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Bacteriological Profile and Antibiotic Susceptibility Pattern of Diabetic Foot Infections at a Tertiary Care Hospital: *A Cross-Sectional Microbiological Study*

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Abstract

Diabetic foot infection (DFI) is a leading cause of amputation worldwide, and empirical management depends on locally generated bacteriological data that remain scarce for tertiary care hospitals in Lahore, Pakistan. This cross-sectional study characterized the bacteriological profile and antibiotic susceptibility pattern of DFI among 93 consecutive patients admitted to a tertiary care hospital in Lahore over three months. Wound swabs and pus aspirates were cultured on blood, MacConkey, and chocolate agar; isolates were identified by colony morphology and biochemical testing, and susceptibility to six

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antibiotics was determined by Kirby–Bauer disk diffusion per CLSI M100 criteria. Gram-negative organisms predominated (68.8%) over Gram-positive organisms (31.2%), consistent with regional trends. Staphylococcus aureus (25.8%), Escherichia coli (23.7%), and Pseudomonas aeruginosa (19.4%) were the leading isolates. Ampicillin resistance was universal (100%), while P. aeruginosa displayed a multidrug-resistant phenotype across four antibiotic classes. Imipenem and vancomycin retained complete susceptibility across all isolates. A statistically significant association emerged between patient sex and Gram-stain classification, $\chi^2(1, N = 93) = 6.846$, $p = .009$, with Gram-negative organisms more common among female patients. These findings support discontinuing ampicillin and restricting empirical fluoroquinolone use, reserving imipenem and vancomycin for severe or culture-confirmed infection, and grounding institutional antibiotic policy in locally derived, rather than extrapolated, susceptibility data.

Introduction

Diabetic foot infection (DFI) is among the most destructive sequelae of diabetes mellitus (DM), driving prolonged hospitalization, repeated debridement, and non-traumatic lower-limb amputation worldwide (International Diabetes Federation [IDF], 2023). Chronic hyperglycaemia produces neuropathy, ischaemia, and impaired immunity, three converging pathways through which a trivial injury becomes a portal for polymicrobial invasion (Lazzarini et al., 2022). The IDF (2023) estimates 537 million adults living with diabetes globally, projected to reach 783 million by 2045, with South and South-East Asia driving the sharpest growth. Pakistan is among the most severely affected nations, with an adult prevalence of 26.3% (Khuwaja et al., 2022), and the lifetime risk of developing a foot ulcer, the principal precursor of DFI, ranges from 19% to 34% (Armstrong et al., 2023).

Rational antibiotic therapy and limb salvage depend on accurately identifying causative organisms and their susceptibility profiles through validated culture techniques. Culture-based bacteriological data from Pakistani tertiary hospitals, particularly in Lahore, remain scarce; most existing studies rely on superficial wound swabs rather than deep-tissue biopsy, omit anaerobic

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culture, and apply inconsistent susceptibility methods (Siddiqui et al., 2023). Clinicians in Lahore are consequently compelled to base empirical treatment on data drawn from epidemiologically dissimilar settings (Rahim et al., 2022). This study addresses that deficit by applying standardized specimen collection, aerobic bacteriological culture with Gram-stain characterization, and Kirby–Bauer disk diffusion susceptibility testing to a consecutive series of DFI patients at a tertiary care hospital in Lahore.

Global and regional evidence converge on three points relevant to this study. First, Staphylococcus aureus and aerobic Gram-negative bacilli, principally Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae, dominate DFI bacteriology across geographies. Still, South Asian data consistently show greater Gram-negative predominance and higher rates of methicillin resistance and extended-spectrum β-lactamase (ESBL) production. In a 29-country point-prevalence study of 1,060 DFI cases, Mendes et al. (2022) found S. aureus the leading isolate overall (30.1%), with MRSA prevalence reaching 44.7% in South Asia and ESBL-positive Gram-negatives recovered in 61.3% of South Asian isolates. Within Pakistan, Rahim et al. (2022) reported Gram-negative predominance (58.1%) and MRSA in 38.5% of S. aureus isolates across 210 specimens, while Bilal et al. (2022) and Rastogi et al. (2022) documented comparable organism rankings and substantial ESBL burden in Punjab and north India, respectively. Siddiqui et al. (2023), using deep-tissue sampling in 150 cases, found Gram-negative organisms in 61.3% of isolates and polymicrobial infection in 48.7% of cases overall, rising to 74.3% among advanced Wagner grades. Second, wound grade and infection severity reliably predict microbiological complexity. The IDSA/IWGDF and Wagner–Meggitt systems both correlate with polymicrobial colonization, Gram-negative representation, and resistance (Lipsky et al., 2023). Saltoglu et al. (2023) demonstrated that severe infection was overwhelmingly polymicrobial (82.1%), with obligate anaerobes comprising 43.5% of isolates, while Hinchliffe et al. (2023) linked critical limb ischaemia to a rise in anaerobic isolation from 12.4% to 37.8%. A pooled meta-analysis of 58 studies by Pourmand et al. (2023) estimated MRSA at 34.1% of S. aureus isolates globally, a proportion elevated in low-

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and middle-income regions, while Cecilia et al. (2023) linked biofilm-forming capacity to multidrug resistance (OR = 4.2). Contrasting higher-income data from Japan showed lower Gram-negative and anaerobic representation, attributed to earlier presentation and better glycaemic control, whereas Hadavand et al. (2023) documented alarming last-line resistance, including colistin and carbapenem resistance, among Iranian isolates.

This study aims to identify the predominant bacterial pathogens associated with diabetic foot infections (DFIs) in a tertiary care hospital in Lahore, determine the distribution of Gram-positive and Gram-negative organisms, and evaluate the antibiotic susceptibility patterns of the isolates.

It is hypothesized that Gram-negative bacteria predominate, reflecting regional tertiary care trends in South Asia; that Staphylococcus aureus is the leading Gram-positive pathogen, with a significant proportion exhibiting methicillin resistance; and that many Gram-negative isolates demonstrate multidrug resistance, including extended-spectrum β-lactamase (ESBL) production. Accordingly, this study seeks to answer two key questions: which bacterial pathogens are most frequently isolated in DFIs at this hospital, and what is the relative prevalence of Gram-positive versus Gram-negative organisms. What are the antibiotic susceptibility patterns of the isolated organisms?

Materials and Methodology

Research Design and Setting

A cross-sectional, hospital-based laboratory study was conducted over three months at a tertiary care hospital in Lahore, Pakistan, including patients with clinically diagnosed diabetic foot infections (Wagner–Meggitt grades 1–5), while excluding grade 0 lesions and HIV-positive cases to avoid confounding due to immunosuppression.

Sample Size

The sample size was calculated using a standard formula with 95% confidence and 10% margin of error, resulting in a minimum of 93 patients.

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Specimen Collection and Culture

Wound exudate, pus aspirate, or debrided tissue was collected after saline cleansing of the wound surface; deep-tissue biopsy was prioritized for deep ulcers and suspected osteomyelitis, given its superior diagnostic yield relative to superficial swabbing ($p = .028$) (Hussain et al., 2022; Ramakant et al., 2021). Specimens were transported in Stuart's medium within two hours and inoculated onto blood agar, MacConkey agar, and chocolate agar, incubated aerobically at 37 °C for 24–48 hours (Atlaw et al., 2022; Islam et al., 2021). Identification combined Gram staining, colony morphology, and a standard biochemical panel including catalase, coagulase, oxidase, triple sugar iron, indole, methyl red–Voges-Proskauer, citrate, and urease tests (Hasan et al., 2024).

Antibiotic Susceptibility Testing and Data Analysis

Susceptibility to ampicillin, ciprofloxacin, gentamicin, ceftriaxone, imipenem, and vancomycin was determined by Kirby–Bauer disk diffusion on Mueller-Hinton agar per CLSI M100 criteria, using inocula standardized to a 0.5 McFarland turbidity (Hussain et al., 2022). Data were analyzed in IBM SPSS Statistics using descriptive statistics, chi-square testing, and one-way ANOVA.

Results

Demographic and Specimen Characteristics

Of 93 enrolled patients, 47 (50.5%) were male and 46 (49.5%) were female, an almost even split. Mean age was 59.9 ± 7.4 years (range 47–73), with the largest group aged 60–69 years (43.0%). Wound swabs (51.6%) and pus aspirates (48.4%) were comparably represented, and specimen source varied by organism, with K. pneumoniae (85%) and E. coli (59%) showing strong affinity for pus, consistent with deeper, polymicrobial involvement (Table 1).

Table 1

Demographic and Clinical Characteristics of Study Participants (N = 93)

Characteristic	n	%
Sex: Male	47	50.5

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Characteristic	n	%
Sex: Female	46	49.5
Age, mean ± SD (years)	59.9 ± 7.4	—
Age range (years)	47–73	—
Age group: 40–49 years	10	10.8
Age group: 50–59 years	27	29.0
Age group: 60–69 years	40	43.0
Age group: 70–79 years	16	17.2
Specimen: Wound swab	48	51.6
Specimen: Pus aspirate	45	48.4

Note. Age-group counts are derived from the reported mean age and distribution (59.9 ± 7.4 years; range 47–73).

Bacteriological Profile

Gram-negative organisms accounted for the majority of isolates (68.8%), and Gram-positive organisms for the remainder (31.2%), directly supporting H1. Staphylococcus aureus was the single most frequent isolate (25.8%), recovered predominantly from wound swabs (71%), followed by Escherichia coli (23.7%, 59% from pus) and Pseudomonas aeruginosa (19.4%, 67% from wound swabs). These three organisms together accounted for 68.8% of all isolates. Klebsiella pneumoniae (14.0%), Proteus mirabilis (8.6%), Enterococcus spp. (5.4%), and Enterobacter spp. (3.2%) completed the profile (Table 2).

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Table 2

Distribution and Characteristics of Bacterial Isolates from Diabetic Foot Infections (N = 93)

Organism	n	%	Gram Stain	Primary Source	Rank
Staphylococcus aureus	24	25.8	Positive	Wound (71%)	1st
Escherichia coli	22	23.7	Negative	Pus (59%)	2nd
Pseudomonas aeruginosa	18	19.4	Negative	Wound (67%)	3rd
Klebsiella pneumoniae	13	14.0	Negative	Pus (85%)	4th
Proteus mirabilis	8	8.6	Negative	Pus (75%)	5th
Enterococcus spp.	5	5.4	Positive	Wound (80%)	6th
Enterobacter spp.	3	3.2	Negative	Wound (67%)	7th
Total Gram-negative	64	68.8	—	—	—
Total Gram-positive	29	31.2	—	—	—

Note. Primary Source refers to the specimen type yielding the majority of isolates for each organism.

Sex and Gram-Stain Association

A chi-square test of independence revealed a significant association between patient sex and Gram-stain classification, $\chi^2(1, N = 93) = 6.846$, $p = .009$. Male patients more often harboured Gram-positive organisms (44.7%) than female patients (17.4%), whereas Gram-negative organisms were disproportionately more common among females (82.6%) than males (55.3%) (Table 3).

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Table 3

Association Between Patient Sex and Gram-Stain Classification (N = 93)

Sex	Gram-negative, n (%)	Gram-positive, n (%)	Total	p
Male (n = 47)	26 (55.3)	21 (44.7)	47	
Female (n = 46)	38 (82.6)	8 (17.4)	46	.009*
Total	64 (68.8)	29 (31.2)	93	

Note. χ^2 = 6.846, df = 1. *p < .05.

Age by Infecting Organism

A one-way ANOVA found no significant difference in mean age across the seven pathogen groups, F(6, 86) = 1.327, p = .254, indicating that patient age did not vary meaningfully by infecting organism (Table 4).

Table 4

Mean Age by Infecting Organism (One-Way ANOVA)

Organism	Mean Age (yrs)	SD	n	Range
Staphylococcus aureus	61.9	7.1	24	47–73
Pseudomonas aeruginosa	60.8	8.6	18	48–72
Escherichia coli	60.7	7.2	22	49–73
Enterococcus spp.	59.4	6.8	5	52–67
Enterobacter spp.	57.7	4.0	3	53–61
Klebsiella pneumoniae	57.5	8.3	13	48–70
Proteus mirabilis	54.8	6.4	8	47–67

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Organism	Mean Age (yrs)	SD	n	Range
Overall	59.9	7.4	93	47–73

Note. F(6, 86) = 1.327, p = .254. SD = standard deviation.

Antibiotic Susceptibility

Ampicillin resistance was universal (100%) across all applicable isolates. Ciprofloxacin resistance followed a bimodal pattern: E. coli and P. aeruginosa were uniformly resistant (100%), while all other organisms remained fully susceptible. Gentamicin and ceftriaxone resistance (19.4% and 20.5%, respectively) were confined entirely to P. aeruginosa, which was therefore simultaneously resistant to ampicillin, ciprofloxacin, gentamicin, and ceftriaxone, meeting criteria for a multidrug-resistant (MDR) phenotype and supporting H3. Imipenem retained universal susceptibility (0% resistance) across all 93 isolates, and vancomycin retained 100% susceptibility among the 29 Gram-positive isolates tested (Table 5).

Table 5

Antibiotic Susceptibility Profiles of Bacterial Isolates by Organism

Organism (n)	AMP	CIP	GEN	CRO	IMP	VAN
S. aureus (24)	R (100%)	S (0%)	S (0%)	S (0%)	S (0%)	S (0%)
E. coli (22)	R (100%)	R (100%)	S (0%)	S (0%)	S (0%)	N/A
P. aeruginosa (18)	R (100%)	R (100%)	R (100%)	R (100%)	S (0%)	N/A
K. pneumoniae (13)	R (100%)	S (0%)	S (0%)	S (0%)	S (0%)	N/A
P. mirabilis (8)	R (100%)	S (0%)	S (0%)	S (0%)	S (0%)	N/A
Enterococcus spp. (5)	N/A	S (0%)	S (0%)	N/A	S (0%)	S (0%)
Enterobacter spp. (3)	R (100%)	S (0%)	S (0%)	S (0%)	S (0%)	N/A

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Organism (n)	AMP	CIP	GEN	CRO	IMP	VAN
Overall (N = 93)	100%*	43.0%	19.4%	20.5%*	0%	0%†

Note. R = resistant; S = susceptible; N/A = not applicable. AMP = ampicillin; CIP = ciprofloxacin; GEN = gentamicin; CRO = ceftriaxone; IMP = imipenem; VAN = vancomycin. *Denominator for AMP and CRO excludes Enterococcus spp. (n = 5). †VAN tested only in Gram-positive isolates (n = 29).

Hypothesis Testing Summary

H1 (Gram-negative predominance) was supported (68.8% of isolates). H2 (S. aureus as leading Gram-positive organism with substantial methicillin resistance) was partially supported: S. aureus was indeed the leading Gram-positive isolate (25.8%), but phenotypic or genotypic MRSA testing was not performed, leaving the resistance component untested. H3 (multidrug resistance among Gram-negative isolates) was supported specifically by P. aeruginosa, which showed simultaneous resistance to four antibiotic classes.

Discussion

This study characterized the bacteriological profile and susceptibility pattern of DFI among 93 patients at a tertiary care hospital in Lahore, directly addressing the surveillance gap identified in the literature. Consistent with H1, Gram-negative organisms predominated (68.8%), aligning with regional findings from Rahim et al. (2022) and Siddiqui et al. (2023) and the broader South Asian pattern of Gram-negative dominance, frequent polymicrobial infection, and elevated resistance driven by late presentation and limited diagnostic capacity.

Staphylococcus aureus (25.8%) remained the leading single organism, recovered chiefly from wound swabs, consistent with its established capacity to colonize superficial wound layers (Javed et al., 2023; Raza et al., 2023). E. coli (23.7%) and P. aeruginosa (19.4%) followed closely, broadly mirroring the global ranking of Mendes et al. (2022), though the Gram-negative proportion observed here exceeded global pooled estimates, consistent with the more advanced

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regional resistance profile described in the literature. Because the culture protocol was restricted to aerobic media, any anaerobic or polymicrobial component of the isolated infections could not be captured, limiting direct comparison with studies reporting substantial anaerobic involvement (Akhi et al., 2015).

Universal ampicillin resistance confirms that this agent no longer has empirical value, consistent with widespread beta-lactamase production documented in Pakistani tertiary hospitals (Akram et al., 2022; Javed et al., 2023). The bimodal ciprofloxacin resistance pattern, confined to E. coli and P. aeruginosa, plausibly reflects gyrA/parC mutations and efflux-pump-mediated resistance selected by fluoroquinolone overuse (Gill et al., 2024), indicating that empirical fluoroquinolone use should be restricted pending culture confirmation. P. aeruginosa's simultaneous resistance to four antibiotic classes is consistent with its recognized AmpC beta-lactamase activity and efflux-pump expression (Bassetti et al., 2021) and echoes, though less severely than, the last-line resistance reported by Hadavand et al. (2023); imipenem retained full activity here, suggesting carbapenem-level resistance had not yet become established at this facility. Vancomycin's complete activity supports its continued empirical use in suspected severe staphylococcal DFI, though the absence of confirmatory MRSA testing limits full evaluation of H2. An unanticipated but statistically robust finding was the association between patient sex and Gram-stain classification, with female patients disproportionately more likely to harbour Gram-negative organisms. Plausible explanations include sex-related differences in host immune response, wound hygiene and care-seeking behaviour, or hormonal influences on microbial colonization (Eleftheriadou et al., 2022; Hobizal & Wukich, 2023). This diverges from the male-predominant case counts reported in several South Asian series (Abbas et al., 2023; Zubair et al., 2024), suggesting that sex-stratified microbiological analysis may reveal patterns not captured by prevalence-only studies; replication in larger, multi-centre cohorts is warranted.

This single-centre, cross-sectional study is limited by its aerobic-only culture protocol, which precluded detection of anaerobic and polymicrobial components; the absence of

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phenotypic or genotypic MRSA and ESBL confirmatory testing; a sample size constrained for organism-specific subgroup comparisons; and a three-month window that may not capture seasonal variation in pathogen prevalence or resistance.

Ampicillin and empirical ciprofloxacin should be discontinued pending culture confirmation, while imipenem and vancomycin should be reserved for severe or culture-confirmed cases under active antimicrobial stewardship. Future institutional studies should incorporate anaerobic culture and MRSA/ESBL confirmatory testing, extend to multiple centres, and further investigate the observed sex-based difference in pathogen distribution. Institution-specific antibiograms of this kind should inform local empirical treatment guidelines in place of extrapolated regional or international data.

Gram-negative organisms predominated over Gram-positive organisms among DFI patients at this Lahore tertiary care hospital, with S. aureus, E. coli, and P. aeruginosa emerging as the three most prevalent pathogens. Universal ampicillin resistance and a multidrug-resistant P. aeruginosa phenotype underscore a considerable resistance burden, while complete susceptibility to imipenem and vancomycin offers reliable therapeutic options for severe infection. A significant association between patient sex and Gram-stain classification suggests that pathogen distribution in DFI may not be uniform across subgroups, warranting further investigation. These locally generated bacteriological and susceptibility data can support more informed, evidence-based empirical treatment decisions for DFI in Lahore and comparable South Asian tertiary care settings.

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