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An Integrated Analysis Of Microbial Profiles, Drug Resistance And Socioeconomic Factors In Women With Diabetic Foot Ulcers

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ABSTRACT

Diabetic foot ulcers (DFUs) can lead to significant health issues, including amputations and mortality. The prevalence of diabetes is notably higher among middle-aged and elderly women, especially those in the 61-70 age group, with socioeconomic disparities significantly impacting access to care. This study highlights the severe complications of DFUs in patients with diabetes mellitus (DM), particularly among women from low-income groups, where limited access to healthcare, lack of awareness, and delayed medical interventions exacerbate the condition. The financial burden of managing chronic wounds, particularly in rural areas, further complicates the already substantial healthcare costs associated with diabetes care. The analysis involved screening 171 patients to identify bacterial pathogens and determine their antibiotic resistance patterns. The results indicated that Gram-positive bacteria, predominantly *Staphylococcus aureus* (72 %), and Gram-negative bacteria, including *Klebsiella* sp., and *Pseudomonas* sp., made up the remaining 28%. These infections are often polymicrobial, posing treatment challenges due to their resistance to commonly used antibiotics. Antibiotic susceptibility tests revealed varying degrees of resistance among the bacterial isolates. Particularly *E. coli* and *Klebsiella* sp. showed sensitivity to tested whereas *Pseudomonas* sp., and *Acinetobacter* sp. exhibited high levels of resistance. These findings emphasize the importance of precise diagnostic testing and the selection of appropriate antibiotic regimens to effectively manage these infections. Overall, this study underscores the critical need for targeted interventions addressing socioeconomic disparities and improving access to healthcare for at-risk populations. It also stresses the necessity for tailored antibiotic treatments to combat the complex bacterial infections associated with DFUs.

KEYWORDS: Diabetes mellitus, Diabetic foot ulcers, antibiotic resistance, bacterial pathogens

INTRODUCTION

Diabetes mellitus (DM) has become a critical public health issue in India, largely due to the country's rapid urbanization. This chronic condition, marked by elevated blood sugar levels, impacts millions globally. Uncontrolled diabetes can lead to severe complications, with diabetic foot ulcers (DFUs) being a major concern due to the high risk of infections and amputations. India has emerged as a global hotspot for diabetes mellitus (DM), with the International Diabetes Federation reporting that 537 million people worldwide were living with diabetes in 2021. Projections indicate that this number will escalate to 643 million by 2030 and further to 783 million by 2045. A significant concern is that 44.7% of individuals with diabetes remain undiagnosed, and a staggering 75% of these cases are found in low- and middle-income countries. India currently ranks second globally in terms of the number of diabetics, with 74.9 million cases reported in 2021, and this figure is expected to rise to 124.9 million by 2045 [1].

DFUs are a prevalent complication in diabetes, leading to considerable morbidity and mortality. These ulcers can impair functional abilities, and increase the risk of infections, hospitalization, amputations, and even death. The lifetime risk of developing foot ulcers in individuals with diabetes ranges from 19% to 34%, a figure that is increasing due to the aging population and the growing complexity of diabetes management [2]. Approximately 60% of diabetic foot ulcers (DFUs) are associated with infections, significantly elevating the risk of complications [3]. A meta-analysis has shown that the mortality rate for patients with DFUs is substantial: 13.1% within one year, 49.1% within five years, and 76.9% within ten years. The primary causes of death among these patients are cardiovascular disease and infection [4].

Several factors elevate the risk of DFUs, including peripheral neuropathy, peripheral artery disease, foot deformities, race, socioeconomic status, and geographic factors. Despite the severe consequences of diabetic foot ulcers (DFUs), research funding for this condition is alarmingly low, accounting for less than 0.2% of total federal diabetes funding [5]. DFUs impose substantial social, psychological, and financial burdens on both patients and the healthcare system. Notably, patients with DFUs have a mortality risk that is 2.5 times higher compared to diabetic patients without ulcers [6]. The global prevalence of DFUs has seen a marked increase, with an estimated rate of 6.4% [7]. Chronic wounds, such as DFUs, are notoriously slow to heal [8]. Studies show that diabetic foot infections (DFIs) are often polymicrobial, with *Staphylococcus aureus* being the most commonly identified pathogen [9]. Misuse of antibiotics in managing DFIs is a persistent issue. Various microorganisms can cause DFIs, including Gram-positive bacteria such as *Staphylococcus epidermidis, Streptococcus pyogenes*, *Staphylococcus aureus*, and Gram-negative bacteria like *Klebsiella pneumoniae, Escherichia coli*, *Acinetobacter* sp., *Pseudomonas aeruginosa*, *Enterococcus* sp., and *Proteus* sp [10]. Its prevalence is influenced by antibiotic use, the host immune response, and the environment of the wound [11]. High resistance to frequently used antibiotics has been documented, highlighting the need for careful antibiotic management in DFI treatment. Certain isolates show greater sensitivity to alternative antibiotics, such as chloramphenicol, aztreonam, amikacin, clindamycin, and vancomycin, making them viable first-line treatments [12].

The increasing global burden of diabetes and its complications underscores the urgency for enhanced prevention, early detection, and better management strategies. Targeted awareness programs, especially in low- and middle-income countries where diabetes remains underdiagnosed, are critical. Addressing this public health challenge requires increased research funding, improving access to care, and promoting lifestyle interventions to prevent the onset of complications like DFUs. The present study aimed to raise awareness of microbial infection among diabetic patients and the emergence of resistant bacteria.

Diabetes mellitus is also the most common cause of chronic kidney disease (CKD) and diabetic nephropathy worldwide. Patients who develop chronic complications such as diabetic foot ulcers often have long-standing hyperglycemia, which is closely linked to microvascular damage including renal impairment. Therefore, understanding the infection profile in DFU patients also has important implications for nephrology and overall renal care.

MATERIALS AND METHODS

Selection of patients and study period

Patients with DFU of different Wagner grades, who visited multispecialty diabetes hospitals in and around Madurai, were selected for the study as either inpatients or outpatients. The study period lasted from January 2021 to September 2023. Patients who had received antibiotics for more than 72 h were permitted to participate in the study, and each patient was included only once. Demographic details were collected from the patients for basic analysis. Specimen collection techniques were designed to exclude superficial or colonizing organisms, including only clinically infected wounds. Basic renal parameters (serum creatinine and eGFR) were reviewed from patient case records to identify any underlying renal impairment commonly seen in long-standing diabetes.

Collection and processing of swabs from DFU patients

A sample for bacterial analysis was collected from the inner region of a diabetic foot ulcer using a sterile swab pre-soaked in glucose broth. The sample was immediately transferred to blood agar (BA), Mannitol salt agar (MSA), and MacConkey agar (MCA) plates, which were then incubated at 37 °C for 24 h. Following incubation, the plates were inspected for bacterial growth. To isolate pure cultures, the bacterial colonies were subcultured onto fresh plates of the same agar types.

Biochemical test of DFU isolates

The pure culture of bacterial isolates was analyzed for their physiological and biochemical features using the methods described in Bergey's Manual of Determinative Bacteriology [13].

Antibiotic Sensitivity Profiles

The antibiotic susceptibility profile of bacterial isolates was assessed with Kirby-Bauer disk diffusion method conferring by CLSI guidelines [14]. Briefly, each pure bacterial culture suspension was inoculated into sterile Muller Hinton broth and adjusted equivalent 0.5 McFarland standard. Each bacterial culture suspension was swabbed on to Mueller-Hinton agar (MHA) plate with cotton swab. Later, selected antibiotic discs were placed on the MHA plates and it were incubated at 37 °C for 24 h. Subsequent incubation, the inhibition zone was measured and recorded for each bacterial strain.

For Gram-positive bacterial strains, the following antibiotics were included Clindamycin (2 µg), Nitrofurantoin (300 µg), Gentamicin (10 µg), Cloxacillin (30 µg), Ampicillin (10 µg),

Chloramphenicol (30 µg), Amoxiclav (30 µg), and Erythromycin (15 µg). The Gram-negative bacterial strains were examined with following antibiotics such as Doxycycline (30 µg), Ofloxacin (5 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Co-Trimoxazole (25 µg), Tobramycin (10 µg), Ceftazidime (30 µg), and Streptomycin (10 µg). All media, antibiotics, and reagents were obtained from HiMedia Laboratories Private Limited, Thane.

RESULTS

A total of 171 patients with DM were screened, with 99 (58%) being male and 72 (42%) being female. The results showed that diabetes was more common among males compared to females (Figure 1).

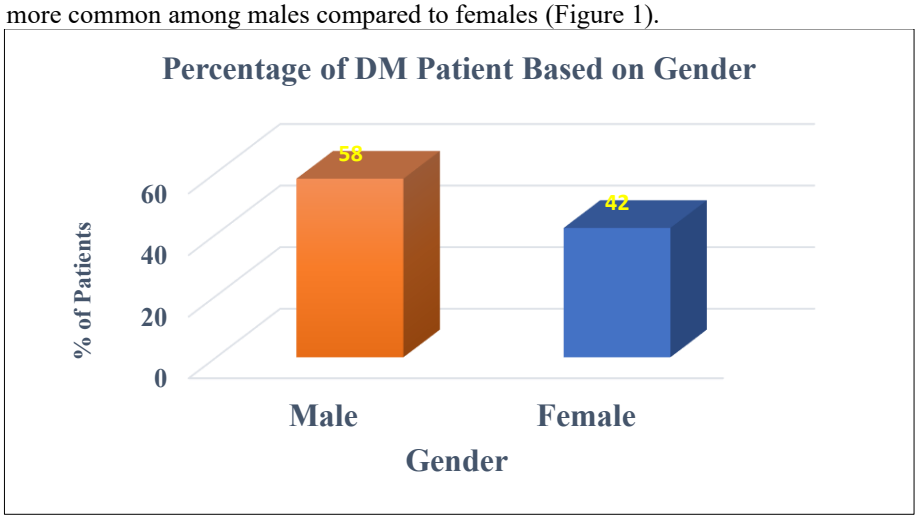


Figure 1. Percentage of diabetic mellitus patients based on gender

Among the female diabetic patients, the highest incidence was found in the 61-70 age group, which comprised 33% of the total sample, representing 24 patients. The next most affected group was those aged 51-60 years, representing 27% of the patients (19 patients). Combined, these two age groups (51-70 years) comprise 60% of the total diabetic population, indicating a significant prevalence of diabetes among middle-aged to older adults (Figure 2).

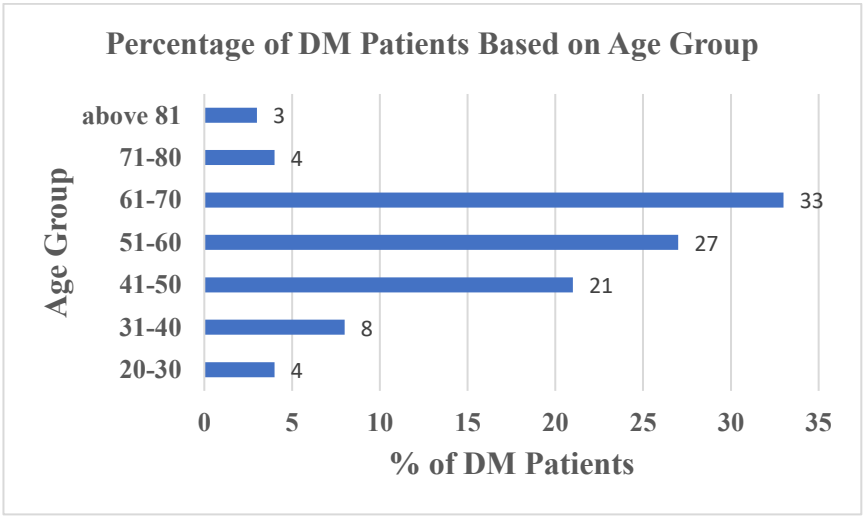


Figure 2. Percentage of DM patients based on age group

The low-income group represents the largest proportion of diabetic patients, comprising 53% of the total sample (38 patients). This suggests a higher prevalence of diabetes among individuals with lower income levels, potentially due to factors such as limited access to healthcare, education, and resources for managing the condition. The lower-middle-income group follows, accounting for 40% of the patients (29 patients), with the combined low and middle-income groups making up 93% of the total diabetic population. In contrast, the upper middle and High-income groups constitute only 7% of the total patients (5 patients), with Rich individuals representing 6% and Rich individuals making up just 1%. This indicates that diabetes is less common in these higher income brackets, likely due to better access to healthcare, healthier lifestyles, or more effective preventive measures available to wealthier individuals (Figure 3).

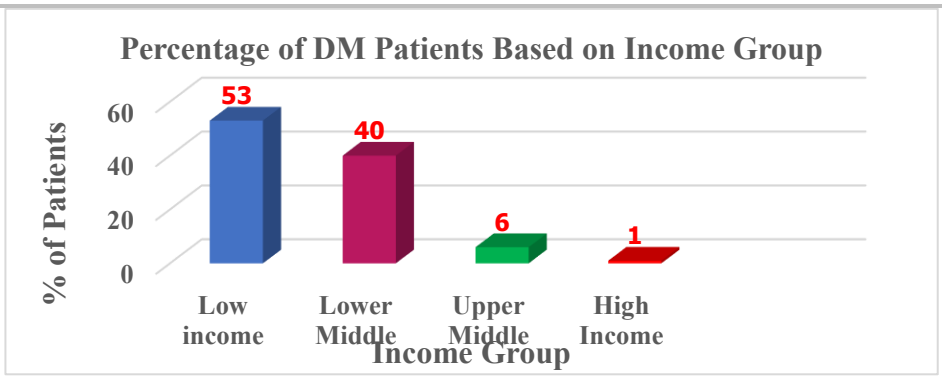


Figure 3. Percentage of DM patients based on income group

This report examined the distribution of diabetic ulcer grades among 72 patients, detailing both the number of cases and the percentage for each grade, ranging from Grade I to Grade V. The majority of patients, 74% (53 patients), fall under Grade III ulcers, indicating that most have moderately severe ulcers. Grade II ulcers account for 9% (7 patients), while Grade IV ulcers comprise 8% (6 patients), representing fewer but still notable cases of either milder or more advanced ulceration. Grade V ulcers, the most severe form, are present in 6% (4 patients), underscoring the existence of advanced complications in a subset of patients. Lastly, only 3% (2 patients) have Grade I ulcers, the mildest stage, suggesting that most patients present with more advanced ulcers, possibly due to delayed diagnosis or inadequate preventive care (Figure 4).

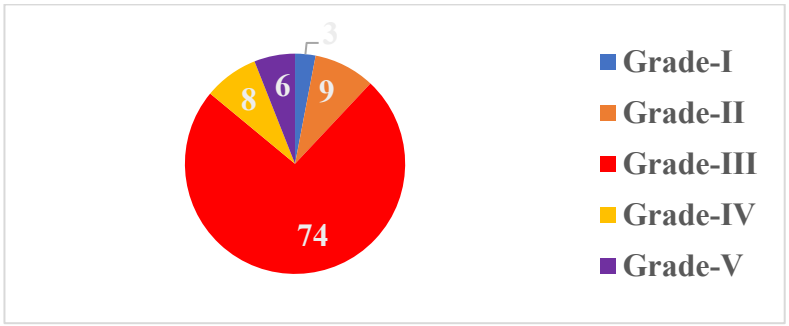


Figure 4. Percentage of DM patients based on Wagner's grade of ulcer

The analysis revealed a significant predominance of DM Type-II among the patients, indicating that this type of diabetes remains the most common. The significant prevalence of Type-II diabetes (84.7%) indicates that lifestyle factors, including diet and physical inactivity, may be crucial contributors to its occurrence. This finding underscores the importance of preventive measures, including lifestyle interventions and public awareness campaigns, to address the risk factors associated with DM Type-II (Figure 5).

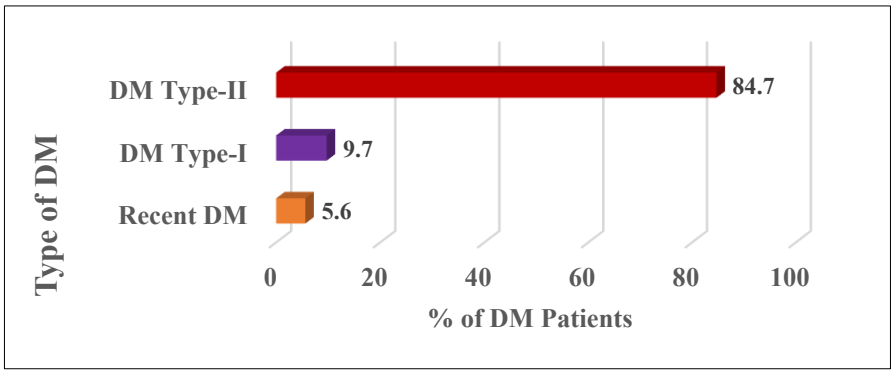


Figure 5. Percentage of DM patients based on type of DM

In contrast, DM Type-I constitutes a smaller proportion (9.7%), reflective of its nature as an autoimmune condition typically diagnosed in childhood or early adulthood. The prevalence of Recent DM, at 5.6%, indicates early-stage or newly diagnosed diabetes cases, which may require targeted interventions to prevent progression to more chronic forms. Clinical records of these diabetic patients also indicated that several individuals, particularly those with long-standing diabetes, showed mild to moderate reductions in renal function (creatinine/eGFR), which is frequently associated with chronic diabetic complications.

The data indicated that out of the 19 bacterial isolates, the majority (72%) are Gram-positive, while the remaining (28%) are Gram-negative. This suggests that Gram-positive bacteria are more prevalent in the sample

population compared to Gram-negative bacteria. The dominance of Gram-positive isolates could be attributed to environmental factors, host characteristics, or specific conditions favouring their growth (Figure 6). The bacterial isolates from diabetic foot ulcers were identified based on physical and biochemical characterization, as shown in Tables 1 and 2.

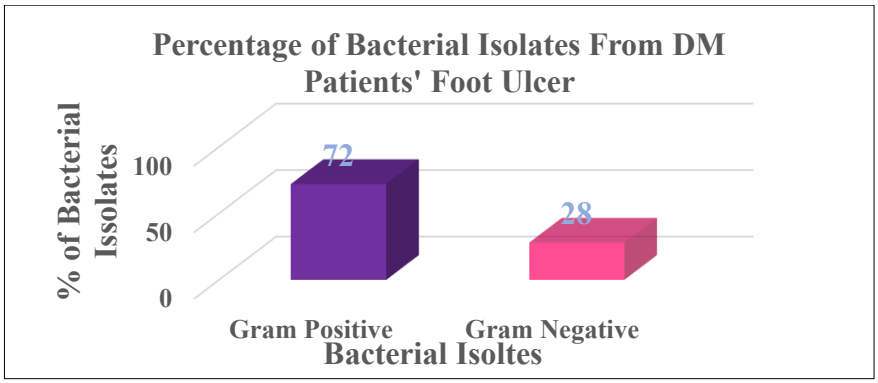


Figure 6. Percentage of bacterial isolates from DM patients' foot ulcer

Table 1. Physical and biochemical analysis of Gram-negative bacteria isolated from DM women patient

S. No.	Physical and Biochemical Test	Inference						
		M1	M9	M17	M25	M30	M45	M47
Physical Characters								
1.	Gram staining	Gram- negative						
2.	Morphology	Bacilli	Cocco-bacilli	Bacilli				
3.	Motility	Mt.	N.Mt.	Mt.	Mt.	N.Mt.	N.Mt.	Mt.
Biochemical Characters								
4.	I Test	-	-	-	-	-	-	+
5.	MR test	+	-	-	-	-	-	+
6.	VP test	-	+	+	-	+	+	-
7.	CU test	+	+	+	+	+	+	-
8.	Glucose	+	+	+	-	+	+	+
9.	Fructose	+	+	+	-	+	+	+
10.	Galactose	+	-	+	-	+	+	+
11.	Lactose	-	-	+	-	+	+	+
12.	Maltose	+	+	+	-	+	+	+
13.	Sucrose	+	+	+	-	+	+	-
14.	Rhamnose	-	-	+	-	+	+	+
15.	Mannitol	-	-	+	-	+	+	+
16.	Oxidase test	-	-	-	+	-	-	-
17.	Catalase	+	+	+	+	+	+	+
18.	Coagulase	-	-	-	-	-	-	-
19.	Starch hydrolysis test	-	-	-	-	-	-	-
20.	Urease test	+	-	-	-	+	+	-
21.	Gelatin utilization test	+	-	+	+	-	-	-
22.	Nitrate reduction tests	+	+	+	-	+	+	+

M1 *Citrobacter* sp. M17 *Enterobacter* sp. M30 *Klebsiella* sp.
M9 *Acinetobacter* sp. M25 *Pseudomonas* sp. M45 *Klebsiella* sp.
M47 *Escherichia coli*

Table 2. Physical and biochemical test of Gram-positive Bacteria isolated from DM women patients

S. No	Physical and Biochemical Test	Inference											
		M3	M4	M6	M10	M13	M14	M16	M18	M20	M23	M26	M28
Physical Characters													
1.	Gram staining	Gram-positive											
2.	Morphology	Cocci (cluster)			Cocci (chain)	Diplococci	Cocci (cluster)		Diplococci	Cocci (cluster)			
3.	Motility	N.Mt.											

Biochemical Characters													
4.	I test	-	-	-	-	-	-	-	-	-	-	-	-
5.	MR test	-	-	-	+	+	-	-	+	-	-	-	-
6.	VP test	+	+	+	-	-	+	+	-	+	+	+	+
7.	CU test	-	-	-	-	-	-	-	-	-	-	-	-
8.	Glucose	+	+	+	+	+	+	+	+	+	+	+	+
9.	Fructose	+	+	+	+	+	+	+	+	+	+	+	+
10.	Galactose	+	+	+	+	+	+	+	+	+	+	+	+
11.	Lactose	-	-	-	-	-	-	-	-	-	-	-	-
12.	Maltose	+	+	+	+	+	+	+	+	+	+	+	+
13.	Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
14.	Rhamnose	-	-	-	+	+	-	-	+	-	-	-	-
15.	Mannitol	+	+	+	-	+	+	+	+	+	+	+	+
16.	Oxidase test	-	-	-	-	-	-	-	-	-	-	-	-
17.	Catalase	+	+	+	-	-	+	+	-	+	+	+	+
18.	Coagulase	+	+	+	-	-	+	+	-	-	-	-	-
19.	Starch hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	-
20.	Urease test	-	-	-	-	-	-	-	-	-	-	-	-
21.	Gelatin utilization test	+	+	+	-	+	+	+	+	+	+	+	+
22.	Nitrate reduction tests	+	+	+	-	-	+	+	-	+	+	+	+

M3-	*Staphylococcus aureus*	M13-	*Enterococcus* sp.	M20-	*Staphylococcus* sp.
M4-	*Staphylococcus aureus*	M14-	*Staphylococcus aureus*	M23-	*Staphylococcus* sp.
M6-	*Staphylococcus aureus*	M16-	*Staphylococcus aureus*	M26-	*Staphylococcus* sp.
M10-	*Streptococcus* sp.	M18-	*Enterococcus* sp.	M28-	*Staphylococcus* sp.

Seven different Gram-negative bacterial strains were identified as follows; *Citrobacter* sp. (M1), *Acinetobacter* sp. (M9), *Enterobacter* sp. (M17), *Pseudomonas* sp. (M25), *Klebsiella* sp. (M30 and M45), and *Escherichia coli* (M47). According to the study, *Staphylococcus aureus* was the most frequently isolated organism, represented by five distinct isolates (M3, M4, M6, M14, M16). This high occurrence suggested that *Staphylococcus aureus* may be the dominant species in the samples analyzed. In comparison, only one isolate of *Streptococcus* sp. (M10) was identified, indicating a much lower presence than *Staphylococcus aureus*. Additionally, two isolates of *Enterococcus* sp (M13, M18) and four isolates of *Staphylococcus* sp. (M20, M23, M26, M28) were found. Although *Staphylococcus* sp. was detected in significant numbers, its prevalence was still lower than that of *Staphylococcus aureus* (Table 3, Figures 7 and 8).

Table 3. Isolation of bacteria from women DM patients' foot ulcer

S. No.	Organism	Isolate Number
Gram Negative Bacteria		
1.	*Citrobacter* sp.	M 1
2.	*Acinetobacter* sp.	M 9
3.	*Enterobacter* sp.	M17
4.	*Pseudomonas* sp.	M25
5.	*Klebsiella* sp.	M30
6.	*Klebsiella* sp.	M45
7.	*Escherichia coli*	M47
Gram-Positive Bacteria		
1.	*Staphylococcus aureus*	M3
2.	*Staphylococcus aureus*	M4
3.	*Staphylococcus aureus*	M6
4.	*Streptococcus* sp.	M10
5.	*Enterococcus* sp.	M13
6.	*Staphylococcus aureus*	M14
7.	*Staphylococcus aureus*	M16
8.	*Enterococcus* sp.	M18
9.	*Staphylococcus* sp.	M20
10.	*Staphylococcus* sp.	M23
11.	*Staphylococcus* sp.	M26
12.	*Staphylococcus* sp.	M28

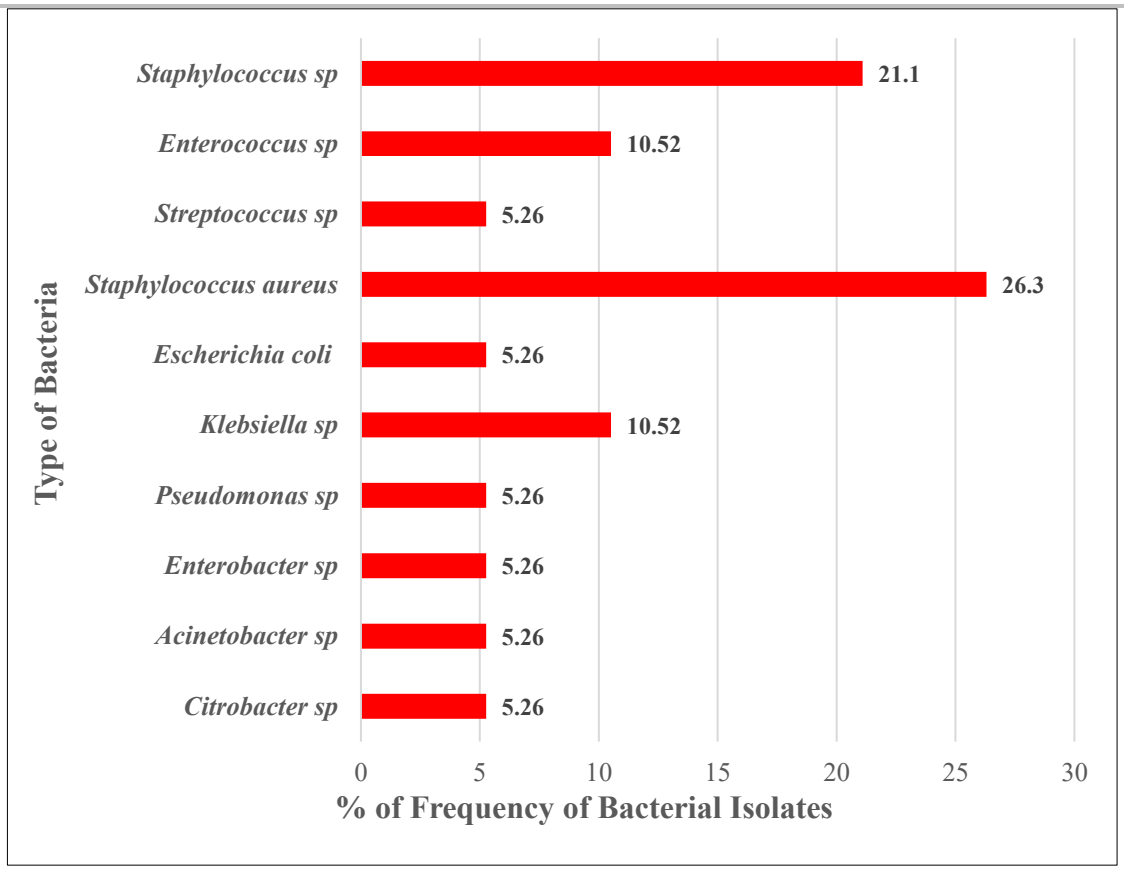
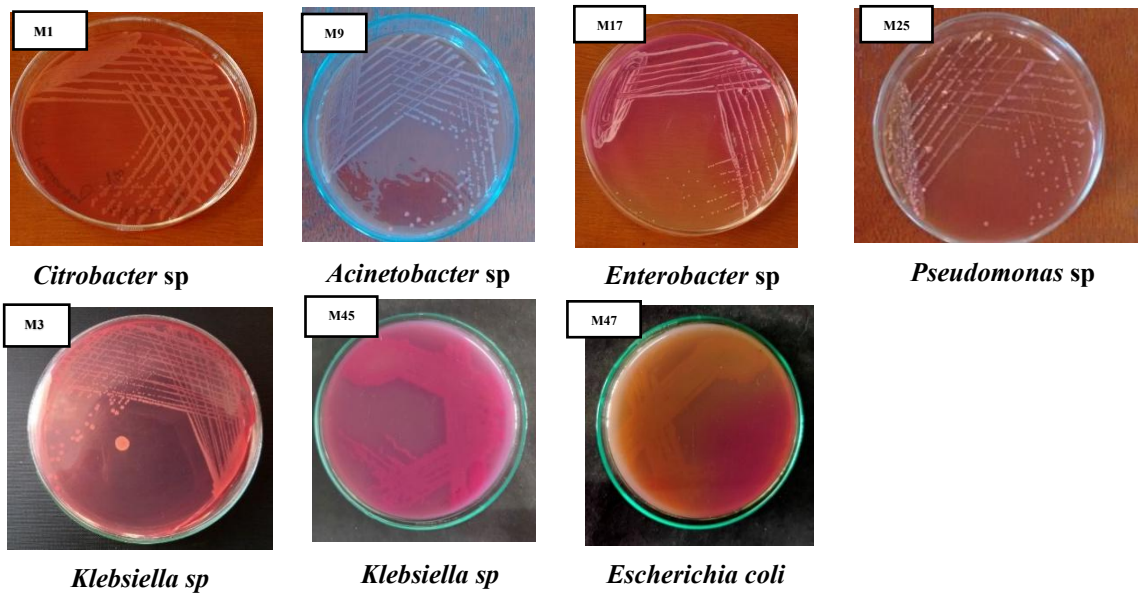


Figure 7. Percentage of frequency of bacteria isolated from DM patient's foot ulcer



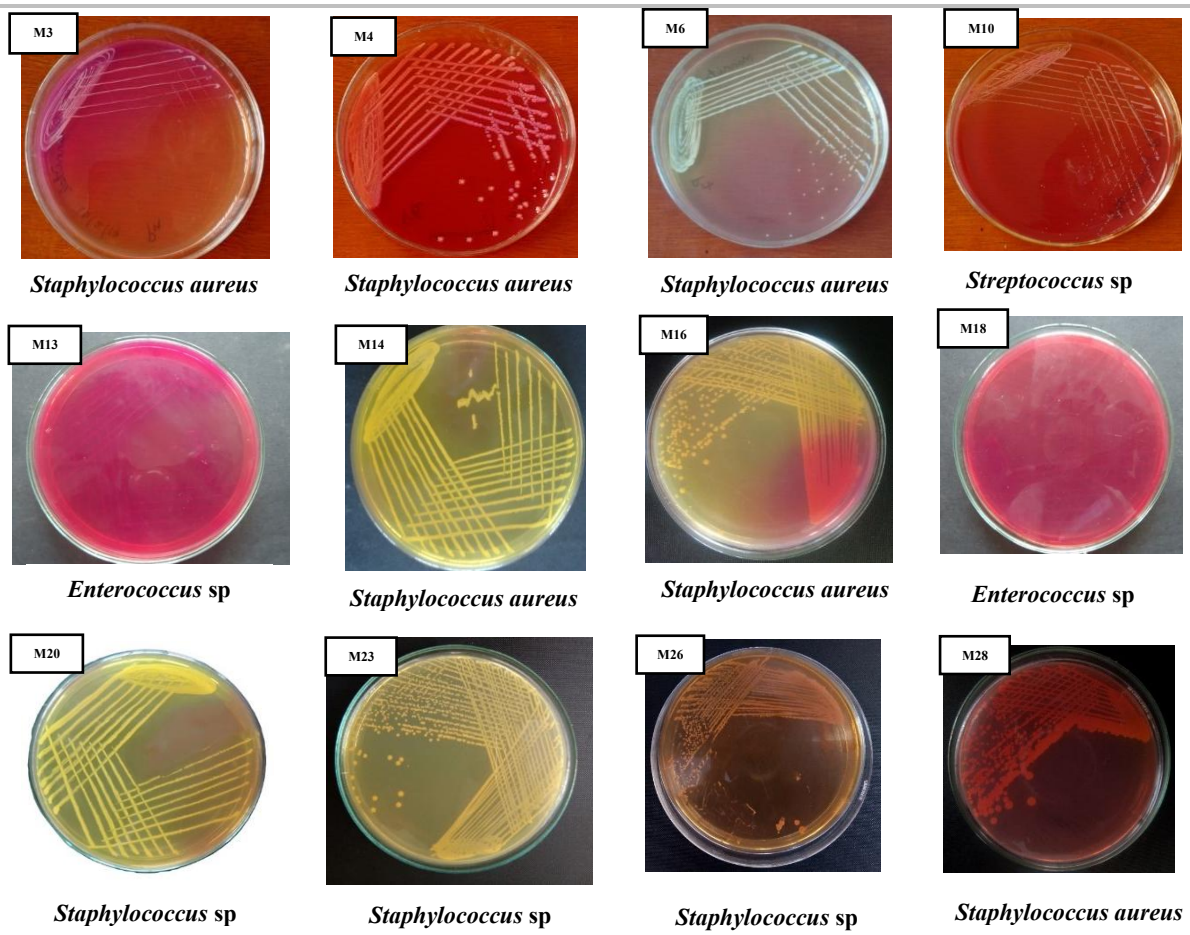


Figure 8. Gram-negative and Gram-positive bacteria isolated from DM patients' foot ulcer

The analysis of the antibiotic susceptibility data of Gram-negative bacterial isolates revealed varying degrees of bacterial resistance and sensitivity across different microorganisms. Doxycycline was generally effective, particularly against *E. coli* (M47), *Klebsiella* sp. (M30), and *Klebsiella* sp. (M45), showing inhibition zones of 27 mm, 24 mm, and 21 mm, respectively. whereas *Acinetobacter* sp. (M9) was resistant to this antibiotic. Cefotaxime was highly effective against *E. coli* (M47) (31 mm) but showed little to no activity against most other bacteria, particularly *Acinetobacter* sp. (M9) and *Pseudomonas* sp. (M25). Ofloxacin exhibited limited effectiveness against *Enterobacter* sp. (M17) showing the only significant sensitivity (16 mm). Ciprofloxacin demonstrated strong activity against *Enterobacter* sp. (25 mm) and *Klebsiella* sp. (20mm), but was ineffective against *Acinetobacter* sp. and *Pseudomonas* sp. Co-trimoxazole was highly effective against *Enterobacter* sp. (25 mm) and *E. coli* (26 mm). Tobramycin showed potent activity against *Klebsiella* sp. (26 mm) and *E. coli* (23 mm). Similarly, Ceftazidime was most effective against *E. coli* (22 mm) and *Enterobacter* sp. (18 mm). Streptomycin was particularly effective against *Klebsiella* sp. (27 mm) and moderately effective against other species (Table 4 and Figure 9).

Table 4. Antibiogram of Gram-negative bacteria isolated from women DM patients

Antibiotics discs	**Bacterial strains / Zone of Inhibition (mm)**						
	M1	**M9**	**M17**	**M25**	**M30**	**M45**	**M47**
Doxycycline (DO) (30 µg)	15 (S)	0 (R)	17 (S)	8 (R)	24 (S)	21 (S)	27 (S)
Ofloxacin (OF) (5 µg)	0 (R)	0 (R)	16 (S)	0 (R)	7 (R)	12 (R)	11 (R)
Cefotaxime (CTX) (30 µg)	8 (R)	0 (R)	0 (R)	0 (R)	0 (R)	15 (I)	31 (S)
Ciprofloxacin (CIP) (5 µg)	8 (R)	0 (R)	25 (S)	0 (R)	20 (I)	21 (S)	11 (R)
Co-Trimoxazole (COT) (25 µg)	0 (R)	0 (R)	25 (S)	0 (R)	14 (I)	8 (R)	26 (S)
Tobramycin (TOB) (10 µg)	0 (R)	0 (R)	19 (S)	0 (R)	0 (R)	26 (S)	23 (S)
Ceftazidime (CAZ) (30 µg)	0 (R)	0 (R)	18 (I)	0 (R)	0 (R)	14 (R)	22 (S)
Streptomycin (S) (10 µg)	15 (S)	7 (R)	13 (I)	9 (R)	27 (S)	16 (S)	15 (S)

M1- *Citrobacter* sp. M17-*Enterobacter* sp. M30- *Klebsiella* sp.

M9- *Acinetobacter* sp. M25- *Pseudomonas* sp. M45- *Klebsiella* sp.

M47- *Escherichia coli*

R- Resistant S- Sensitive I- Intermediate

Acinetobacter sp. and *Pseudomonas* sp. showed high resistance to multiple antibiotics, while *E. coli* and *Klebsiella* sp. were more susceptible, particularly to doxycycline, ciprofloxacin, and co-trimoxazole. These findings underscore the need to select antibiotics based on specific susceptibility patterns.

The antibiotic susceptibility test results of Gram-positive bacterial isolates revealed significant variations in the effectiveness of different antibiotics against *Staphylococcus aureus*, *Enterococcus* sp., *Staphylococcus* sp., and *Streptococcus* sp. isolates. Gentamicin (10 µg) emerged as the most potent antibiotic, exhibiting large inhibition zones, particularly 24 mm for *Staphylococcus* sp. (M23) and 18 mm for *Staphylococcus aureus* (M16) and *Staphylococcus* sp. (M28). Cloxacillin (30 µg) also showed strong efficacy, particularly against *Staphylococcus aureus* (M16) with a 31 mm inhibition zone and moderate effectiveness against *Staphylococcus* sp. (M26) with 17 mm.

However, Clindamycin (2 µg) was the least effective, showing no inhibition for five isolates, including *Staphylococcus aureus* (M16), *Enterococcus* sp. (M18), *Staphylococcus* sp. (M20, M26, M28), and only minimal activity against *Staphylococcus* sp. (M23). Ampicillin (10 µg) demonstrated strong activity against *Enterococcus* sp. (M18) with a 24 mm inhibition zone, but it was ineffective against most other isolates. Chloramphenicol (30 µg) showed high activity against *Staphylococcus* sp. (M23) with 28 mm inhibition and moderate activity against *Staphylococcus aureus* (M16).

Nitrofurantoin (300 µg) had moderate activity, particularly against *Staphylococcus aureus* (M16) and *Enterococcus* sp. (M18). Amoxyclav (30 µg) was effective against *Enterococcus* sp. (M18) and *Staphylococcus* sp. (M28), with inhibition zones of 25 mm and 19 mm, respectively. In comparison, Erythromycin (15 µg) showed good activity against *Staphylococcus aureus* (M16) and *Staphylococcus* sp. (M23), but did not affect *Staphylococcus* sp. (M20, M26, M28). *Staphylococcus* sp. (M26) was the most resistant isolate, while *Staphylococcus aureus* (M16) was the most susceptible, responding well to multiple antibiotics, especially Cloxacillin, Gentamicin, and Erythromycin.

Gentamicin showed the largest and most consistent inhibition zones across multiple isolates, particularly

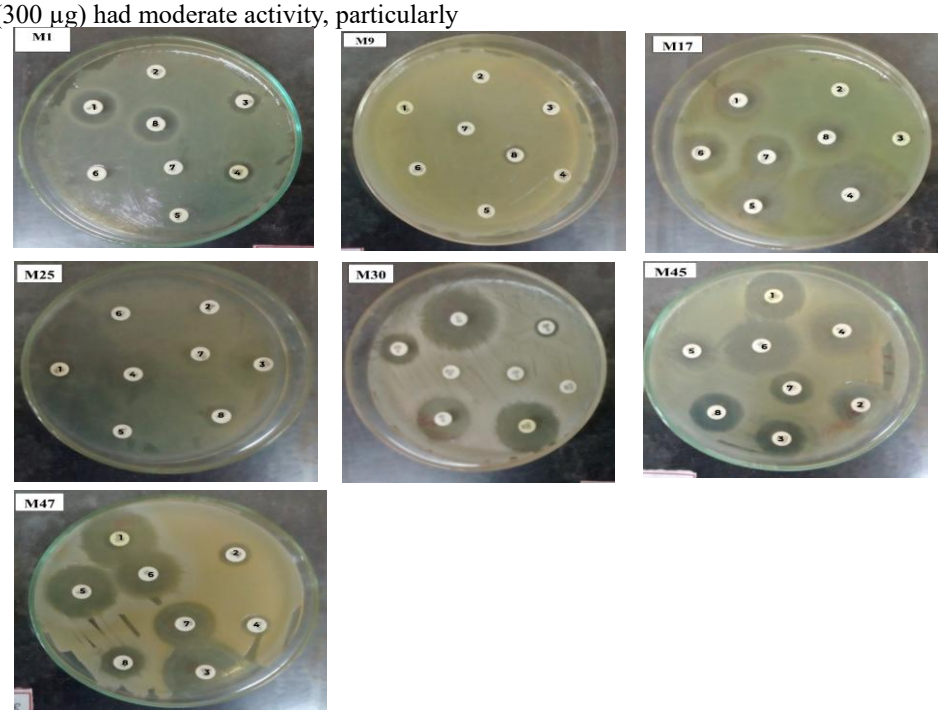


Figure 9. Antibiogram of Gram-negative bacteria isolated from DM women patients

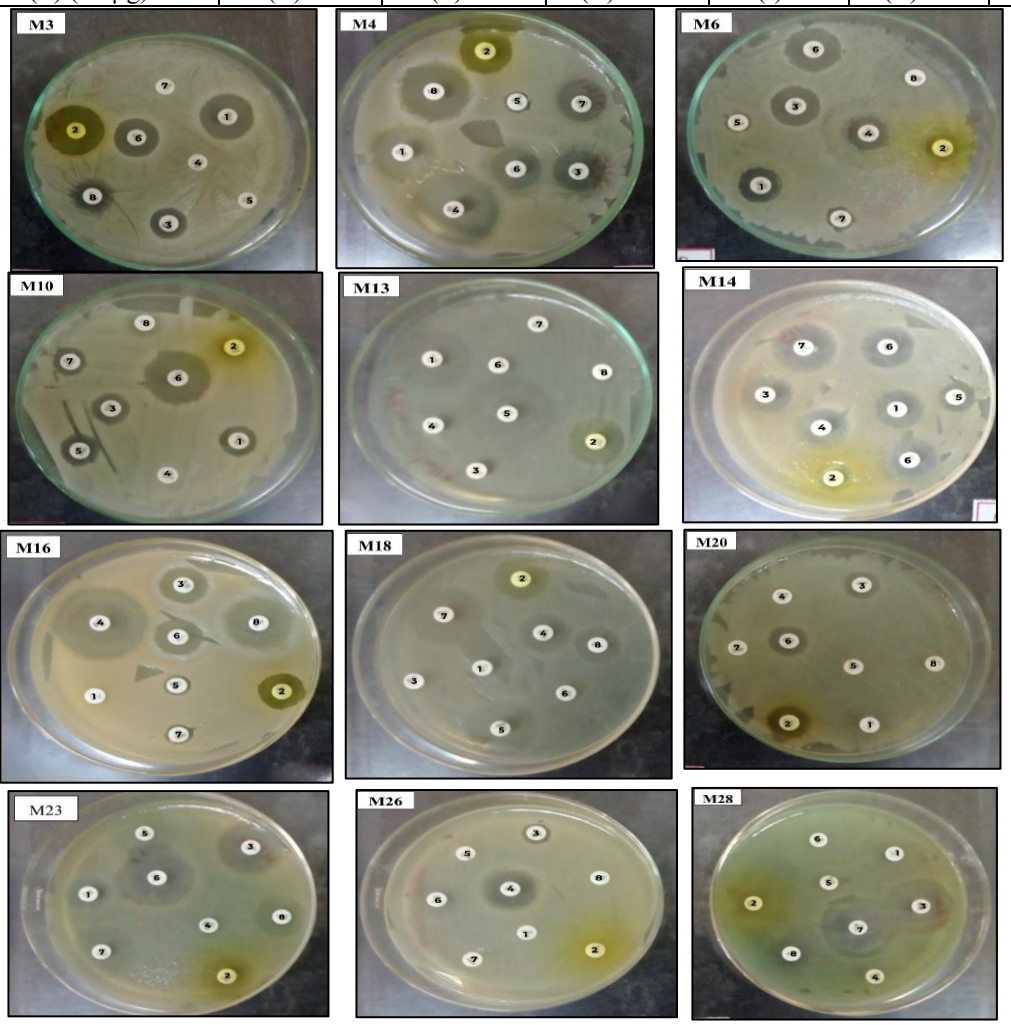
M1- *Citrobacter* sp. M17- *Enterobacter* sp. M30- *Klebsiella* sp.
M9- *Acinetobacter* sp. M25- *Pseudomonas* sp. M45- *Klebsiella* sp.
M47- *Escherichia coli*

1- Doxycycline (DO) 4- Ciprofloxacin (CIP) 7- Ceftazidime (CAZ)
2- Ofloxacin (OF) 5- Co-trimoxazole (COT) 8- Streptomycin (S)
3- Cefotaxime (CTX) 6- Tobramycin (TOB)

against *Staphylococcus* sp. (M23) with 24 mm, *Staphylococcus aureus* (M16) with 18 mm, and *Staphylococcus* sp. (M28) with 18 mm. The least effective antibiotic was Clindamycin, which demonstrated limited effectiveness, with no inhibition observed in five isolates—*Staphylococcus aureus* (M16), *Enterococcus* sp. (M18), *Staphylococcus* sp. (M20, M26, M28)—and only minimal activity (8 mm) against *Staphylococcus* sp. (M23). The most resistant isolate was *Staphylococcus* sp. (M26), which showed no inhibition against Clindamycin, Nitrofurantoin, Gentamicin, Ampicillin, Chloramphenicol, Amoxyclav, and Erythromycin, exhibiting only moderate susceptibility to Cloxacillin (17 mm). Conversely, *Staphylococcus aureus* (M16) was the most susceptible isolate, displaying high susceptibility to multiple antibiotics, including Cloxacillin (31 mm), Erythromycin (23 mm), and Gentamicin (18 mm) (Tables 5 and 6 and Figure 10).

Table 5. Antibiogram of Gram-positive bacteria isolated from women DM patients

Antibiotics discs	**Bacterial strains / Zone of Inhibition (mm)**					
	M3	**M4**	**M6**	**M10**	**M13**	**M14**
Clindamycin (CD) (2 µg)	18 (I)	16 (I)	13 (R)	13 (R)	22 (S)	16 (I)
Nitrofurantoin (NIT) (300 µg)	20 (I)	11 (R)	11 (R)	8 (R)	18 (I)	10 (R)
Gentamicin (GEN) (10 µg)	12 (R)	17 (R)	16 (R)	13 (R)	0 (R)	13 (R)
Cloxacillin (COX) (30 µg)	0 (R)	28 (S)	13 (S)	0 (R)	0 (R)	16 (S)
Ampicillin (AMP) (10µg)	0 (R)	0 (R)	0 (R)	13 (R)	0 (R)	11 (R)
Chloramphenicol (C) (30 µg)	14 (I)	20 (I)	17 (I)	22 (S)	0 (R)	15 (I)
Amoxiclav (AMC) (30 µg)	0 (R)	16 (I)	7 (R)	11 (R)	0 (R)	18 (S)
Erythromycin (E) (15 µg)	12 (R)	23 (S)	0 (R)	15 (I)	0 (R)	12 (R)



1- Clindamycin (CD)
2- Nitrofurantoin (NIT)
3- Gentamicin (GEN)
4- Cloxacillin (COX)
5- Ampicillin (AMP)
6- Chloramphenicol (C)
7- Amoxiclav (AMC)
8- Erythromycin (E)

Figure 10. Antibiogram of Gram-positive bacteria isolated from DM women patients

M03-	*Staphylococcus aureus*	M13-	*Enterococcus* sp.	M20	*Staphylococcus* sp.
M04	*Staphylococcus aureus*	M14	*Staphylococcus aureus*	M23	*Staphylococcus* sp.
M06	*Staphylococcus aureus*	M16	*Staphylococcus aureus*	M26	*Staphylococcus* sp.
M10	*Streptococcus* sp.	M18	*Enterococcus* sp.	M28	*Staphylococcus* sp.

Table 6. Antibiogram of Gram-positive bacteria isolated from women DM patients

Antibiotics Discs	**Bacterial strains / Zone of Inhibition (mm)**					
	M16	**M18**	**M20**	**M23**	**M26**	**M28**
Clindamycin (CD) (2 µg)	0 (R)	0 (R)	0 (R)	8 (R)	0 (R)	0 (R)
Nitrofurantoin (NIT) (300 µg)	16 (I)	18 (I)	16 (I)	0 (R)	0 (R)	0 (R)
Gentamicin (GEN) (10 µg)	18 (S)	0 (R)	14 (R)	24 (S)	8 (R)	18 (S)
Cloxacillin (COX) (30 µg)	31 (S)	14 (S)	0 (R)	0 (R)	17 (S)	0 (R)
Ampicillin (AMP) (10 µg)	8 (R)	24 (S)	0 (R)	0 (R)	0 (R)	0 (R)
Chloramphenicol (C) (30 µg)	16 (I)	0 (R)	15 (I)	28 (S)	0 (R)	0 (R)
Amoxyclav (AMC) (30 µg)	8 (R)	25 (R)	0 (R)	0 (R)	0 (R)	19 (S)
Erythromycin (E) (15 µg)	23 (S)	14 (I)	0 (R)	16 (I)	0 (R)	11 (R)

M03	*Staphylococcus aureus*	M13	*Enterococcus* sp.	M20	*Staphylococcus* sp.
M04	*Staphylococcus aureus*	M14	*Staphylococcus aureus*	M23	*Staphylococcus* sp.
M06	*Staphylococcus aureus*	M16	*Staphylococcus aureus*	M26	*Staphylococcus* sp.
M10	*Streptococcus* sp.	M18	*Enterococcus* sp.	M28	*Staphylococcus* sp.
S-	Sensitive	I-	Intermediate	R-	Resistant

Discussion

Several surveys have revealed that in the Indian population, the prevalence of diabetes is marginally higher among males with random blood glucose levels exceeding 140 mg/dL (30.1% versus 25.9%) compared to females (10.8% versus 10.2%). Additionally, the Indian Council of Medical Research–India Diabetes (ICMR-INDIAB) study observed a significant difference in diabetes prevalence between males and females, particularly in the 35 to 65 age group, where males had a higher incidence.

Nevertheless, females beyond this age group exhibit a slightly higher prevalence, possibly explained by survivor bias [15]. Differences in diabetes risk between sexes can be linked to biological variations such as chromosomal differences, autosomal gene expression, and the impact of sex hormones on body systems. Gender, being a multifaceted concept, includes various traits that can affect health-related behaviours and factors like susceptibility to stress. Behavioural, environmental, and lifestyle factors, including physical activity levels, dietary habits, and stress management, also contribute to gender-based disparities in diabetes risk [16]. Recognising and understanding these differences is crucial for developing targeted awareness campaigns and addressing the unique challenges faced by women with diabetes. In rural Puducherry, the incidence rate of diabetes was determined to be 21.5 cases per 1,000 person-years [17]. Interestingly, males had a significantly higher risk, with an incidence rate of 28.7 cases per 1,000 person-years, nearly twice that of females, who had a rate of 14.6 cases per 1,000 person-years [18].

Additionally, about 57% of adults in India with diabetes remain undiagnosed, which translates to approximately 43.9 million individuals [19]. Type 2 diabetes, accounting for 90% of all diabetes cases, was once primarily associated with affluent Western nations. However, it has now become a global health crisis, significantly impacting younger populations and contributing to disability and mortality worldwide [20]. It is also well established that long-standing diabetes increases the risk of diabetic nephropathy, and patients presenting with complications such as DFU often have evidence of underlying renal impairment [21]. Reduced kidney function weakens host immunity, slows wound healing, and increases susceptibility to severe infections. Although renal parameters were not the primary focus of this study, clinical records indicate that some patients had mildly reduced kidney function, supporting the known microvascular relationship between DFU progression and diabetic nephropathy [22].

The findings of previous research, which has consistently shown a high prevalence of Grade III and IV ulcers among patients with diabetes. The presence of Grade V ulcers underscores the importance of early diagnosis and intervention to prevent the progression of diabetic foot ulcers and reduce the risk of amputation. The limited number of Grade I ulcers suggests that there is a need for improved education and awareness regarding foot care among patients with diabetes, as well as enhanced access to podiatry services. According to Tripathy (2017) , India has 69.1 million people with Type 2 Diabetes Mellitus (T2DM), ranking second globally after China. In this study, the overall prevalence of diagnosed T2DM cases was found to be 4.7%. Among individuals under 40 years of age, the prevalence was 35%, whereas it was 66.7% for those aged 50 and above [23].

There was a significant presence of *Klebsiella* sp. among the isolates, suggesting a potentially higher occurrence in patients with diabetes mellitus. Previous studies showed that 79% of Gram-positive and 21% of Gram-negative bacteria, respectively in northeast India, which is consistent with the current findings of the current study. In contrast to the current study, previous research identified common Gram-negative isolates as

P. aeruginosa (9.4%), followed by *Proteus* sp. (4.6%) and *P. mirabilis* (2.5%) [24].

A prior study in Ethiopia identified *Klebsiella* sp. as the most prevalent bacteria at 23.9%, followed by *Proteus* sp. at 18.47% (17/92) [31]. The most frequently isolated bacterium was *P. mirabilis* (16.8%) in Egypt, whereas in Saudi Arabia, *Pseudomonas* sp. accounted for 15.6% [32]. Similarly, *Pseudomonas* sp. was the most common in South America, making up 18.8% of the isolates [33]. These findings indicate that the predominant bacterial agents responsible for DFU infections can differ by region. Variations may stem from factors such as differences in sample sizes and the specific conditions of each study site [25].

The analysis showed a clear predominance of Gram-positive bacteria, comprising 72% of the isolates. This finding is significant because Gram-positive bacteria, like *Staphylococcus* sp. and *Streptococcus* sp., are frequently related with skin and soft tissue infections, respiratory infections, and other clinical conditions. Their predominance suggests a need for targeted empirical treatment protocols that prioritize Gram-positive coverage, especially in settings where these infections are prevalent.

Research from Western nations has consistently identified Gram-positive bacteria as the primary culprits in diabetic foot infections (DFIs). The earlier studied reported *S. aureus* stands out as the most common pathogen, followed by *Streptococcus agalactiae*, methicillin-resistant *S. aureus* (MRSA), and *Enterococcus* sp. Although DFIs often involve multiple microorganisms, particularly in Western countries the *S. aureus* remains most recurrently isolated pathogen. This Gram-positive bacterium, commonly found on human skin and causes a wide range of infections, including bloodstream, heart, skin, bones, lungs, and infection through medical devices.

E. coli and *P. aeruginosa* are less prevalent Gram-negative bacteria significantly contributors to DFIs. These bacteria frequently display resistance to multiple antibiotics, which complicates treatment. As a result, thorough diagnostic testing and the use of either broad-spectrum or targeted antibiotics are essential for the effective management of diabetic foot infections (DFIs).

Conclusion

This study screened 171 diabetic patients and identified a high prevalence of moderate to severe diabetic foot ulcers, with Grade III ulcers being the most common. Gram-positive bacteria—particularly *Staphylococcus aureus*—were the predominant isolates, while Gram-negative organisms such as *Klebsiella* and *Pseudomonas* also contributed to infections. Antibiotic susceptibility testing showed that *E. coli* and *Klebsiella* were more sensitive to doxycycline and co-trimoxazole, whereas *Acinetobacter* and *Pseudomonas* exhibited high resistance. These findings emphasise the need for accurate microbial diagnosis and appropriate antibiotic selection to effectively manage DFU infections. Since diabetic foot ulcers usually occur in individuals with long-standing diabetes, many of these patients are also at risk for developing diabetic nephropathy. Although renal function was not the primary focus of this study, integrating routine kidney assessment in DFU patients may assist in early detection of renal impairment and improve overall management outcomes.

Declaration Statements

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Conflict of Interest Disclosure

The authors declare that there is no conflict of interest

Authors' Contribution

Emmanuel Rajkumar S. conducted the experimental work and data collection. Nimithap S. conceptualized the study. V. R. Anjana performed statistical and microbiological analyses. Ravindran Jaganathan contributed to data interpretation and validation of results. Muthukrishnan Pallikondaperumal prepared figures and visualized the data. Subha Chandraraj assisted in manuscript review and critical editing. Manivannan Govindasamy contributed to literature review and scientific writing. Venkatesan Srinivasan supervised the research, coordinated the project, and finalized the manuscript for publication.

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Data Availability

All data obtained from the corresponding author by mail requesting.

Ethics Statement

This study did not involve the use of human tissue, body fluids, or animal subjects.

Informed Consent Statement

Not applicable.

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