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Bacteriological profile and antimicrobial susceptibility patterns of diabetic foot ulcers: a prospective cross-sectional study.

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Abstract

Background

Diabetic foot ulcers are a major source of infection-related morbidity, prolonged hospitalisation and lower-limb amputation. Their microbial spectrum varies across settings, while antimicrobial resistance increasingly limits empirical treatment.

Objectives

To characterise the bacteriological profile of clinically infected diabetic foot ulcers and determine the antimicrobial susceptibility patterns of recovered bacterial isolates in a tertiary-care hospital.

Methods

This prospective cross-sectional study included 71 adults with type 2 diabetes and clinically infected foot ulcers presenting from January to December 2024. Deep wound swabs, aspirated pus or tissue specimens were collected after wound preparation and processed by conventional culture and biochemical methods. Antimicrobial susceptibility was assessed by Kirby-Bauer disk diffusion using contemporaneous Clinical and Laboratory Standards Institute criteria. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

Results

Significant bacterial growth was obtained from 62 of 71 specimens (87.3%). *Staphylococcus aureus* was the most frequent isolate (24.2%), followed by *Escherichia coli* (17.7%), Proteus species (16.1%) and *Pseudomonas aeruginosa* (12.9%). Polymicrobial infection occurred in 21.0% of culture-positive cases. Five of 15 *S. aureus* isolates (33.3%) were methicillin resistant. Resistance among *E. coli* was highest to amoxicillin-clavulanate (72.7%) and cotrimoxazole (54.5%), whereas meropenem and levofloxacin showed comparatively lower resistance (27.3% each). Overall, 19 isolates (30.6%) met the multidrug-resistance definition.

Conclusion

Clinically infected diabetic foot ulcers in this setting contained mixed bacterial flora with a substantial antimicrobial-resistance burden. Culture-directed therapy should accompany timely debridement, glycaemic optimisation and structured wound care.

Recommendations

Local antibiograms should be updated periodically, and empirical regimens should be reviewed promptly after culture results become available.

Keywords: *antimicrobial resistance; bacterial culture; diabetic foot infection; diabetic foot ulcer; multidrug resistance; Staphylococcus aureus*
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Introduction

Diabetic foot ulcers (DFUs) are among the most serious and resource-intensive complications of diabetes mellitus. Neuropathy, repetitive trauma, foot deformity, peripheral arterial disease and impaired host defence interact to initiate ulceration and impede healing. Once an ulcer develops, infection increases the likelihood of hospital admission, operative intervention and limb loss. Recurrence is also frequent after apparent healing, so each episode contributes to cumulative disability,

reduced quality of life and sustained healthcare expenditure.[1,2]

The clinical diagnosis of diabetic foot infection is based on local or systemic inflammatory findings rather than culture positivity alone. Nevertheless, microbiological assessment is central to the management of moderate or severe infection, recurrent disease and ulcers that fail to respond to initial therapy. Current guidance favours tissue or deep specimens obtained after cleansing and debridement because superficial swabs are more susceptible to contamination by colonising flora.[3] Early infections are often dominated by aerobic Gram-positive cocci, especially *Staphylococcus aureus*, whereas chronic, deep, necrotic or previously treated ulcers can contain Gram-negative bacilli and multiple organisms.[4–8]

The organism distribution reported from Indian hospitals is heterogeneous. Several studies have identified *S. aureus* as a leading pathogen, while others have documented a substantial or predominant contribution from Enterobacterales and *Pseudomonas aeruginosa*.[4–6,9] This variation reflects differences in ulcer chronicity, prior antimicrobial exposure, referral patterns, specimen quality and local prescribing practices. Consequently, empirical regimens derived from distant populations can provide inadequate coverage or unnecessarily broad treatment.

Antimicrobial resistance further complicates diabetic foot infection. Methicillin-resistant *S. aureus*, extended-spectrum beta-lactamase-producing Enterobacterales and resistant non-fermenting Gram-negative bacilli are increasingly encountered in tertiary-care practice.[6,8–12] Inappropriate empirical therapy can delay source control and wound recovery, whereas indiscriminate broad-spectrum therapy intensifies selection pressure. Reliable local antibiograms are therefore essential for balancing timely treatment with antimicrobial stewardship.

The present study was undertaken to describe the bacteriological profile of clinically infected DFUs managed at a tertiary-care hospital in Suryapet, Telangana, and to determine the antimicrobial susceptibility patterns of the recovered organisms. A secondary objective was to quantify the prevalence of methicillin-resistant *S. aureus*, polymicrobial infection and multidrug resistance in the study population.

Materials and Methods

Study design and setting

This hospital-based prospective cross-sectional study was conducted in the Department of Microbiology, Government General Hospital, Suryapet, Telangana, India, from January to December 2024. The hospital receives patients from Suryapet and surrounding districts and provides coordinated surgical, medical and microbiology services. Reporting was structured in accordance with the observational nature of the study.

Study population and sampling

Consecutive adults with type 2 diabetes mellitus and a clinically infected foot ulcer were assessed during the study period. Because the investigation was designed as one-year local microbiological surveillance, all eligible consenting patients encountered during the defined period were included; no separate probability-based sample was drawn. The final sample comprised 71 participants.

Eligibility criteria

Patients aged 18 years or older with a Wagner grade 1 or higher DFU and at least one clinical feature of infection, including purulent discharge, erythema, local warmth, oedema, tenderness or cellulitis, were eligible. Patients with traumatic, venous or non-diabetic arterial ulcers, those who had received systemic antibiotics for more than 72 hours before sampling, and those who declined consent were excluded.

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Specimen collection and transport

After cleansing the ulcer and removing superficial debris or devitalised material, a deep wound specimen was obtained according to the wound characteristics. Specimens consisted of deep swabs, aspirated pus or tissue biopsy material. Samples were placed in appropriate sterile containers, transported promptly to the microbiology laboratory and processed within one hour. Anaerobic and fungal cultures were not performed routinely.

Culture and bacterial identification

Direct Gram staining was performed for preliminary assessment of inflammatory cells and bacterial morphology. Specimens were inoculated on 5% sheep blood agar and MacConkey agar and incubated aerobically at 37°C for 24–48 hours. Isolates were identified from colony morphology, haemolysis, Gram-stain appearance and conventional biochemical reactions, including catalase, coagulase, oxidase, indole, citrate, urease and triple-sugar-iron tests, as appropriate.

Antimicrobial susceptibility testing

Clinically significant isolates underwent Kirby-Bauer disk diffusion testing on Mueller-Hinton agar. Inhibition zones were interpreted using the Clinical and Laboratory Standards Institute M100 criteria applicable during 2024.[14] The antibiotic panel was selected by organism group and included penicillins, beta-lactam/beta-lactamase inhibitor combinations, cephalosporins, aminoglycosides, fluoroquinolones, carbapenems, glycopeptides, clindamycin and linezolid. Cefoxitin screening was used to identify methicillin resistance in *S. aureus*. Appropriate reference strains were included for internal quality control.

Outcome definitions and statistical analysis

The primary outcomes were culture positivity, organism distribution and antibiotic resistance proportions. Polymicrobial infection denoted recovery of more than one clinically significant bacterial species from a specimen. Multidrug resistance was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[13] Data were analysed descriptively. Categorical variables are presented as numbers and percentages; no inferential comparisons were planned for this microbiological surveillance dataset.

Ethical considerations

The Institutional Ethics Committee of Government Medical College, Suryapet, approved the study. Written informed consent was obtained before enrolment. Participation was voluntary, confidentiality was maintained and clinical care was not altered by refusal to participate.

Results

Culture positivity

A total of 71 specimens from clinically infected DFUs were processed. Significant aerobic bacterial growth was obtained from 62 specimens (87.3%), while 9 (12.7%) showed no growth after standard incubation (Table 1).

Table 1: Culture results among diabetic foot ulcer Specimens: (n = 71)

Culture result	Number	Percentage
Culture positive	62	87.3
No growth	9	12.7
Total	71	100.0

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Bacterial spectrum

Among the 62 bacterial isolates tabulated, *S. aureus* was the most common organism (15/62; 24.2%). *E. coli* accounted for 11 isolates (17.7%), *Proteus* species for 10 (16.1%) and *P. aeruginosa* for 8 (12.9%). *Enterococcus faecalis*, *Klebsiella* species and *Enterobacter freundii* were less frequent. Eight isolates were retained as an aggregated 'other bacterial isolates' category because species-level identities were not available in the supplied study dataset. Thirteen of the 62 culture-positive specimens (21.0%) were reported as polymicrobial; this specimen-level characteristic is not added to the organism counts in Table 2.

Table 2: Distribution of bacterial isolates (n = 62)

Organism	Number	Percentage
Staphylococcus aureus (including MRSA)	15	24.2
Escherichia coli	11	17.7
Proteus spp.	10	16.1
Pseudomonas aeruginosa	8	12.9
Enterococcus faecalis	5	8.1
Klebsiella spp.	4	6.5
Enterobacter freundii	1	1.6
Other bacterial isolates	8	12.9
Total	62	100.0

MRSA: methicillin-resistant Staphylococcus aureus. Polymicrobial growth was recorded in 13/62 culture-positive specimens (21.0%) and is reported separately because it is not an organism category.

Antibiotic resistance in Staphylococcus aureus

Resistance among *S. aureus* isolates was highest to penicillin and ciprofloxacin (60.0% each). Cefoxitin resistance identified 5 MRSA isolates, representing 33.3% of all *S. aureus*. No linezolid resistance was detected. Clindamycin resistance was present in 4 isolates (26.7%) (Table 3).

Table 3: Antibiotic resistance among Staphylococcus aureus isolates (n = 15)

Antibiotic	Resistant, n	Resistant, %
Penicillin	9	60.0
Ciprofloxacin	9	60.0
Erythromycin	6	40.0
Cotrimoxazole	6	40.0

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Antibiotic	Resistant, n	Resistant, %
Piperacillin-tazobactam	6	40.0
Cefoxitin (MRSA)	5	33.3
Clindamycin	4	26.7
Linezolid	0	0.0

Cefoxitin resistance was used as a surrogate marker for methicillin resistance.

Antibiotic resistance in Escherichia coli

E. coli showed the highest resistance to amoxicillin-clavulanate (72.7%), followed by cotrimoxazole (54.5%). Resistance to ceftazidime, gentamicin, amikacin and piperacillin-tazobactam was 45.5% for each agent. Meropenem and levofloxacin had the lowest observed resistance proportions (27.3% each), although resistance to these agents was still clinically relevant (Table 4).

Table 4: Antibiotic resistance among Escherichia coli isolates (n = 11)

Antibiotic	Resistant, n	Resistant, %
Amoxicillin-clavulanate	8	72.7
Cotrimoxazole	6	54.5
Ceftazidime	5	45.5
Gentamicin	5	45.5
Amikacin	5	45.5
Piperacillin-tazobactam	5	45.5
Cefepime	4	36.4
Levofloxacin	3	27.3
Meropenem	3	27.3

Other isolates and multidrug resistance

Proteus and *Klebsiella* species showed resistance to several commonly used beta-lactams and cotrimoxazole. *P. aeruginosa* retained activity to some antipseudomonal beta-lactams, although fluoroquinolone resistance was observed. All *E. faecalis* isolates were susceptible to linezolid. Overall, 19 of 62 isolates (30.6%) met the MDR definition. The principal microbiological indicators are summarised in Table 5.

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Table 5: Principal microbiological indicators

Indicator	Numerator/denominator	Percentage
Culture-positive specimens	62/71	87.3
Polymicrobial culture-positive specimens	13/62	21.0
MRSA among S. aureus isolates	5/15	33.3
MDR isolates	19/62	30.6

MDR: multidrug resistant; MRSA: methicillin-resistant Staphylococcus aureus.

Discussion

This prospective surveillance study found a high culture-positivity rate of 87.3% among clinically infected DFUs. *S. aureus* was the leading isolate, but Gram-negative bacilli collectively constituted a substantial component of the microbial spectrum. Polymicrobial infection was identified in approximately one-fifth of culture-positive specimens, MRSA represented one-third of *S. aureus* isolates and almost one-third of all isolates were multidrug resistant. These findings indicate that empirical treatment in this setting must address a heterogeneous pathogen distribution without losing sight of local resistance pressure.

The predominance of *S. aureus* accords with hospital studies from India and with broader reviews of diabetic foot infection microbiology.[4–8] Skin colonisation, adherence to damaged tissue and persistence within chronic wounds favour this organism. The MRSA proportion of 33.3% is clinically important and is within the broad range reported from tertiary-care cohorts.[6,7] MRSA coverage should not be automatic for every ulcer; it should be determined by infection severity, previous colonisation or infection, recent healthcare exposure and the local antibiogram, followed by de-escalation when culture results are available.[3]

E. coli, *Proteus* species and P. aeruginosa were prominent Gram-negative organisms. Similar Gram-negative contributions have been reported in chronic or previously treated DFUs in India.[5,6,9,10,12] Chronicity, repeated antibiotic courses, moist wound environments and referral of complicated ulcers to tertiary centres can shift the spectrum away from uncomplicated Gram-positive infection. The 21.0% polymicrobial rate also supports careful specimen collection and source control, although the absence of anaerobic culture probably reduced the measured complexity. Studies that systematically process debrided tissue for aerobic and anaerobic organisms frequently report higher polymicrobial yields.[11]

Resistance patterns narrowed several commonly used options. Sixty per cent of *S. aureus* isolates were resistant to ciprofloxacin, while *E. coli* resistance was high to

amoxicillin-clavulanate and cotrimoxazole. Meropenem and levofloxacin showed lower resistance than the other tested agents in *E. coli*, but the observed meropenem resistance argues against indiscriminate carbapenem use. The MDR prevalence of 30.6% reinforces the need for tissue-based culture, timely review of laboratory results and stewardship-guided narrowing of therapy. The

internationally accepted MDR definition used in this study improves comparability, but complete testing across the relevant antimicrobial categories remains important for correct classification.[13]

Clinical management should integrate microbiology with debridement, pressure off-loading, vascular assessment, glycaemic optimisation and treatment of systemic illness.[2,3] Antibiotics alone cannot compensate for retained necrotic tissue, undrained collections or critical ischaemia. The present results are most useful as a local guide for initial therapy in clinically infected ulcers and as a baseline for repeat surveillance. Organism-specific treatment decisions should still incorporate ulcer severity, renal function, tissue penetration, allergy history, prior microbiology and current susceptibility breakpoints.[3,14]

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Generalizability

The findings are most applicable to adults with clinically infected diabetic foot ulcers presenting to comparable tertiary-care hospitals in Telangana and similar Indian settings. Referral patterns, prior antibiotic exposure, specimen techniques and local antimicrobial use can alter both organism distribution and resistance. Extrapolation to uncomplicated community ulcers, paediatric patients or centres using routine anaerobic and molecular testing should therefore be undertaken cautiously. Periodic local surveillance remains necessary before these proportions are used to guide empirical protocols.

Conclusion

Diabetic foot infections in this tertiary-care setting showed a mixed bacterial spectrum and a substantial antimicrobial-resistance burden. *Staphylococcus aureus* was the leading isolate, while *Escherichia coli*, *Proteus* species and *Pseudomonas aeruginosa* formed an important Gram-negative component. One-third of *S. aureus* isolates were methicillin resistant, and 30.6% of all isolates met the multidrug-resistance definition. These findings limit the reliability of uniform empirical regimens. Deep-specimen culture and susceptibility testing should guide definitive therapy, supported by prompt debridement, wound care, off-loading, vascular assessment and glycaemic optimisation. A coordinated multidisciplinary approach and periodic local antibiogram review are essential to reduce treatment failure, prolonged hospitalisation and avoidable limb loss.

Limitations

The single-centre design and modest sample size restrict external validity. Anaerobic and fungal cultures were not routinely performed, and molecular confirmation of resistance mechanisms was unavailable. Clinical variables such as ulcer duration, Wagner grade, glycaemic control, peripheral arterial disease, previous antibiotic exposure and patient outcomes were not analysed against the microbiological findings. Species-level identities for eight isolates were unavailable in the supplied dataset, and organism-specific MDR subgroup counts were therefore not presented.

Recommendations

Deep tissue or aspirate sampling should be prioritised after cleansing and debridement, especially for moderate, severe, recurrent or non-responsive infections. Empirical therapy should follow a periodically updated hospital antibiogram and be narrowed as soon as reliable culture results are available. Antimicrobial stewardship teams should audit fluoroquinolone, broad-spectrum beta-lactam and carbapenem use. Future multicentre studies should incorporate ulcer severity, vascular status, glycaemic control, prior antibiotic exposure, anaerobic and fungal culture, molecular resistance testing and clinical follow-up. Linking microbiological patterns with healing, reoperation, length of stay and amputation will produce stronger evidence for treatment pathways and prevention strategies.

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Abbreviations

ATCC-American Type Culture Collection;
CLSI-Clinical and Laboratory Standards Institute;
DFU, diabetic foot ulcer; DFI-diabetic foot infection;
MDR-multidrug resistant;
MRSA-methicillin-resistant *Staphylococcus aureus*

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Conflicts of interest

The authors declare no conflicts of interest.

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Data availability

De-identified data can be made available by the corresponding author on reasonable request, subject to institutional and ethics requirements.

Author contributions

Dr Thannuri Fanindra contributed to conceptualisation, methodology, investigation, data curation, literature review and preparation of the original draft. **Dr S Ruth Ramya** contributed to methodology, laboratory supervision, validation, interpretation and manuscript review. **Dr Aditi Tomer Thakur** contributed to investigation oversight, validation, formal analysis and manuscript editing. **Dr Taruni** provided senior supervision, resources, methodological guidance and critical intellectual revision. **Dr R Kondal Rao** contributed to supervision, data interpretation, formal analysis and critical revision. **Dr Nagasaikaran Sade** assisted with clinical data coordination, literature review, data verification and manuscript editing. All authors reviewed and approved the final manuscript and accept accountability for the integrity of the work.

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