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Diabetes-related lower limb wounds: Antibiotic susceptibility pattern and biofilm formation

Raha Orfali [a,*], Safina Ghaffar [a,*], Lateefa AlAjlan [a], Shagufta Perveen [a], Eman Al-Turki [a], Fuad Ameen [b]

[a] Department of Pharmacognosy, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia
[b] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

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ABSTRACT

The expeditious incidence of diabetes mellitus in Riyadh, Saudi Arabia, there is a significant increase in the total number of people with diabetic foot ulcers. For diabetic lower limb wound infections (DLWs) to be effectively treated, information on the prevalence of bacteria that cause in this region as well as their patterns of antibiotic resistance is significant. Growing evidence indicates that biofilm formers are present in chronic DFU and that these biofilm formers promote the emergence of multi-drug antibiotic resistant (MDR) strains and therapeutic rejection. The current study targeted to isolate bacteria from wounds caused by diabetes specifically at hospitals in Riyadh and assess the bacterium's resistance to antibiotics and propensity to develop biofilms. Totally 63 pathogenic microbes were identified from 70 patients suffering from DFU. Sixteen (25.4%) of the 63 bacterial strains were gram-positive, and 47 (74.6%) were gram-negative. Most of the gram-negative bacteria were resistant to tigecycline, nitrofurantoin, ampicillin, amoxicillin, cefalotin, and cefoxitin. Several gram-negative bacteria are susceptible to piperacillin, meropenem, amikacin, gentamicin, imipenem, ciprofloxacin, and trimethoprim. The most significant antibiotic that demonstrated 100% susceptibility to all pathogens was meropenem. *Serratia marcescens* and *Staphylococcus aureus* were shown to have significant biofilm formers. MDR bacterial strains comprised about 87.5% of the biofilm former strains. To the best of our knowledge, Riyadh, Saudi Arabia is the first region where *Serratia marcescens* was the most common bacteria from DFU infections. Our research findings would deliver information on evidence-based alternative strategies to develop effective treatment approaches for DFU treatment.

1. Introduction

Diabetes is becoming more ubiquitous worldwide every year. Due to this rapid expansion, the World Health Organization (WHO) currently recognized Type 2 diabetes as an epidemic that is expanding extensively throughout the world (Ong et al., 2023). An epidemiologic investigations estimate that 700 million individuals worldwide will be affected by the disease by 2045, a 51 % rise if the current trend prevails. There is a significant financial impact in addition to this risk to worldwide overall wellness. From USD 760 billion in 2019 to USD 845 billion in 2045, the comparable annual international health spending is projected to rise by 11 % (Liang et al., 2023). The International Diabetes Federation's latest information highlights the extent to which the issue remains. It is concerning to take into account that diabetes caused 6.7 million deaths in 2021 or a fatality every five seconds.

Saudi Arabia is ranked seventh globally and has the second-highest incidence of type 2 diabetes mellitus (DM) among Middle Eastern countries, at a rate of 25.4 % (Jarrar et al., 2023). Additionally, 82 % of diabetic neuropathy cases, 31 % of retinopathy cases, and 32 % of kidney cases are among the highest rates of diabetes-related morbidities globally (Zhou et al., 2023). Foot ulcers (2.05 %), amputations (1.06 %), and gangrenes (0.19 %) were the most common diabetic foot problems in this cohort, with a prevalence of 3.30 %. Greater incidence levels of diabetes mellitus, ranging from 26.0 % to 61.8 %, have been documented among Saudi individuals (Alshaikh et al., 2023). Around 7 million people in the region suffer from diabetes at the moment, and an additional 3 million are pre-diabetic. A high proportion of diabetic patients will inevitably result in a higher number of individuals

* Corresponding authors.
E-mail addresses: rorfali@ksu.edu.sa (R. Orfali), sghafar.c@ksu.edu.sa (S. Ghaffar), shagufta792000@yahoo.com (S. Perveen), emalturki@ksu.edu.sa (E. Al-Turki), fuadameen@ksu.edu.sa (F. Ameen).

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experiencing issues related to their feet, placing significant strain on the country's health sector and economy (El-Kebbi et al., 2021).

According to a retrospective population investigation performed in Saudi Arabia, the fatality incidence among diabetes patients with foot ulcers was elevated by nearly twice as much as that of diabetic patients without foot ulcers. This indicates that DFU enhances the rate of death among diabetic patients. After acquiring a diabetic foot ulcer and enduring an amputation, death rates are high: up to 70 % of individuals die within five years after the surgery, and approximately 50 % die within five years of the ulcer's inception (Jarrar et al., 2023). In communities with low income, it is one of the diabetic consequences that can lead to an expense on the economic growth of the community, and overall health (Cuddapah et al., 2022).

The lifetime incidence of diabetic foot ulcers (DFU) is 15–25 %. Diabetes wound infections are most frequent, serious and expensive infections (Chen et al., 2023). DFU condition linked to lower limb amputation that carries a high risk of death and morbidity (McDermott et al., 2023). Diagnosis of diabetic foot infections (DFIs) can be challenging, which might result in the overuse of drugs. Innovative antimicrobial strategies are required due to the microbial organization in DFU and the existence of multidrug-resistant (MDR) bacteria (Turzańska et al., 2023).

DFUs tend to spread quickly to an infection, which increases the severity and fatality incidence tremendously in patients with diabetes. Numerous aerobes and anaerobes, as well as Gram-positive and Gram-negative bacteria, potentially invade DFUs. Chronic DFUs that are inhabited by a variety of aerobic bacterial species, including *Pseudomonas* species, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and anaerobic pathogens, might result in polymicrobial DFU infections (Wada et al., 2023). There are significant differences in the abundance of common pathogens obtained from DFUs between researches conducted across various regions of the world. Several variables, including regional characteristics, the severity of the infection, information about the patient, and the consumption of antibiotics, might affect the bacterial diversity in Diabetic wound infections (Afonso et al., 2021).

Bacterial formations in a biofilm are present in 60–80 % of chronic wounds. Because the colonizing bacteria interact in concert to produce a symbiotic habitat that is advantageous for the propagation of the infection and consequently, the development of a chronic wound, biofilms play a critical role in diabetic foot infections (Pouget et al., 2021). Differentiation between colonizing and infecting microorganisms presents a major challenge for the clinician. Faulty recommendations for antibiotics resulting from misinterpretation can accelerate the expansion of MDR microorganisms which poses significant risks for DFU patients (Pouget et al., 2020).

Enhanced knowledge of the bacterial arrangement in biofilms in long-term wounds would facilitate the creation of specialized antibacterial approaches and enhance the process of wound recovery. In this regard, the significantly large quantities of recent scientific research on DFUs concentrate on the effects of the surrounding milieu on pathogens as well as bacterial interaction (Goswami et al., 2023).

The host-microorganism interaction is consequently pivotal to the emergence of DFI. According to Dowd et al., (Dowd et al., 2008) bacteria in DFU are arranged typically into functionally equivalent pathogroups, where commensal and pathological microbes co-aggregate mutually beneficial relationships in a pathogenic biofilm to sustain a persistent infection. Since the bacteria in biofilms are 100–1,000 times more resistant to antibiotics than other bacteria, novel methods of delivering high dosages of antibiotics into the biofilm have been established.

Polymicrobial biofilms have been documented in DFU research on patients as well as pre-clinical investigations employing animal models. They are the primary factor for the delay in recovery. To manage infections, certain strategies have recently focused on biofilm formation. To limit the dissemination of multidrug-resistant bacteria, novel treatment strategies that consider the biofilm must be developed, which requires a stronger knowledge of the host-bacterial dynamics (Salah et al., 2022).

In this study, we designed and carried out a hospital-based study for Saudi patients with diabetic foot illness, drawing on the prior awareness campaigns executed in Riyadh, Saudi Arabia. The purpose of this study was to determine the bacteria responsible for diabetic foot ulcers as well as the patterns of antibiotic sensitivity exhibited by the bacterial isolates and biofilm formers.

2. Materials and methods

2.1. Sample collection

This prospective study was conducted at the King Fahad Medical City. The study took place from March 2023 to September 2023.70 diabetic patients with foot ulcers or wounds in outpatient departments of the hospital that were included by the research. Informed consent from the subjects was obtained in their native tongue, and institutional ethics approval was acquired. The study included all adult diabetic patients who visited the DM clinics at the study sites during the study period, provided informed consent, and had diabetic foot ulcers that measured up to or equivalent to the Wagner first-degree grading scale. Every patient was older than eighteen years of age. Pregnant women, children (less than 18 years) and patients with other co-morbid conditions like Hepatitis and HIV infections, chronic venous insufficiency, and osteomyelitis were excluded. Semi-structured questionnaires were used to collect socio-demographic and other clinical data.

The patients were evaluated with a thorough clinical examination and history. After doing an assessment of the ulcers by physician, sterile cotton-tipped swabs were used to collect material for culture from the deeper areas of the foot ulcers. The swabs were transported immediately in a transport medium to the lab for culturing and assessing sensitivity and biofilm formation.

To categorize ulcers, the Wagner Diabetic Foot Ulcer Classification System was used in this study. If there was neither an open lesion nor cellulitis; the classification was Grade 0-Pre-ulcerative. Superficial ulcers were Grade 1, deep ulcer up to tendons and joint tissue were Grade 2 and deep ulcer accompanied by joint infection, osteomyelitis and an abscess considered as Grade 3. Grade 4: Heel or forefoot gangrene that is localized and Grade 5: Global gangrene that affects the entire foot.

2.2. Ethical consideration

The King Fahad Medical City Riyadh's ethics and review committee granted ethical approval (FWA00018774). The Department sent an official letter of support to the study locations. Before enrolling potential participants in the study, an information sheet with all the study-related details was handover to them, and their voluntary consent was obtained. During the course of the study, their identifiers were kept anonymous to preserve confidentiality.

2.3. Bacterial identification and antimicrobial susceptibility analysis

Swabs sample from patients were cultured on blood agar and Mac Conkey agar and the plates were incubated at 37 °C overnight. To identify gram-positive and gram-negative bacterial strains gram staining was performed. Antibiotic identification and susceptibility were identified by VITEK® 2 system. The bacteria were identified using the Vitek 2 Compact 60 (AES software) Gram-Positive Identification test (GPI) cards and Gram-Negative Identification test (GNI) (BioMérieux, France). The automated reader-incubator was packed with cards that had been inoculated with the suspension vials at the Smart Carrier Station™. The manufacturer's instructions were followed for the inoculation and interpretation of the identification and susceptibility cards (Clinical and Laboratory Standards Institute, 2017).

2.4. Congo red agar (CRA) method

The Congo red agar (CRA) method is a qualitative assay that uses color changes in colonies inoculated on CRA media to identify bacteria that produce biofilms. About 38 g/L of Brain Heart Infusion (BHI) agar, which was prepared with 0.8 g of Congo red and 36 g of sucrose, is combined to produce the CRA medium. The colonies that experience color changes during 24-hour incubation period at 37 °C can be used to determine whether they produce biofilm or not. Colored colonies that form biofilm are characterized by a dry black, crystalline quality, while pink colonies do not produce biofilm (Asghari et al., 2021).

2.5. Tube method (TM)

The tube technique (TM) is a qualitative test used to identify microorganisms that produce biofilms by observing a visible film. A polystyrene test tube containing TSB is inoculated with isolates, and the tube is then incubated for 24 h at 37 °C. Planktonic cells on PBS are released from the sessile isolates of biofilms that formed on the walls of polystyrene test tubes after an hour and two rinses with phosphate-buffered saline. After safranine staining the polystyrene test tube, the stain is removed by rinsing it twice with PBS. Air drying the test tube procedure subsequently resulted in the visible film lining. The walls and bottom of the tube indicate the formation of biofilm (Kırmusaoğlu, 2019).

2.6. Statistical analysis

The tests were performed in triplicate and the average of results was calculated. Strains were classified as biofilm producers and non-biofilm producers. Data was compiled and descriptive statistics were applied using Microsoft Excel 2016 Edition (Microsoft, Seattle, WA).

3. Results

3.1. Patient's socio-demographic and clinical characteristics

The current study includes seventy (70) King Fahad Medical City study patients. Among 42/70 (60 %) of these were men, and 28/70 (40 %) were women. The majority of the patients ranged in age from 40 to 80 years old, with a mean age of 54 ± 7SD. Of the survey individuals, 65/70 (92.8 %) were urban inhabitants of Riyadh, whereas only 5/70 (7.1 %) were from rural areas. According to Table 1, type II diabetes impacted 51/70 (72.8 %) of the individuals, type I diabetes 19/70 (27.1 %), hypertension 52/70 (74.27 %), and kidney diseases 17/70 (24.28 %) of the individuals studied.

Table 1
Socio-demographic and clinical characteristics of the study participants.

Characteristics	Categories	Frequency (n)	(%)
Sex	Male	42	60
	Female	28	40
Age	<40	2	2.85
	41–50	19	27.1
	51–60	22	31.3
	61–70	14	20
	71–80	5	7.14
	≥ 80	3	4.2
Residence	Urban	65	92.85
	Rural	05	07.14
Diabetes	Type 1	19	27.1
	Type 2	51	72.8
HBA1c	1.6 to 8.0	4	05.7
	8 to 16	56	80
	16–24	10	14.28
Kidney disease	Yes	17	24.28
	NO	53	75.57
Hypertension	Yes	52	74.27
	No	18	25.7
Wagner's Classification	Grade 1	13	18.47
	Grade 2	10	14.28
	Grade 3	32	45.57
	Grade 4	14	20
	Grade 5	1	1.4
Total		**70**	**100**

Table 2
Bacterial isolates from study participants with diabetic foot ulcer.

Gram-Positive	No.	%	Gram-Negative	No.	%
Staphylococcus aureus	7	11.1	*Serratia marcescens*	13	20.6
Enterococcus faecalis	4	6.3	*Escherichia coli*	6	9.5
Staphylococcus warneri	1	1.5	*Proteus mirabilis*	6	9.5
Staphylococcus caprae	1	1.5	*Pseudomonas aeruginos*	5	7.9
Staphylococcus lentus	1	1.5	*Enterobacter aerogenes*	3	4.7
Dermacoccus nishinomiyaensis	1	1.5	*Klebsiella pneumoniae*	3	4.7
Kocuria Kristina	1	1.5	*Citrobacter koseri*	2	3.1
			Klebsiella oxytoca	2	3.1
			Sphingomonas paucimobilis	2	3.1
			Enterobacter cloacae complex	2	3.1
			Proteus penneri	1	1.5
			Morganella morganii	1	1.5
			Alcaligenes faecalis	1	1.5
Total	**16**	**25.4**		**47**	74.6

3.2. Magnitude of bacterial isolates from diabetic foot ulcer infections

Totally 63 distinct bacterial isolates were found in 70 patients suffering from diabetic foot ulcers. 47 (74.6 %) of these isolates were Gram-negative, and 16 (25.3 %) were Gram-positive. Higher Gram-negative bacteria (74.6 %) than Gram-positive bacteria (25.3 %) were identified. *Staphylococcus aureus* accounted for the majority of the isolated Gram-positive bacteria (11.1 %), followed by *Enterococcus faecalis* (6.3 %), *Staphylococcus warneri* (1.5 %), *Staphylococcus lentus*, *Dermacoccus nishinomiyaensis*, and *Kocuria kristinae*.

The most common isolated Gram-negative bacteria were *Serratia marcescens* (20.6 %), *Escherichia coli* (9.5 %), *Proteus mirabilis* (9.5 %) and *Pseudomonas aeruginosa* (7.9 %). *Sphingomonas paucimobilis*, *Enterobacter cloacae*, *Morganella morganii*, *Alcaligens faecalis*, *Proteus penneri*, *Citrobacter koseri*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were among the other isolates (Table 2).

The Wagner grade 3 of diabetic foot ulcers had the greatest percentage of culture-positive cases (45.57 %) (32/70), followed by Wagner grade 4 (20 %) (14/70), Wagner grade 1 (18.47 %) (13/70), Wagner grade 2 (14.28 % (10/70), and Wagner grade 5 (1.4 %) (1/70).

3.3. Antimicrobial susceptibility profiles of gram-positive isolates

Among the sixty-three bacterial strains, sixteen (25.3 %) were classified as gram-positive. Six out of seven (85.7 %) *S. aureus* species showed resistance to both fusidic acid and benzylpenicillin. 4/7 (57.1 %) of the *S. aureus* samples showed resistance to moxifloxacin, levofloxacin, and oxacillin. 100 % resistance to erythromycin and 50 % resistance to levofloxacin, linezolid, teicoplanin, vancomycin, and nitrofurantoin were demonstrated by *Enterococcus faecalis*. However, all S. aureus species have demonstrated 100 % sensitivity to tigecycline, vancomycin, clindamycin, and linezolid. Except for fusidic acid, *S. warneri* was sensitive to all antibiotic tested. Two out of four (50 %) and four out of six (57.1 %) gram-positive isolates of *E. faecalis* and *S. aureus* were multidrug-resistant (MDR). As indicated in Table 3, the MDR classification was assessed by the CLSI classification (three or more types of antibiotics were resistant).

Table 3
Antimicrobial susceptibility pattern of the Gram-positive pathogens.

Antibiotics	Sensitive/ Resistant	*S. aureus* n = 7	*E. faecalis* n = 4	*S. warneri* n = 2
Benzylpenicillin	S	1	–	0
	R	6		2
Oxacillin	S	3	–	2
	R	4		0
Gentamicin	S	6	–	2
	R			0
Tobramycin	S	4	–	2
	R	3		0
Levofloxacin	S	3	2	2
	R	4	2	0
Moxifloxacin	S	3	–	2
	R	4		0
Erythromycin	S	5	0	0
	R	2	4	2
Clindamycin	S	7	–	2
	R	0		0
Linezolid	S	7	2	2
	R	0	2	0
Teicoplanin	S	7	2	2
	R	0	2	0
Vancomycin	S	7	2	2
	R	0	2	0
Tetracycline	S	4	1	2
	R	3	3	0
Tigecycline	S	7	2	2
	R	0	0	0
Nitrofurantoin	S	_	2	2
	R		2	0
Fusidic Acid	S	1	–	2
	R	6		2

3.4. Antimicrobial-susceptibility of the gram-negative isolates

Serratia marcescens was resistant to the majority of antibiotics, including amoxicillin, cefalotin, cefoxitin, and nitrofurantoin. While all of the gram-negative isolates were resistant to this drug. Ampicillin, Amoxicillin, Piperacillin, Cefalotin, Cefoxitin, Tigecycline and Nitrofurantoin were resistant to more than half of the gram-negative bacteria isolates. Each antibiotic had the same effect on *C. koseri*. The majority of bacteria are sensitive to piperacillin, meropenem, amikacin, gentamicin, imipenem, ciprofloxacin, and trimethoprim/sulfamethoxazole. The best antibiotic, according to Table 4, was meropenem, which showed 100 % sensitivity to all microorganisms.

3.5. Biofilm formation

A total of 32 isolates (or 57 %) with bacterial foot infections had visible biofilm formation. The two most common bacteria that formed biofilms were *Serratia marcescens* and *Staphylococcus aureus*. 85.7 % (6/7) of the *S. aureus* and 84.6 % (11/13) *Serratia marcescens* isolates were biofilm formers. Following *Enterococcus faecalis*, *P. aerogenosa*, *E. coli*, *E. aerogenes*, *C. koseri*, and *P. mirabilis*, *Serratia marcescens* was the predominant gram-negative biofilm producer (Fig. 1).

4. Discussion

The deadliest severe chronic illness is diabetes mellitus, which has a high prevalence of 9.3 % worldwide and is continually increasing 10.2 % by 2030. Diabetes mellitus patient's foot ulcers have a high risk of severity and death rates, which globally results in non-traumatic limb amputation (Anita et al., 2023). This study examined the diagnostic prognosis and microbial cause of foot ulcers in individuals with diabetes diagnosed at King Fahad Medical City in Riyadh, Saudi Arabia. In the current investigation, men were shown to have a higher prevalence of diabetes mellitus than women. To the best of our knowledge, no previous studies have described the prevalence of diabetic foot ulcers by using VITEK system in Saudi Arabia. The majority of patients in this study were male, which is in line with results from previous studies carried out in Ethiopia, Indonesia, and India. Men are more likely to engage in outdoor activities, which raise their incidence of injury and ulcer progression (Murshed, 2020; Shah et al., 2021). According to research performed in Saudi Arabia, Indonesia, Ethiopia, India, and other countries, most individuals with DFU infections were found to be between the ages of 51 and 60 (Al Ayed et al., 2018; Atlaw et al., 2022; Ismail et al., 2021; Van Netten et al., 2020).

Glycated hemoglobin (HbA1c) was measured in all patients with diabetes in this study to screen for glycemic control. HbA1c data ranged from 5.7 % to 80 %, at the lowest and highest, respectively. An intrinsic risk indicator for cardiovascular disease as well as stroke in diabetics, HbA1c is an index of long-term glycemic management and is often elevated (Chen et al., 2023).

According to the Wagner Diabetic Foot Ulcer Classification System, ulcers were classified in this study as follows: grade 3 was most common, accounting for 45.57 percent (32/70), followed by grade 4. These results are consistent with research conducted in Ethiopia and Egypt, where grade 4 was found in 20 % of participants (14/70), Wagner grade 1 in 18.47 percent (13/70), Wagner grade 2 in 14.28 percent (10/70), and Wagner grade 5 in 1.4 % of participants (1/70) (Atlaw et al., 2022; Ismail et al., 2021). However, the results of a study from India revealed that grade 2 is higher than grade 3 (5.1 %). Similarly, the results of a study from Saudi Arabia revealed that Wagner grade 2 ulcers were predominant for bacterial infections followed by Wagner grade III, which is in contrast with our current findings (Al Ayed et al., 2018).

According to our research, only 12.5 % of the samples were poly-microbial isolates, whereas the majority of the samples were mono-microbial isolates. Contrary to the majority of earlier publications, that demonstrated the poly-microbial nature of DFUs. An estimated 23 % of cultures are mono-microbial, which makes treating these infections more challenging and extends the duration of hospitalization, whereas 66 % of cultures are poly-microbial, indicating they comprise both aerobic and anaerobic bacteria as well as fungus (Ismail et al., 2021). In contrast, every diabetic foot isolate found in research (Banu et al., 2015) was mono-microbial. Antimicrobial therapy and ulcer length are related to the mono-microbial characteristics of the disease. An early stage of infection is characterized by the mono-microbial condition, which gives rise to a poly-microbial state as the infection continues. Mono-microbial ulcers are also more common in those that are shallower and have less necrosis (Macdonald et al., 2021).

Overall, compared to gram-positive isolates (25.4 %), gram-negative bacteria (74.6 %) were more frequently isolated. *Pseudomonas aeruginosa* was the most common gram-negative bacteria from diabetic foot infections in the same location as this investigation, which showed comparable findings (Al Ayed et al., 2018). However, gram-negative bacteria were found more frequently than gram-positive pathogens in the majority of investigations (Amogne et al., 2011). Research conducted in Egypt revealed that 56 % of the samples were gram-negative and 27.7 % were positive, however in northeast India, 79 % of the samples were positive and 21 % of the samples were negative (Dwedar et al., 2015; Ismail et al., 2021).

When the ulcer developed progressively chronic, correspondingly increased the degree of bacterial isolation and the variety of bacteria found inside the ulcer. This illustrates the degree to which organisms influence the DFU recovery process, a point reinforced by other studies conducted in other countries, including Nigeria (Oates, 2002), China (Xie et al., 2017) and India (Shah et al., 2021).

The gram-negative *Serratia marcescens* (20.6 %) was the most common isolate in the current study. However, the previous research performed in Ethiopia, found that *Klebsiella* species accounted for 23.9 % of the total bacteria, followed by *Proteus* species (18.47 %) (Amogne et al., 2011). The most frequent strain in Egypt was *P. mirabilis* (16.8 %) (Dwedar et al., 2015); in Saudi Arabia, the most abundant strain was *Pseudomonas* species (15.6 %) (Al Ayed et al., 2018) and in South

Table 4
Antimicrobial susceptibility pattern of the Gram-negative pathogens.

Antibiotics	Sensitive/ Resistance	*S. marcescens*	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*E. aerogenes*	*C. koseri*	*Morganella* spp.	*P. penneri*	*E. cloacae compl*	*S.paucimobilis*
		n = 13	n = 6	n = 5	n = 6	n = 3	n = 3	n = 2	n = 1	n = 1	n = 2	n = 2
Ampicillin	S	–	0	–	6	_	3	–	0	0	–	–
	R		6		0		0		1	1		
Amoxicillin	S	1	6	–	6	_	0	2	0	1	0	–
	R	12	0		0		3	0	1	0	2	
Piperacillin	S	–	6	5	6	_	3	2	1	1	2	–
	R		0	0	0		0	0	0	0	0	
	S	1	2	5	6	0	0	2	0	1	0	–
Cefalotin	R	12	4	0	0	3	3	0	1	0	2	
Cefoxitin	S	1	3	5	6	–	0	2	0	1	0	–
	R	12	3	0	0		3	0	1	0	2	
Ceftazidime	S	12	2	5	6	0	3	2	1	1	2	_
	R	1	4	0	0	3	0	0	0	0	0	
Ceftriaxone	S	12	3	–	6	0	3	2	1	1	2	_
	R	1	3		0	2	0	0	0	0	0	
Cefepime	S	12	4	5	6	0	3	2	1	1	2	_
	R	1	2	0	0	2	0	0	0	0	0	
Imipenem	S	12	6	5	2	_	3	2	1	1	_	_
	R	0	0	0	4		0	0	0	0		
Meropenem	S	12	6	5	–	_	3	2	1	1	_	_
	R	0	0	0			0	0	0	0		
Amikacin	S	_	–	4	–	_	3	2	1	1	_	_
	R			1			0	0	0	0		
Gentamicin	S	13	6	5	–	_	3	2	1	1	_	2
	R	0	0	0			0	0	0	0		0
Ciprofloxacin	S	13	3	5	–	_	3	2	1	1	_	2
	R	0	3	0			0	0	0	0		0
Tigecycline	S	12	6	5	1	_	3	2	0	0	_	_
	R	1	0	0	5		0	0	1	1		
Nitrofuranton	S	0	1	–	0	0	–	2	0	0	1	–
	R	13	5		6	3		0	1	1		
Trimethoprim	S	_	1	–	_	_	3	2	1	1	_	_
	R		5				0	0	0	0		

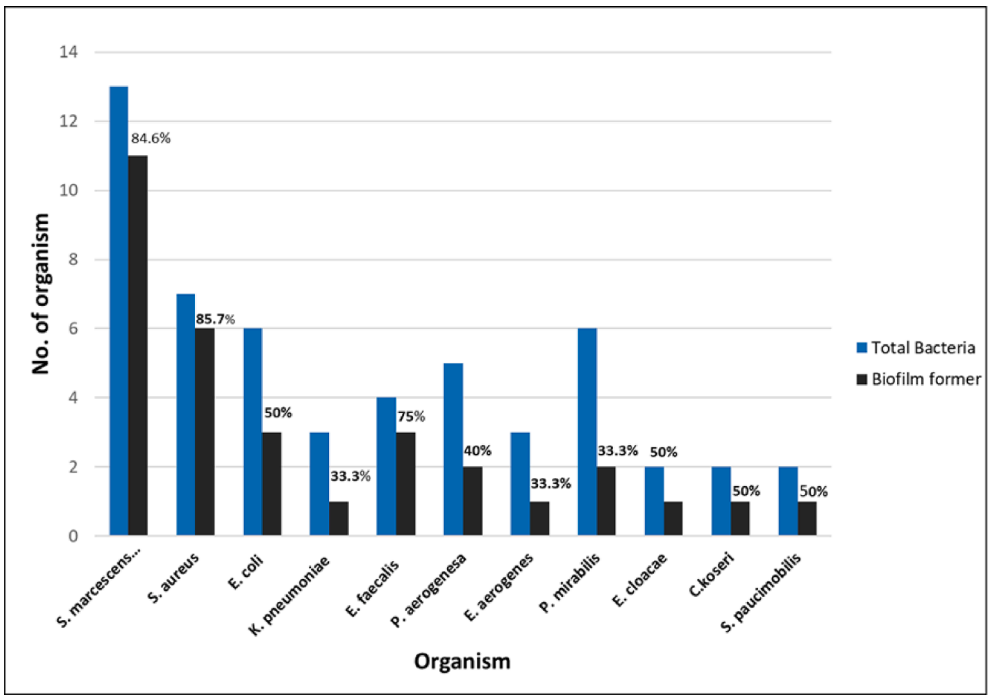

Fig. 1. Comparison of biofilm-forming organisms from diabetic foot ulcers.

America, the most prevalent isolate was *Pseudomonas* species (18.8 %) (Ponce de Leon et al., 2020). These studies demonstrate how different environments may have different prevalent bacteria that cause DFU illnesses. The current study indicated a very high rate of multidrug resistance, which is in line with research from Nigeria, India, and other countries (Adeyemo et al., 2021; Thanganadar Appapalam et al., 2021).

Consistent with the results of this investigation, similar research (Małecki et al., 2021) found that *S. aureus* was the most commonly found bacteria in the diabetic foot, followed by *P. aeruginosa, Escherichia coli*, *Streptococcus* spp, *Proteus* spp, *Enterococcus* spp, and *Klebsiella pneumoniae*. In addition, based on a distinct recent investigation, *P. aeruginosa* was the most frequently reported isolated bacterium, followed by *S. aureus* and methicillin-resistant *S. aureus* (Al Ayed et al., 2018). Still, there is a lack of research on antibiotic sensitivity in the various DFU locations.

All *S. aureus* species showed 100 % sensitivity against clindamycin, linezolid, teicoplanin, vancomycin, and tigecycline but most isolated *S. aureus* in this study were resistant to benzylpenicillin, fusidic acid, oxacillin, levofloxacin, and moxifloxacin. *Enterococcus faecalis* showed 100 % resistance against erythromycin and levofloxacin, while 50 % of the isolates of *S. aureus* were resistant to most of the mentioned drugs. Conversely, it was discovered that 100 % of *Staphylococcus aureus* and *Enterococci* were susceptible to chloramphenicol (Jain and Barman, 2017). There could be a variety of factors contributing to this high level of resistance. It might be explained by the patient's interactions with the hospital surroundings during their frequent follow-up visits, incorrect antibiotic usage, self-medication, and recurrent courses of antibiotics linked to the chronic nature of the DFU.

Studies have revealed that bacteria linked to biofilms can display antibiotic resistance up to 1,000 times higher than that of planktonic bacteria that float freely. Among the 32 biofilm former isolates, 28 (87.5 %) contained MDR bacterial strains. However, biofilm formation was seen in 85.7 % (6/7) of poly-microbial samples on agar plates and 37 (46.3 %) of the MDR isolates also developed biofilms. Swarna et al. (Swarna et al., 2013) stated that 80.4 % of the MDR microorganisms were biofilm formers, which is contrary to the current finding.

In our investigation, biofilm development was seen in 46.3 % of the isolates. Six percent of acute wounds and sixty percent of chronic wounds were reported in research by James et al. (James et al., 2008). Such variations from the standard may be brought about by patients lowered ulcer duration or by efficient debridement techniques. In this study, 38.8 % of the isolates tested positive for biofilm formation, with *Serratia marcescens* and *Staphylococcus aureus* being the most common biofilm formers (Fig. 1).

This study has certain limitations because it was conducted in one hospital with a small patient population, anaerobic culturing was not utilized, and there were never many cases of foot infection. Thus, it might not be feasible to draw a valid conclusion about the distribution of pathogens in every part of the foot. To assess the anaerobic distribution and medication sensitivity in the various grades of DFUs, extensive experimental research is therefore recommended.

The present investigation proved that aerobic pathogenic bacteria, both gram-positive and gram-negative are responsible for DFU infection in the study sites. These bacteria exhibit a propensity for antibiotic resistance, which poses a challenge for patient management and may result in additional complications like osteomyelitis and potentially limb amputation.

5. Conclusion

Numerous infections, including multidrug-resistant bacteria, can cause diabetic foot ulcers. The biofilm former *Serratia marcescens*, a gram-negative bacterium was the most common pathogen in this investigation, followed by *Staphylococcus aureus*. The significant incidence of resistance to widely used antibiotics identified in the current study highlights the need for caution when using antibiotics to treat illnesses. Piperacillin, Meropenem, Amikacin Gentamicin, Imipenem, Ciprofloxacin and Trimethoprim/Sulfamethoxazole showed greater sensitivity in certain isolates in the present investigation. First-line treatment for these infections can be achieved with Meropenem, the best drug that exhibits 100 % sensitivity to all bacteria. In current study 87.5 % biofilms contained MDR bacterial strains. *Staphylococcus aureus* and *Serratia marcescens* were the most common biofilm formers. An overall increase in bacterial resistance to antimicrobial drugs and high biofilm formation in MDR bacteria was demonstrated by the findings, which highlight the significance of microbiological examination and

antimicrobial susceptibility testing of biofilms with planktonic bacteria before undertaking antibiotic treatment for diabetic foot ulcer infections.

Institutional review board statement

Ethical approval was attained from the ethics and review committee of the King Fahad Medical City, Riyadh (FWA00018774) and informed approval was achieved from the subjects in their native language.

Informed consent statement

In this study the Written informed consent has been obtained from the patient is as following**: "I**, the undersigned, hereby acknowledge that I have been fully informed and understand the nature and purpose of the study entitled "**Diabetes-Related Lower Limb Wounds: Antibiotic Susceptibility Pattern and Biofilm Formation"** conducted by Dr. Raha Orfali at King Saud University. I have had the opportunity to ask questions and have received satisfactory answers regarding the study.

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CRediT authorship contribution statement

Raha Orfali: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Safina Ghaffar:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Lateefa AlAjlan:** Conceptualization, Data curation, Resources, Validation. **Shagufta Perveen:** Conceptualization, Investigation, Methodology. **Eman Al-Turki:** Conceptualization, Investigation, Methodology, Validation. **Fuad Ameen:** Writing – review & editing, Visualization, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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