

Microbiological and antimicrobial pattern of diabetic foot ulcers (DFUs) at a tertiary care center in North East, Punjab

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Abstract

Objective: To determine the pattern of antimicrobial sensitivity of common microorganisms isolated from diabetic foot infections.

Method: The cross-sectional study was conducted from January to August 2019 at the Diabetes and Footcare Clinic, Mayo Hospital, Lahore, Pakistan, and comprised deep-seated tissue samples from the ulcer bed that were tested for culture and sensitivity. Antimicrobial susceptibility was determined in accordance with Clinical and Laboratory Standards Institute guidelines. Data was analysed using SPSS 26.

Results: There were 389 bacterial isolates collected from 201 patients who had a mean age of 53.05 ± 10.70 years. Of the isolates, 238(61.11%) were gram-negative and 151(38.89%) were gram-positive organisms. Methicillin-resistant *Staphylococcus aureus* was found in 62(64.58%) isolates.

Conclusion: Early identification of diabetic foot infections can help in timely initiation of empirical treatment and prevention of unwanted amputations.

Keywords: Diabetic foot, Diabetic foot infections, Complications of diabetes, Antimicrobial sensitivity, Microorganisms.

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Introduction

The overall age-adjusted prevalence of glycaemic dysregulation in Pakistan, including diabetes and pre-diabetes, is 26.3% and 14.4% respectively. This prevalence is almost twice compared to that in the first National Diabetes Survey of Pakistan (NDSP; 1994-98).¹ Diabetes-related complications represent the major cause of premature deaths in the world^{2,3} with a death occurring every 6 seconds due to diabetes.^{2,5}

With lifetime incidence of diabetic foot ulcers (DFUs) up to 12-25%^{6,7} diabetes-related morbidity rates are staggeringly high; the five-year mortality rate after lower extremity amputation (LEA) is second only to lung cancer.⁸ More than half of all DFUs become infected, requiring hospitalisation, and 20% of lower extremity infections result in amputation.^{8,9} In countries like Pakistan, about 14-20% patients with diabetes who develop DFUs undergo amputation.¹⁰

Diabetic foot infections (DFIs) are usually poly-microbial.^{6,10} Most of the studies report mild to moderate DFIs as mono-

microbial, and severe DFIs as poly-microbial.¹¹⁻¹³ Almost all studies agree upon *staphylococcus (S.) aureus* as the most common organism isolated from infected DFUs^{7,9,10,14} along with β -haemolytic *streptococci*, including groups A, B, C and G.

Studies in this regard are designed keeping certain factors in view. These include the diversity in geographical distribution of organisms^{15,16} the prevalence of nosocomial infections¹⁵ clinical significance of regional data in developing guidelines regarding decisions of choice, route, duration and dosage of antibiotics; constantly evolving local antibiotic resistance patterns due to injudicious use;¹⁷ and the perpetual dynamic trend observed with gram-negative pathogens replacing gram-positive isolates as the commonest agents involved in DFIs.¹⁰

The current study was planned to determine the pattern of antimicrobial sensitivity of common microorganisms isolated from DFIs.

Materials and Methods

The observational, cross-sectional study was conducted at the Diabetes and Footcare Clinic (DFC), Mayo Hospital, Lahore, Pakistan, from January to August, 2019. After approval from the ethics review board of the King Edward Medical University, Lahore, the sample size was calculated using estimated prevalence of foot ulcers as 25%^{6,7} with a confidence interval of 95% and absolute precision of 6%. The sample was raised using non-probability consecutive sampling technique, and comprised diabetic patients of all

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ages and genders with DFUs. Patients with history of antibiotic use 90 days prior to presentation, as well as those with limb infections without history of diabetes were excluded. After taking informed consent from the subjects, data was collected using a predesigned proforma which is routinely used for patient registration to record demographic details, such as age, gender, DFU stage and type, outcome and details of follow-up.

DFU grades were determined based on the University of Texas Classification (UTC)¹⁸ with Grade 0 = epithelialised ulcer; Grade 1 = superficial ulcer; Grade 2 = ulcer penetrating to tendon or capsule; and Grade 3 = ulcer penetrating to bone or joint.

DFIs were graded using the International Working Group on the Diabetic Foot (IWGDF) classification¹⁸ with Grade 1 = uninfected; Grade 2 = mild infection having at least two symptoms from among local swelling/induration; erythema <0.5 cm² around ulcer; tenderness; warmth; and purulent discharge; with no systemic signs of infection; Grade 3 = moderate infection, invading deeper than skin/subcutaneous tissues, or >0.5 cm² erythema around ulcer, with no systemic signs or symptoms; and Grade 4 = severe infection, meaning any foot infection with systemic inflammatory response syndrome (SIRS).

Sample collection was carried out under universal aseptic measures, with samples collected after surgical debridement of slough and necrotic tissue, as well as thorough ulcer washing with normal saline. Deep-seated tissue samples, approximately 0.5 x 0.5cm were taken from the ulcer bed, collected in sterile containers with normal saline, and immediately sent for culture and sensitivity (C&S) testing to the microbiology laboratory. Antimicrobial susceptibility was determined in accordance with Clinical and Laboratory Standards Institute (CLSI) standards¹⁹ and reports were stored in the clinic's database.

Data was analysed using SPSS 26, and was expressed as mean±standard deviation (SD) for continuous variables, and as frequency with percentage for categorical variables.

Results

Of the 201 patients with a mean age of 53.05±10.70 years, 134(66.7%) were male. The most common site of DFUs was forefoot 129(64.2%), followed by hindfoot 49(24.4%) and midfoot 23(11.4%). DFU Grade 3 was the most prevalent 104(51.7%), followed by Grade 1 62(30.8%). Further, 179(89.1%) of DFUs were neuropathic, while 22(10.9%) were neuroischaemic.

As for DFIs, Grade 2 was the most prevalent 126(62.7%). Majority of the ulcers 122(60.7%) recovered, 12(6%) had an amputation and the fate of 67(33.3%) remained

Table-1: Frequency of Bacterial Pathogens isolated from patients with diabetic foot infections (DFIs) (n=389).

	n (%)
Name of the organism	
Gram Positive Organisms	151 (38.76%)
Staphylococcus aureus	97 (24.93%)
Streptococcus pyogenes	20 (5.14%)
Enterococcus spp.	28 (7.19%)
Gram Negative Organisms	238 (61.24%)
Proteus mirabilis	58 (14.91%)
Pseudomonas aeruginosa	47 (12.1%)
Escherichia coli	41 (10.53%)
Klebsiella pneumoniae	32 (8.22%)

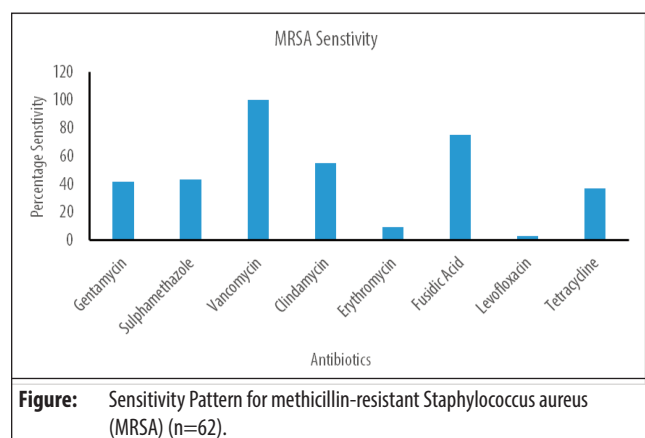
Table-2: Cumulative antimicrobial sensitivity pattern of gram-negative and gram-positive bacteria in diabetic foot infections (DFIs) (antibiogram).

Antibiotics	Proteus	E. coli	Klebsiella	Pseudomonas
Gram Negative Organisms				
Amoxicillin/Clavulanate	55.17	31.71	48.39	-
Ampicillin	12.07	7.692	0	-
Amikacin	74.14	95	93.55	76.19
Imipenem	96.67	97.44	93.33	50
Piperacillin/ Tazobactam	94.83	80	80.65	54.55
Oxacillin	-	-	-	69.57
Ceftriaxone	49.12	29.27	34.38	-
SMX/TMP	10.53	35.9	32.26	-
Cefixime	36	31.43	30.77	-
Ciprofloxacin	43.1	37.5	51.61	48.89
Meropenem	94.83	97.5	92.86	53.33
Colistin	-	-	100	100
Gram Positive Organisms				
Antibiotics	Staph	Strep	Enterococcus	
Amikacin	85.71	-	-	
Oxacillin	35.42	-	-	
SMX/TMP	57.73	-	-	
Vancomycin	100	100	100	
Clindamycin	61.05	60	-	
Fusidic Acid	79.35	-	-	
Levofloxacin	14.61	-	-	
Linezolid	100	-	66	

unknown. This invariably coincided with follow-up, with 132(65.7%) patients reporting regular follow-up till complete healing.

Of the 389 bacterial isolates, 8(4%) showed no growth. Monomicrobial infection was present in 57(28.4%) specimens, and 136(67.6%) were polymicrobial, and, among them, the frequency of 2 organisms present in the specimen was the greatest at 81(40.3%). Among the isolates, 238(61.11%) were gram-negative, while 151(38.89%) were gram-positive organisms. Also, 27 fungal isolates were present, with aspergillus (A.) flavus 18(66.7%) dominating over the others (Table 1).

Patterns of antibiotic sensitivity were determined for all the



isolated organisms based on microbiology reporting (Table 2). Pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) sensitivity was also noted (Figure).

Discussion

The present study showed a predominance of polymicrobial foot infections, which is in concordance with several earlier studies.^{6,9,10} One study showed a higher percentage of monomicrobial infections¹⁷ which can be attributed to its smaller sample size.¹⁷ Some studies have reported increased monomicrobial cultures when patients were treated with antibiotics before sampling.²⁰ In the current study, this bias was also eliminated as all patients with prior history of antibiotic therapy were excluded.

In the present study, 38.81% bacterial isolates were gram-positive. The most common among these was *S. aureus*, accounting for 24.93% of the isolates. Such a finding is common in literature.^{7,9,10} The present study found a high incidence of MRSA at 64.58%. Highest susceptibility of *S. aureus* was reported with vancomycin, fusidic acid and sulphamethoxazole/trimethoprim (SMX/TMP) at 100%, 79.35% and 61.05% respectively. Among MRSA isolates, the susceptibility pattern was observed with vancomycin, fusidic acid and clindamycin at 100%, 75% and 55% respectively. These results report a higher incidence compared to studies conducted in other countries in which MRSA incidence ranged from as low as 12.2%¹⁰ to as high as 49%²⁰. In the current study, the unusually high incidence of MRSA can be attributed to delayed referral, improper duration and injudicious use of antibiotics, and advanced stage of ulcer at presentation. The high prevalence of *S. aureus* in DFIs can be attributed to contiguous spread from overlying soft tissue.²¹

The current study found a high overall incidence of gram-negative infections, at 61.19%. Among these, proteus (14.91%) and pseudomonas (12.1%) accounted for the majority of isolates. The antimicrobial pattern revealed

Table-3: Recommendations for empirical antibiotic use according to diabetic foot infection (DFI) grade.

Grade of DFIs	Recommendations
Grade I	No antibiotics. Effective glycaemic control, proper off-loading of DFU, adequate debridement, ulcer care and regular follow up are effective strategies, among others, for management.
Grade II	Narrow spectrum, oral and single antibiotic therapy is recommended. β -lactam antibiotics such as anti staphylococcal beta lactams, clindamycin, fusidic acid, and trimethoprim/sulfamethoxazole (TMP/SMX) are the drugs of choice.
Grade III (Limb threatening infections)	Require double antibiotic therapy, preferably by the intravenous route. 3rd and 4th generation cephalosporins along with one oral agent (fusidic acid/clindamycin/newer fluoroquinolones/linezolid) are considered sufficient if no complicating features are present. In case of contraindications, vancomycin/carbapenems can be added, while clindamycin and anti-pseudomonal penicillins are recommended if there is clinical evidence of gram negative or anaerobe infection.
Grade IV (Life threatening infections)	Require similar treatment as grade III DFIs. In cases of suspected MRSA, antibiotics such as vancomycin and linezolid should be considered in addition to or as substitution for other agents used previously.

overall good sensitivity, especially against the two mentioned above, for carbapenems, piperacillin/tazobactam and colistin. There was a dynamic trend of increasing resistance for penicillin, cephalosporins, and fluoroquinolones (Table 2) which is consistent with other studies.^{21,22}

Fungal infections are often overlooked in the context of DFIs. In the current study, 27 fungal isolates were obtained; 9 *A. flavus*, 5 *A. niger*, and 4 candida (*C. tropicalis*), and some other less significant organisms. The reported incidence and type of fungal infection has varied.²³ Usually fungal infections are associated with high grade of ulcer and infection²⁴ and careful attention needs to be paid regarding their management while treating DFIs.

The trend of gram-negative predominance in the current study is reflective of what has already been reported by major studies carried out in temperate climates.²⁵ A systematic review of 39 studies carried out across the Middle East and North Africa (MENA) region failed to find the reason for discrepancy in geographical trends, thereby highlighting the need for further large-scale microbiological and epidemiological studies on DFIs.²⁶

Based on the findings and in line with IWGDF guidelines,¹⁸ it is recommended that a regimen of empirical antibiotic use for various grades of DFIs must be followed (Table 3). Empirical treatment should commence after diagnosis till definite antimicrobial sensitivity report of tissue isolate is available, following which antibiotics should be changed

or adjusted. Definite therapy guided by C&S report should only be initiated if clinical response to empirical regimen is observed as slow, inadequate and incomplete. Moreover, it should be done within as short a time period as possible.

Conclusion

DFIs are a leading cause of morbidity and mortality in diabetic patients. These infections are mostly polymicrobial, with gram-negative predominance in temperate climates. Early, narrow spectrum, oral and single empirical therapy can help prevent progression of DFIs, and should always be supplemented or substituted by definite antibiotic therapy based on tissue C&S. In the backdrop of rising multiple drug-resistant organisms (MDROs), health policy-makers, institutions/facilities and healthcare professionals (HCPs) should collaborate to design and execute effective antibiotic stewardship strategies to combat this problem at all levels of the healthcare system.

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