higher MAR index of 0.54 with 76/87 isolates (87%) identified as MDR (Table 5). The Gram-negative bacteria were highly resistant to antibiotics and had MAR indices of 0.51–0.57; about 95% of the Gram-negative isolates were MDR.

Most *S. aureus* isolates were resistant to 4–11 antibiotics with a median resistance of 9 out of the 21 antibiotics tested. In contrast, CoNS isolates exhibited broader resistance, with many resisting up to 18 antibiotics with a median resistance of 11 (Fig. 1A). The Gram-negative isolates were resistant to 9–14 antibiotics with a median resistance of 11 or 13 (Fig. 1B). The Gram-positive bacteria *S. aureus* and CoNS exhibited high levels of resistance (70–100%) to beta-lactam antibiotics, including amoxycillin, ampicillin, aztreonam, methicillin, oxacillin, and penicillin. Additionally, CoNS demonstrated notable resistance (~ 80%) to doxycycline (tetracycline class) and clindamycin (lincomycin class) as well (Fig. 1C).

S. aureus showed high susceptibility (70–90%) to several antibiotics, including amikacin, gentamycin, tobramycin, imipenem, chloramphenicol, co-trimoxazole, linezolid and vancomycin (Fig. 1D). CoNS, on the other hand, had lower susceptibility (~ 60%) to amikacin, gentamycin, tobramycin, imipenem, chloramphenicol, ciprofloxacin and co-trimoxazole (Fig. 1E). *S. pyogenes* exhibited limited susceptibility (50–70%) to aminoglycosides but was relatively more sensitive (70–90%) to amoxycillin, imipenem, chloramphenicol, ciprofloxacin, co-trimoxazole, linezolid and vancomycin (Fig. 1F).

All Gram-negative bacterial isolates were highly susceptible (60–80%) to aminoglycoside antibiotics (amikacin, gentamycin, tobramycin), the beta-lactam imipenem and the fourth-generation cephalosporin

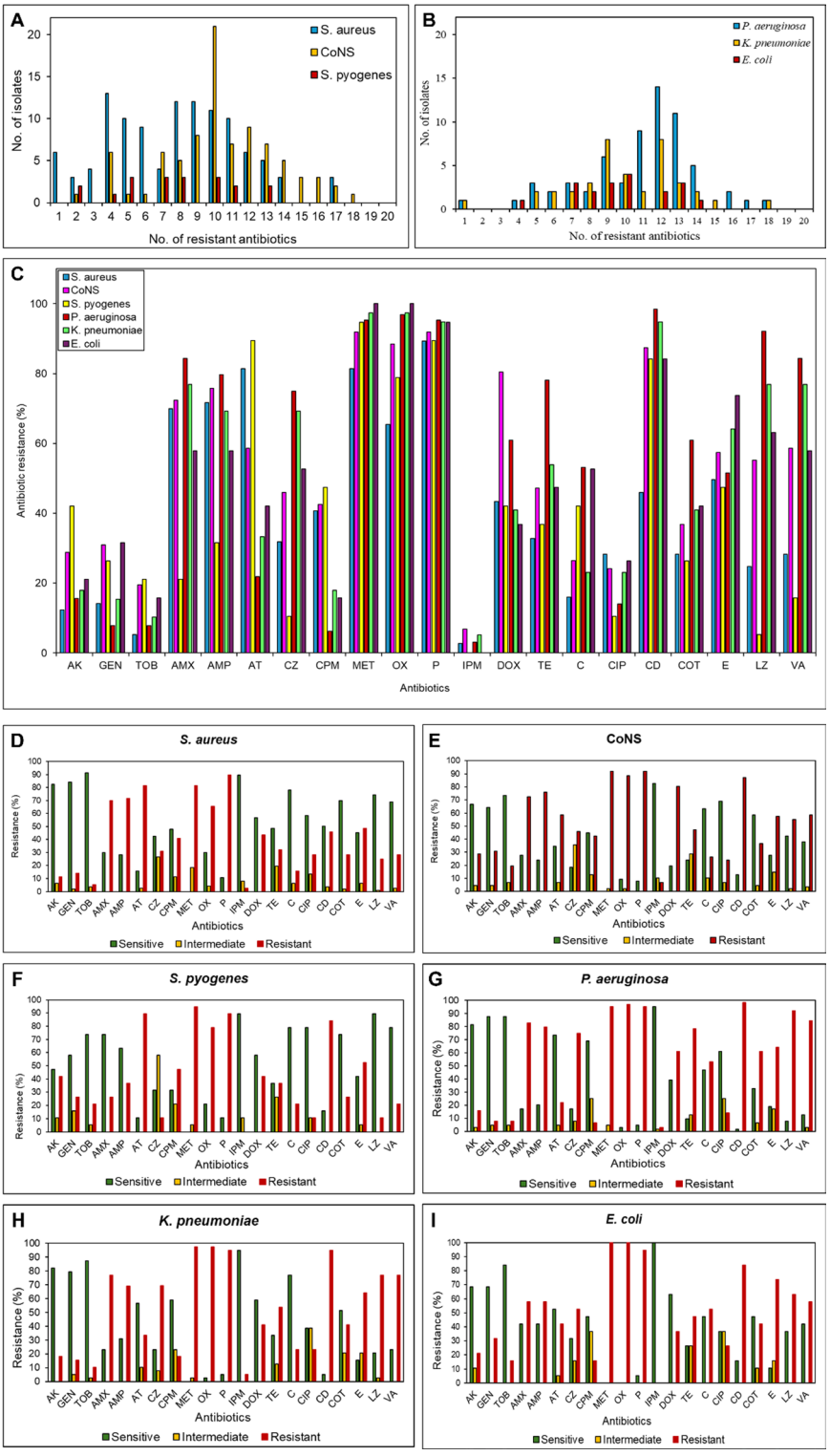

Fig. 1. Antibiotic resistance pattern of DFU bacteria. Graphs showing antibiotic resistance depicted in terms of the number of antibiotics versus the number of isolates of (**A**) Gram-positive and (**B**) Gram-negative bacteria. (**C**) Antibiotic resistance percentage (sum of antibiotic-resistant isolates/total no. of isolates) of three Gram-positive and three Gram-negative bacteria against the different antibiotics. **D-I**) Graphs depicting percentage of susceptibility (green) or resistance (red) or intermediate resistance (orange) to the different antibiotics for the Gram-positive bacteria *S. aureus* (**D**), CoNS (**E**) and *S. pyogenes* (**F**) and Gram-negative bacteria *P. aeruginosa* (G), *K. pneumoniae* (**H**) and *E. coli* (**I**). The antibiotics used are: amikacin (AK), amoxicillin (AMX), ampicillin (AMP), aztreonam (AT), chloramphenicol (**C**), clindamycin (CD), cefepime (CPM), cefazolin (CZ), ciprofloxacin (CIP), co-trimoxazole (COT), doxycycline (DOX), erythromycin (E), gentamicin (GEN), methicillin (MET), oxacillin (OX), imipenem (IPM), linezolid (LZ), penicillin G (P), tetracycline (TE), tobramycin (TOB) and vancomycin (VA).

cefepime. However, they exhibited high resistance (60–100%) to beta-lactam antibiotics such as amoxycillin, ampicillin, methicillin, oxacillin and penicillin, as well as to clindamycin, erythromycin, linezolid and vancomycin (Fig. 1G-I).

This study highlights the bacterial profile and prevailing antibiotic resistance patterns of clinically significant bacteria in diabetic foot infections.

Discussion

Globally, the leading pathogens identified to be responsible for deaths associated with antibiotic resistance in 2019 were *E. coli*, *S. aureus*, *K. pneumoniae*, *S. pneumoniae*, *Acinetobacter baumannii*, and *P. aeruginosa*[21], most of which are present in our patients. The Institute for Health Metrics and Evaluation has identified *E. coli*, *S. aureus*, *K. pneumoniae* as the top causative organisms for deaths associated with antimicrobial resistance (AMR)[22]. The most prevalent pathogenic bacteria identified in this study were *S. aureus* (113 isolates) and CoNS (87 isolates) in the Gram-positive organisms, and *P. aeruginosa* (64 isolates) and *K. pneumoniae (*39 isolates) among Gram-negative organisms. Studies conducted in Germany and Portugal report a similar predominance of Gram-positive bacteria, especially *S. aureus* in DFU[10,11]. Contrarily, in Bangladesh, Gram-negative bacteria contribute to 83% of the isolates with *K. pneumoniae* being the most common isolate and in Peru, 63% of the isolates were Gram-negative, predominated by *S. aureus* and *E. coli*[12,23]. A meta-analysis from China with 11,483 patients showed a prevalence of ~ 52% Gram-negative and ~ 43% Gram-positive bacteria in DFI[9]. Other studies from China also report a high prevalence of Gram-negative bacteria (54–57%) with a predominance of Enterobacteria, Staphylococcus, Klebsiella and Pseudomonas[13, 24]. The antibiotic resistance pattern varies widely geographically probably in accordance with the prevailing antibiotic use in the country. A 40% prevalence of MDR bacteria in DFU was reported in Southern China while a study from Bangladesh reported that majority of the isolates were multidrug resistant[12,13]. In contrast, a study from Germany depicted very low prevalence of multidrug resistant species[10].

The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report highlights that beta-lactams penicillin, amoxicillin and ciprofloxacin were among the most frequently prescribed antibacterials globally[25]. This is reflected in the antibiotic resistance pattern observed in our study whereby, bacteria show a high level of resistance to beta-lactam family of antibiotics. Another study from Malaysia highlights similar beta-lactam resistance observed in bacteria causing upper respiratory infections[26]. The beta-lactams used in our study viz. amoxicillin, ampicillin, cefazolin, methicillin, oxacillin are all classified under "Access" group based on the AWaRe (Access (A), Watch (W), Reserve (R)) classification developed by the World Health Organization (WHO)[27]. The "access" group is the first line of antibiotics that are supposedly safe to use, but we observed very high resistance of DFU bacteria to many of these antibiotics.

We observed most bacteria to be sensitive to the aminoglycosides, which prove to be better treatment option as amikacin and gentamycin are in the "Access" (A) group. The only beta-lactam antibiotic that almost all bacteria were sensitive to was imipenem, which is placed under the "Reserve" (R) category of AWaRe classification and therefore is to be used only against multidrug resistant bacteria when other options are not available[27]. Clindamycin (A), co-trimoxazole (A), amoxycillin (A), ampicillin (A), ciprofloxacin (W), cefepime (W), vancomycin (W) and linezolid (R) are commonly recommended for management of diabetic foot as per the clinical practice guidelines[28]. Out of these, a high level of resistance is observed in our study against clindamycin, amoxycillin, and ampicillin by all the bacteria. Only ciprofloxacin is effective against all bacteria while cefepime is more effective against Gram-negative bacteria. Linezolid and vancomycin are only effective against *S. pyogenes* and *S. aureus* while the other bacteria show very high resistance to them. There is moderate level of resistance against co-trimoxazole.

The National Antibiotic Resistance Surveillance Report, 2023 details increased resistance rates of *S. aureus* for penicillin, rifampicin, clindamycin, erythromycin, gentamicin, and linezolid with majority of MRSA strains from medical, surgical, and orthopedic wards[29]. Our study shows S. aureus has about 90% resistance for penicillin, ~ 40% resistance to clindamycin and erythromycin and ~ 20% or less resistance to linezolid and gentamycin. The National Surveillance on Antimicrobial Utilization Report, 2023 highlights increased use of antibiotics in the hospitals especially, cephalosporins (mainly 3rd generation) and penicillin[30]. In our study, we observed moderate to high resistance against cefazolin, the first generation of cephalosporins while the fourth generation, cefepime has moderate resistance. Imipenem is not widely used, and this corresponds directly to the lowest resistance (< 10%) against this antibiotic by all the bacteria tested in our study. Similar effectiveness of imipenem has been reported from China as well[24]. Malaysian utilization of antibiotics is reported to be 10-times more in private sector for primary care compared to public sector. Private sector used < 70% of antibiotics in the Access category with increased use of antibiotics in the Reserve category[31]. As an increase in antibiotic usage drives an increase in resistant bacteria, stewardship strategies need to be designed to control local utilization of antibiotics to prevent the emergence of MDR and XDR strains of bacteria.

Our cross-sectional study presents the dynamic clinical profile of the prevailing bacteria in DFU and local resistance patterns highlighting the need for routine microbiological surveillance coupled with appropriate management. This study provides a basis for re-evaluation of the therapeutic regimen for DFU bacteria periodically as per the prevailing antibiotic resistance pattern. Measures to reduce general antibiotic usage, following the AWaRe classification and prudent prescription of antibiotics would help control the emergence of MDR bacteria. Periodical monitoring of the resistance-sensitivity profile of the DFU bacteria coupled with effective antimicrobial stewardship programs would be valuable in guiding effective treatment strategies to improve patient outcomes.

Limitations:

The study was conducted on a single site, but the patient population was a mixed group representing the major ethnicities in the country.

Methods

Aim, design and study setting

The aim of this cross-sectional study was to isolate and characterize the bacteria prevalent in DFU patients and study their antibiotic resistance pattern. The study was conducted on patients from the orthopedic clinic in a tertiary hospital in Klang valley, Malaysia. All methods were performed in accordance with the Declaration of Helsinki and National ethical guidelines for biomedical research involving human subjects. Ethics approval for this study was obtained from the National Medical Research Register (NMRR), Malaysia (KKM/NIHSEC/P18-2188(R)) and the International Medical University (IMU) Joint Committee on Research Ethics (MMHS 1/2018 (01)).

Patient recruitment

A total of 153 patients were recruited for this study during 2019–2020, after obtaining informed consent. The sample size of this study was derived based on the incidence rate obtained from the National Diabetes Registry Report[32] and was calculated using the equation, n = [DEFF*Np(1-p)]/[(d2/Z21-α/2*(N-1) + p*(1-p) where n = sample size; N = total population size (1527); p = probability of disease or exposure in the population (0.5); cl = confidence level (95%); d = Absolute precision on either side of the proportion (0.05); DEFF = design effect. The inclusion criteria applied were diabetes, age above 18, ulcer duration of at least one month and exclusion criteria were patients less than 18 years age, pregnant women and patients with unrelated skin diseases around the wound.

Sample collection and bacterial characterization

The wound was assessed according to the guidelines obtained from the Clinical Practice Guidelines: Management of Diabetic Foot by the Ministry of Health, Malaysia[28]. A swab sample was collected from the active ulcer site using a sterile cotton-tip applicator. The cotton swab was immediately placed in a transport medium and transferred to the laboratory, where it was stored at −20 °C until further processing. The bacteria isolated from the swabs were cultured on different selective media, bacterial isolation and characterization was done by Gram staining and standard biochemical tests. Patient history and demographic details were obtained from the medical records.

Biofilm formation

The bacterial isolates were cultured in plain tryptic soy broth (TSB) with 1% glucose till they reached a turbidity of 0.5 McFarland units. Then 100 µl of the bacterial inoculum was added per well of a 96-well flat-bottomed plate (Eppendorf, Germany) and incubated in a shaking incubator at 37 °C at 150 rpm for 24 h to form biofilms. The wells were then gently washed with ultrapure water thrice and the biofilms formed were stained with 0.1% crystal violet solution at 25 °C for 15 min. The wells were washed and 100 µl of absolute ethanol was added to release the crystal violet and the optical density was measured at 595 nm using a microplate reader.

Bacterial isolates were also inoculated in brain heart infusion (BHI) agar with 5% sucrose and 0.08% Congo red and incubated for 24 h at 37 °C. Biofilm producers formed black or dark red colonies, whereas non-biofilm producers formed red colonies.

Antibiotic sensitivity test

The bacterial isolates were tested for antibiotic susceptibility by the Kirby–Bauer disc diffusion method on Tryptic Soy Agar (TSA) (CRITERIONTM) following standard protocols (CLSI, 2020)[33]. The antibiotic discs used were (HiMedia Laboratories*, India) amikacin (AK) (30 µg), amoxicillin (AMX) (10 µg), ampicillin (AMP) (25 µg), aztreonam (AT) (30 µg), chloramphenicol (C) (30 µg), clindamycin (CD) (2 µg), cefepime (CPM) (30 µg), cefazolin (CZ) (30 µg), ciprofloxacin (CIP) (5 µg), co-trimoxazole (COT) (25 µg), doxycycline (DOX) (10 µg), erythromycin (E) (15 µg), gentamicin (GEN) (10 µg), methicillin (MET) (10 µg), oxacillin (OX) (1 µg), imipenem (IPM) (10 µg), linezolid (LZ) (30 µg), penicillin G (P) (10 units), tetracycline (TE) (30 µg), tobramycin (TOB) (10 µg) and vancomycin (VA) (30 µg). The bacterial isolates (0.5 McFarland units) were swabbed on the TSA plates and antibiotic discs were placed on them. The plates were incubated at 37 °C for 18–24 h and the zones of inhibition were measured. The isolates were classified as resistant, intermediate or sensitive to antibiotics on the basis of the size of the zone of inhibition according to the Clinical Laboratory Standard Institute (CLSI) guidelines[33]. In this study, 21 antibiotics belonging to 11 classes were used. Multiple antibiotic resistance (MAR) index was calculated as: (Number of resistant antibiotics)/(Total number of antibiotics tested)[34]. Multi drug resistance (MDR) is calculated as resistance to at least one agent in three or more antimicrobial categories[35]. Antibiotic resistance percentage was calculated as: (Sum of antibiotic-resistant isolates)/(Total no. of isolates).

Statistical analysis

Data was analyzed using IBM SPSS Statistics software (Version 25). Descriptive statistics for the demographic data were calculated as mean, median and percentages. Association between different categories was calculated using independent t-test. p-values < 0.05 were considered as statistically significant.

Data availability

The data used during the current study are available from the corresponding author on reasonable request.

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Author contributions

FDA: Conception, Planning, Supervision, Data analysis, Finalizing the manuscript; ECF: Planning, Supervision, Data analysis, Funding, Manuscript writing; NNMS: Sample collection, Carrying out experiments, Data analysis; OJR and WT: Data analysis, Literature search; VKN: Supervision, Planning; AHH: Clinical supervision, Planning. All authors reviewed and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Ethics approval for this study was obtained from the National Medical Research Register (NMRR) (KKM/NIHSEC/P18-2188(R)) and the International Medical University (IMU) Joint Committee on Research Ethics (MMHS 1/2018 (01)). The patients were recruited after obtaining informed consent to participate in the study.

Additional information

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