ORIGINAL ARTICLE

Bacterial Diversity and Their Antimicrobial Susceptibility Patterns in Diabetic Foot Infections, a Tertiary Care Hospital Study

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ABSTRACT

Objective: To determine the frequency of bacterial pathogens and their antimicrobial profile in diabetic foot infections.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was conducted at the Department of Microbiology, Combined Military Hospital (CMH) Lahore, Pakistan from January 2022 to December 2022.

Methods: Three hundred and forty-one samples with a history of diabetic foot infections were processed. Antibiotic susceptibility testing was done using the Kirby-Bauer Disk Diffusion technique for the commonly used antibiotics. Clinical and Laboratory Standard Institute Guidelines (CLSI) 2022 were used to interpret the result of susceptibility testing.

Results: Three hundred and forty-one clinical samples with bacterial isolates causing diabetic foot infections were processed. The most common organism isolated was *Pseudomonas aeruginosa* (25.5%). Gram-positive isolates where found most susceptible to vancomycin and linezolid while gram negative was most sensitive to meropenem.

Conclusion: In the current study gram-negative bacteria were found to be the main pathogens. Effective antibiotic therapy based on microbiological profiles will definitely improve clinical outcomes.

Keywords: *Antibiotics, Diabetes Mellitus, Gram Negative Bacteria, Methicillin-Resistant Staphylococcus Aureus (MRSA).*

How to cite this: *Hussain C, Farooque MA, Ain Q, Khan SH, Younas A, Huma Z. Bacterial Diversity and Their Antimicrobial Susceptibility Patterns in Diabetic Foot Infections, a Tertiary Care Hospital Study. Life and Science. 2024; 5(4):* 433-438. *doi: http://doi.org/10.37185/LnS.1.1.633*

Introduction

Diabetic foot infection (DFI) is defined as soft tissue or bone infection below the knee often incorporated with peripheral arterial disease or neuropathy in diabetic patients.[1] It is rated as the second most common complication of diabetes mellitus (DM) after cardiovascular complications.[2] According to estimates, 15% of diabetics will experience a DFI at some point in their lives.[3] In Pakistan 14-20 % of amputations of lower limbs were found due to DFI.[4]

The pathophysiologys of the disease is complex. Several factors like glycemic control, general hygiene, wound care, and peripheral vascular status aid in the management of the patient. The disease may range from a minor ulcer, cellulitis, and carbuncles to severe necrotizing fasciitis and osteomyelitis.[5] Both mono-microbial and poly-microbial infections contribute to the wound microbiology of DFI's. Etiological agents most commonly associated are Staphylococcus aureus, beta-hemolytic streptococcus, aerobic Gram-negative rods, and Pseudomonas aeuroginosa.[6] In addition the emergence of antimicrobial resistance also increases the treatment challenges. Understanding the microbiology of DFI thus plays a key role in tackling these cases.

Prompt antimicrobial therapy should be initiated to

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Received: Feb 19, 2024; 1st Revision: Jun 01, 2024
2nd Revision: Sep 15, 2024; Accepted: Sep 25, 2024

improve limb-saving probabilities as many DFIs are true emergencies.[7] The aim of this study was to further strengthen understanding of the bacteriology of diabetic foot ulcers alongside the assessment of the *in vitro* antimicrobial susceptibility of the offending pathogens.

Methods

The study was conducted at the Department of Microbiology, Combined Military Hospital (CMH) Lahore, Pakistan from January 2022 to December 2022 after obtaining approval from the Ethical Committee of the hospital held on dated: 30th September 2021 vide letter no: ERC # 309/2021. The sampling technique used was non-probability convenience sampling.

All three hundred and forty-one clinical specimens including pus, tissue, and pus swabs sent to the laboratory from indoor and outdoor patients with diabetic foot infections were included in the study. Duplicate samples and samples of patients without diabetes were excluded from the study. Data was collected and analyzed on a daily basis. Specimens of pus and pus swabs were collected after thoroughly washing the area with normal saline while tissue specimens were collected from deep portions of the wound margins.

The clinical specimens having pure single morphological type were inoculated onto an appropriate culture medium in accordance with their particular requirements.[8] Using common microbiological procedures such as Gram staining, catalase, coagulase, and oxidase tests, as well as the morphological appearance of the colonies, important pathogens linked to DFI were identified. Using API 10S, API 20E, and API 20NE (BioMérieux, France), isolates were identified up to the genus and species level. Using Muller Hinton agar (Oxoid, UK) and the Kirby-Bauer Disk Diffusion technique, the susceptibility of bacterial isolates to various antibiotics was ascertained in accordance with the guidelines suggested by the Clinical and Laboratory Standards Institute (CLSI) 2022.[9] Data was analyzed using SPSS 23 and was expressed as frequency with percentages for categorical variables while mean ± standard deviation (SD) for continuous variables.

Results

A total of 341 patients were included in the study having a mean age of 58.85±10.17 years with a minimum age of 34 and a maximum of 89 years, 263 (77.1%) were males and 78 (22.9%) were females. There were 118 (34.6%) samples received from outdoors and 223 (65.4%) from indoor patients.

Out of the specimens, 135 (39.6%) pus, 133 (39%) tissue, and 73 (21.4%) pus swabs were processed which yielded growth of 243 (71.3%) gram-negative and 98 (28.7%) gram-positive organisms. The most common organisms are isolated were *Pseudomonas aeruginosa* (25.5%) *Klebsiella pneumoniae* (17%), *Methicillin Resistant Staphylococcus aureus (MRSA)* (14.7%) followed by *Escherichia coli* (12.9%) as shown in table-1.

MRSA and Enterococcus sp. showed 100% sensitivity

Table-1: Distribution Of Pathogens Isolated

Name Of Organism	Frequency n=341(%)
Gram Positives Organism	**n=98**
Staphylococcus aureus	37 (10.9%)
MRSA	50 (14.7%)
Enterococcus feacium	6 (1.8%)
Enterococcus faecalis	8 (2.3)
Gram Negative Organisms	
Pseudomonas aeruginosa	87 (25.5%)
Klebsiella pneumoniae	58 (17%)
Acinetobacter baumanii	18 (5.3%)
Escherichia coli	44 (12.9)
Proteus mirabilis	27 (7.9%)
Serratia marsecens	1 (0.3%)
Citrobacter sp.	5 (1.5%)

Table-2: Resistance Pattern of Gram Positive Isolates

Antibiotics	Organism Name			
	S.aureus n=37 (%)	MRSA n=50 (%)	E.feacium n=6 (%)	Faecalis n=8 (%)
Ampicillin	35 (94.6%)	50 (100%)	6 (100%)	1 (12.5%)
Augmentin	0 (0%)	50	6 (100%)	1 (12.5%)
Linezolid	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vancomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ciprofloxacin	22 (59.5%)	35 (70%)	3 (50%)	5 (62.5%)
Doxycycline	10 (27%)	18 (36%)	nt*	nt*
Tetracycline	16 (43.2%)	37 (74%)	nt*	nt*
Levofloxacin	21 (56.8%)	38 (76%)	3 (50%)	5 (62.5%)
Erythromycin	16 (43.2%)	40 (80%)	nt*	nt*
Clindamycin	10 (27%)	33 (66%)	nt*	nt*
Cotrimazole	13 (35.1%)	39 (78%)	nt*	nt*
Cefoxitin	0 (0%)	50 (100%)	nt*	nt*

nt=Not Tested*

Table-3: Resistant Pattern Of Gram Negative Isolates

Antibiotics	Organism Name				
	P.aeruginosa n=87(%)	Kleb. pneumoniae n=58(%)	Acinetobacter spp. n=18(%)	E.coli n=44(%)	P.mirabilis n=27(%)
Ampicillin	nt*	nt*	nt*	44 (100%)	27 (100%)
Amc**	nt*	49 (84.5%)	nt*	42 (95.5%)	23 (85.2%)
Piperacillin-Tazobactam	42 (48.3%)	35 (60.3%)	14 (77.8%)	27 (61.4%)	4 (14.8%)
Ciprofloxacin	60 (69%)	50 (86.2%)	16 (88.9%)	41 (77.8%)	15 (55.6%)
Doxycycline	nt*	46 (79.3%)	3 (16.7%)	28 (63.6%)	2 (7.4%)
Tetracycline	nt*	46 (79.3%)	15 (83.3%)	30 (68.2%)	2 (7.4%)
Levofloxacin	59 (67.8%)	49 (84.5%)	16 (88.9%)	40 (90.9%)	16 (59.3%)
Cotrimoxazole	nt*	45 (77.6)	1 (5.6%)	27 (61.4%)	13 (48.1%)
Meropenem	33 (37.9%)	30 (51.7%)	4 (22.2%)	14 (31.8%)	6 (22.2%)
Ceftazidime	50 (57.5%)	nt*	nt*	nt*	nt*
Cefipime	56 (48.4%)	nt*	nt*	nt*	nt*
Ceftriaxone	nt*	42 (72.4%)	nt*	36 (81.8%)	9 (33.3%)

nt=not tested amc=amoxicillin-clavulanate*

against vancomycin and linezolid. *MRSA* showed 70% resistance against ciprofloxacin, 80% against erythromycin and 74% against tetracycline while *Enterococcus feacium* was found 100 % resistant to ampicillin, 50% resistant to ciprofloxacin. The pattern of resistance of gram-positive organisms is shown in table-2. In gram-negative isolates 16 (88.9 %) *Acinetobacter sp*. 50 (86.2%) *Klebsiella pneumoniae,* 60 (69*%) Pseudomonas aeruginosa*, 41 (77.8%) *Escherichia coli* were resistant to ciprofloxacin. Table-3 depicts the resistant pattern of gram-negative isolates.

Discussion

DFI is a serious and common complication among diabetic patients. The microbiological aspect thus plays a key role in influencing treatment strategies. This article give an insight of the various etiological agents involved in DFI and their antimicrobial susceptibility pattern in our tertiary care hospital setup thus aiding the clinicians in choosing the correct empirical therapy for both indoor and outdoor patient management.

Our study shows male predominance with a percentage of 77.1%. This finding is similar to

another study conducted in Belgium showing a prevalence of 76% in males.[10] Another study observed a similar findings with a percentage of 60.70% males carried out in a tertiary care setting of Peshawar.[11] The exact reason for gender difference is not known but male gender can be considered as one of the risk factor in development of diabetic foot ulcers owing to increase in physical activity and lack of self-care.[12]

In our current study gram-negative pathogens were in majority, with *Pseudomonas aeruginosa* the most prevalent pathogen followed by *Klebsiella pneumoniae*. Several local studies conducted in our country observed a similar trend.[13,14] A systemic review of 73 studies of 12 Asian countries showed gram-negative bacteria as the dominant pathogens.[15] However, a study conducted in diabetic foot care Centre of Germany observed gram-positive species with *Staphylococcus aureus* as the dominant pathogen.[16] Other studies conducted in western countries also showed gram positive bacteria as the prevalent microbiological agents.[17] This difference may be associated with more recurrent infections and the inappropriate use of antibiotics in developing countries. Furthermore, the geographical difference in microbiological profile of DFI highlights the utter need to perform additional local studies on our patient population.

MRSA was found to be the predominant pathogen among gram-positive isolates showing 100% susceptibility to vancomycin and linezolid. This susceptibility pattern was also found in a study done in a tertiary care hospital in Iran where MRSA was 100% sensitive to vancomycin.[18] This finding emphasized the limited use of vancomycin in our hospital setting to prevent future resistance against this drug. All gram-positive isolates showed poor activity against ampicillin, clindamycin, erythromycin, and ciprofloxacin. Similar resistance was noticed by Sannathimmappa MB et al. in their study.[19] This explains how extensive use of these antimicrobials in clinical settings leads to the development of resistance to these drugs thus, limiting only a few antimicrobials for empirical therapy.

In the current study gram negative organisms were resistant to multiple antibiotics. Resistant to ceftriaxone, ciprofloxacin, levofloxacin, was dominant among *Klebsiella pneumoniae*, *Escherichia coli and Proteus mirabilis*. This trend of resistance is consistent with several other studies conducted in tertiary care hospitals of our country.[14,20] This is in contrast to a meta-analysis carried out in Africa which showed good activity of ciprofloxacin, levofloxacin, and gentamicin for gram-negative isolates.[21]

In the present study, Meropenem and piperacillin-tazobactam were found to be most effective against all gram-negative organism including *Pseudomonas* and *Acinetobacter* sp. Another study conducted at Bahawalpur found a similar susceptibility profile.[22] Based on this finding these agents can be used for empirical therapy of DFI's underscoring the importance of this study.

Our study thus adds valuable information regarding the microbiological aspect of DFI and their antimicrobial profile. However, it has certain limitations. Firstly, anaerobic cultures are not described in our study. Secondly, the study focuses on the patient population only in a single medical setting. Despite these limitations, it does offer important insights into the management of DFI.

Conclusion

The present study highlighted the local pathogen distribution. The antibiogram observed will be fruitful for physicians for better patient management. Presence of resistance against multiple antibiotics in gram negative organisms demand effective infection control strategies and antimicrobial stewardship policies in our settings.

Acknowledgment: None.

Conflict of Interest: The authors declare no conflict of interest.

Grand Support and Financial Disclosure: None.

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Authors Contribution

CH: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading
MAF: Manuscript writing and proofreading
QA: Data collection, data analysis, results and interpretation
SHK: Idea conception, study designing
AY: Data collection
ZH: Data collection