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Bacteriological Profile and their Drug Sensitivity Profile in Diabetic Foot Ulcer, a Report from a Tertiary Care Center

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study aims to identify different microorganisms involved inDiabetic foot ulcers (DFU), compare their antibiotic sensitivity, and find the best combination of empirical antibiotics to treat patients.
Study Design: This is a Prospective observational study of patients treated at Tertiary Health Care Centre, Pune.
Type of Study: Prospective and observational hospital-based study.
Period of Study: From February 2021 to January 2022.
Sample Size: Tissue culture samples were collected from 100 patients.
Results: 81 male and 19 female patients participated in this research.
In this study, according to Wagner's grading system, 6 patients have Grade 1 ulcers, 21 patients have grade 2 ulcers, 48 patients have grade 3 ulcers, 21 patients have grade 4, and 4 patients have grade 5 ulcers.

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Out of 100 cases, 62 patients had neuropathic conditions, 18 patients had neuropathic cases combined with sepsis, 11 patients had neuro ischemic conditions, and 9 had neuro ischemia plus sepsis.
In our study, there were 31 (31%) polymicrobial cases, 65 (65%) monomicrobial cases, and 4 (4%) cases in which the culture was sterile.
Gram-negative bacterial growths were present in 59 (59%) cases compared to 41 (41%) cases where Gram-positive bacterial growths were present.
S. aureus (26%) was the most common bacteria isolated, followed by *E. coli* (20%) and Enterococcus spp (15 percent). Extended-spectrumbeta-lactamase (ESBL) producers made up 53% of the Gram-negative bacteria, Methicillin-resistant staphylococcus aureus (MRSA) made up 41%, and Vancomycin-resistant enterococci (VRE). made up 19%.
Discussion: Most of the patients (63%) in this study were over 45 years old This could be due to a higher incidence of comorbidities.
Higher male prevalence is comparable with a study by Harrison and Lederberg. This might be because men engage in more outdoor physical activity than women, especially in hot, humid environments, with poor foot care.
While GPC was more prevalent in Grades 1 and 2, Gram-negative bacilli and mixed infections were common in Grades 3 and 4, suggesting that Gram-negative infections were associated with severity in DFU and some cases needing limb amputation.
Frequent hospitalization, frequent use of broad-spectrum antibiotics, insufficient surgical source reduction, chronic wounds, irrational use of antibiotics, and the transmission of resistance genes via transport methods are possible causes of MDR. Clinicians should use antibiotics judiciously, on time, and in sufficient amounts, and the relevant organizations should periodically monitor drug intake.
Conclusion: This study demonstrated that among the isolates from the DFUs, multidrug-resistant bacteria predominated. Determining the antibiotics for the empirical therapy of diabetic ulcers will be made easier with knowledge of the pattern of antibiotic resistance among the isolates. Thus, the likelihood of subsequent development of antibiotic resistance as well as the indiscriminate use of antibiotics can be reduced.

Keywords: Diabetic foot ulcers; bacteriological profile; the drug sensitivity profile.

1. INTRODUCTION

A diabetic foot ulcer isa common complication of diabetes Mellitus (DM) that significantly increases the disease burden on patients [1-3].

Diabetic foot ulcers were found in 4.54% of patients newly diagnosed with type 2 diabetes mellitus in India; 46.1% had neuropathic, 19.7% had ischemic, and 34.2% had neuro ischemic foot ulcers [4].

Infections, which account for 40% to 80% of instances of DFU morbidity and mortality, are the most frequent complication in DFU [5].

Poor microvascular circulation prevents phagocytic cells from reaching the infected location, which impairs the effectiveness of antibiotics in the infected tissue [6].

Deep-seatedinfections seldomget treated with conservative antibiotic treatment and usually require surgery. Available surgical options are incision and drainage, wound debridement, bone resection, tissue revascularization, and amputation [7-10].

The infection causes the development of microthrombi, which aggravate ischemia, necrosis, and progressive gangrene necessitating limb amputation [11].

Diabetes patients with severe soft tissue infections, significant osteomyelitis, extensive peripheral artery occlusion, extensive gangrene, and non-healing ulcers may necessitate lower-limb amputations [12-14].

Because DFUs are chronic, repeated hospital stays are usually necessary. Hencethe chance of contracting a multidrug-resistant infection increases with repeated antibiotic exposure [15].

Generally, the DFU infections arepolymicrobial, and the proper antibiotic selection is important for the treatment of these infections, based on the

culture and the antimicrobial susceptibility testing results [16].

1.1 Aim

This study aims to identify different microorganisms involved inDiabetic foot ulcers (DFU), compare their antibiotic sensitivity, and find the best combination of empirical antibiotics to treat patients.

2. METHODS

2.1 Inclusion Criteria

All diabetic patients with foot ulcers or infections visited the study center's outpatient division.

2.2 Exclusion Criteria

Nondiabetic foot infections and ulcers.

The study was started after receiving approval from the institutional ethical committee.

A detailed history was obtained of the Patient's demographics, the duration of diabetes and foot condition, the type of diabetes treatment previously received, and the existence of any systemic disorders.

The foot ulcers on PWD were graded according to Wagner's grade (Wagner and Meggitt): [17].

Diabetic neuropathy is a result of chronic microvascular malfunction, oxidative stress, and systemic inflammation all causing nerve damage.

Based on associated neuropathy, ischemia, and infection, ulcer foot type was determined. For this, investigations such as monofilament nerve conduction velocity testing, biothesiometry, and Doppler-based ankle-brachial index estimation were done along with the clinical history and examination [18].

After the debridement, tissue samples were taken [19]. Before obtaining a tissue sample, no antibiotic or antiseptic agent was used. Empirical broad-spectrum antibiotic coverage was started for every patient with DFU according to institutional protocol.

Anaerobic and fungal cultures were not performed for this study.

The organisms were identified based on their Gram-staining properties, and further analysis was done in VITEK® 2 Compact system (BioMérieux) [20].

2.3 Antibiotic Susceptibility Testing

A bacterial suspension was matched with the McFarland standard of 0.5ml in 2.5 ml of a 0.45% sodium chloride solution with a VITEK® 2 DensiChek instrument (BioMérieux) with the incubation temperature kept at 35.5°C.

The isolates were subjected to a colorimetric measurement using a fresh optical reading head every 15 minutes for a maximum incubation time of 10 hours.

Table 1. Characteristics of ulcer

Grade	Ulcer Characteristics
0	No ulceration in high-risk foot
1	Ulcer involving skin and subcutaneous tissue
2	Ulcer extending into tendon,bone, and capsule
3	Deep ulcers with changes of osteomyelitis
4	Localized gangrene involving toes and forefoot
5	Extensive gangrene requiring major amputation

Table 2. Details of the method of study

Specimen type	Deep tissue specimen. like fat, fascia, muscle, bone
The system used for the identification of organisms	VITEK 2 Compact system (BioMérieux) and a few isolates were identified manually using Gram-stained smears
Culture media used	Blood agar, chocolate agar, Mac Conkey's agar, and thioglycollate medium
Temperature for inoculation	37°C overnight

VITEK® 2 database version 4.01 was used to analyze the data for organism identification in kinetic mode after 2 h of incubation. The interpretations provided were then considered for the analysis [21].

3. RESULTS

In our study, out of 100 cases, 37 were below 45 years and 63 patients were above 45 years.

81 male and 19 female patients participated in this research.

Table 3. Cases distribution according to ulcer grade

Ulcer grade	Number of cases
1	6
2	21
3	48
4	21
5	4

Out of 100 cases, 62 patients had neuropathic conditions, 18 patients had neuropathic cases combined with sepsis, 11 patients had neuro ischemic conditions, and 9 had plus sepsis.

In our study, there were 31 (31%) polymicrobial cases, 65 (65%) monomicrobial cases, and 4 (4%) cases in which the culture was sterile.

Gram-negative bacterial growths were present in 59 (59%) cases compared to 41 (41%) cases where Gram-positive bacterial growths were present.

S. aureus (26%) was the most frequent single bacterial growth, followed by *E. coli* (20%) and Enterococcus specimen (15 percent). Extended-spectrumbeta-lactamase (ESBL) producers made up 53% of the Gram-negative bacteria, Methicillin-resistant staphylococcus aureus (MRSA) made up 41%, and Vancomycin-resistant enterococci (VRE) made up 19% [22,23].

Table 4. The bacterial sensitivity pattern

Antibiotics	Bacterial isolates along with *Staphylococcus aureus*	Sensitivity pattern (%) Enterococcus	Enterobacteri-aceae	Pseudomo-nas
Ampicillin			11	
Amoxicillin-clavulanic acid			64	
Piperacillin-tazobactam			73	74
Cefalotin			24	
Ceftriaxone			56	
Cefoxitin.			26	
Cefixime			11	
Ertapenem			76	
Ofloxacin			42	
Ticarcillin-clavulanic acid			14	67
Ceftazidime			64	72
Cefoperazone-sulbactam				68
Cefepime				74
Doripenem				87
Imipenem			89	72
Meropenem			84	70
Amikacin			90	90
Aztreonam				43
Gentamicin	83		89	66
Ciprofloxacin	73	74	65	67
Minocycline				54
Tigecycline				72
Trimethoprime-sulfamethoxa		47	39	22
Levofloxacin	74	68		68
Colistin				100

Antibiotics	Bacterial isolates along with *Staphylococcus aureus*	Sensitivity pattern (%) Enterococcus	Enterobacteri-aceae	Pseudomo-nas
Oxacillin	72			
Erythromycin	78	70		
Clindamycin	71	58		
Linezolid	100	100		
Daptomycin	100	100		
Teicoplanin	84	89		
Vancomycin	100	67		
Benzylpenicillin	24	11		
Tetracycline		82		
Tigecycline	89	74		

Most of the Enterobacteriaceae culture isolates in the current investigation were susceptible to amikacin (90%), imipenem (89%), and meropenem (84%) [24,25,2].

Most of the Pseudomonas culture isolates were sensitive to amikacin (90%), imipenem (72%), and meropenem (70%) [2].

Most of the Staphylococcus culture isolates were sensitive to linezolid (100%), daptomycin (100%), tigecycline (89%).

In our study, most of the Enterococcus culture isolates were sensitive to linezolid (100%), daptomycin (100%), teicoplanin (89%), and tigecycline (74%).

4. DISCUSSION

Most of the patients (63%) in this study were over 45 years old This could be due to a higher incidence of comorbidities."This is similar to a study by King et al. in 1998 also mentioned that the majority of people with diabetes foot were in 45–64 years" [1].

Higher male prevalence is comparable with a study by Harrison and Lederberg [26]. This might be because men engage in more outdoor physical activity than women, especially in hot, humid environments, with poor foot care.

Our study found that the majority of DFI patients reported having an advanced grade of infection Wagner Grade III and above. This is frequently ascribed to the public's and medical professionals' lack of knowledge of foot care [27].

S. aureus was the single most frequent pathogen (26%) followed by *E. coli* (20%). A study by Abdulrazaq et al. also found the same) [23]. A contrary study carried out by Ako-Nai et al. showed *E. coli* as the frequent bacterial pathogen, whilea study by Shankar et al reported P. aeruginosaas the most common pathogen. "Source of infection, useof the antibiotic drug for treatment, sample collection method, and different types of infection can influence pathogen diversity in DFI [28-31,2].

While GPC was more prevalent in Grades 1 and 2, Gram-negative bacilli and mixed infections were common in Grades 3 and 4, suggesting that Gram-negative infections were associated with severity in DFU and some cases needing limb amputation [32].

These days, the rising threat of MDR pathogens and related consequences in developing nations worries clinical microbiologists and doctors [33]. In the current investigation, 91 percent of the bacteria (VRE 34%,MRSA 48%, and ESBL 78%) were resistant to three or more antibiotics. In contrast to an Iranian study by Japoni et al. these rates are much higher. These isolated infections are more challenging to treat [34-36].

Frequent hospitalization, frequent use of broad-spectrum antibiotics, insufficient surgical source reduction, chronic wounds, irrational use of antibiotics, and the transmission of resistance genes via transport methods are possible causes of MDR [2].

Clinicians should use antibiotics judiciously, on time, and in sufficient amounts, and the relevant organizations should periodically monitor drug intake to improve the condition and lower the rate of amputation.

Clinicians should switch to using narrower spectrum therapy depending on the culture report. To reduce infection sources, sufficient and prompt surgical intervention is necessary [36] These aid in lowering the excessive and careless use of antibiotics.

5. CONCLUSION

This study demonstrated that Gram-negative aerobes like *S. aureus* were commonest in diabetic foot ulcers.

In the DFI cases, monomicrobial infection was more prevalent than polymicrobial infection [37].

MDR organisms were alarmingly prevalent in the PWD and in people with foot ulcers.

According to local sensitivity patterns, the ideal empirical antibiotics combination for Diabetic foot ulcers in our institution is Linezolid and Amikacin which is most effective in cohorts of patients with the presentation of infections associated with DFU [7].

In the present study, 91% of the bacteria were resistant to three or more antibiotics. Thus, indiscriminate use of antibiotics and chances of subsequent development of antibiotic resistance can also be reduced with proper knowledge of antibiotics sensitivity [2].

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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