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Title:	**Microbial Profile and Antimicrobial Resistance among the Most Common Grades of Diabetic Foot Ulcers at Mayo Hospital, Lahore, Pakistan**
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Microbial Profile and Antimicrobial Resistance among the Most Common Grades of Diabetic Foot Ulcers (DFUs) at Mayo Hospital, Lahore, Pakistan

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ABSTRACT

The current study was aimed to determine the pattern of antimicrobial resistivity of common microorganisms isolated from the most common grades of diabetic foot ulcers (DFUs) presenting at Mayo Hospital, Lahore, Pakistan. A total of 293 patients were included in the study conducted from January 2019 to December 2020. Deep-seated tissue samples of dimensions 0.5×0.5 cm were taken from the ulcer after surgical debridement, washed with saline, and immediately sent to the laboratory for culture and sensitivity (C&S). Data were analyzed by using SPSS 26. Grade 2 and Grade 3 DFUs (26.62% and 49.14%, respectively) were the most abundant of all DFUs, while *Staphylococcus aureus* was the most abundant bacteria. The polymicrobial growth pattern was found to be dominant in the current study. The incidence of MRSA was 68.4%(26/38) and 66.2%(49/74) in grades 2 and 3, respectively. The overall incidence of gram-negative microbes increased with an increase in the grade of ulcer. Hence, it can be concluded that early identification and grading of the disease can assist in the early initiation of empirical treatment and may reduce the unwanted morbidity of the disease. Furthermore, ultimate treatment should always be based on culture and sensitivity reported to minimize the disease's socioeconomic burden and morbidity.

Keywords: antibiotics resistance, diabetic foot, diabetic foot ulcers (DFUs), diabetic foot infections (DFIs), microorganisms

1. INTRODUCTION

Diabetes is a metabolic disorder characterized by an escalating prevalence and has been predicted to become a pandemic by 2030, doubling the number of patients since the year 2000 AD [1]. Complications related to diabetes have been a significant challenge for healthcare professionals (HCPs) and remain the cause of mortality for the patients [2]. One of the complications of diabetes, affecting the quality of life and resulting in increased morbidity and mortality, is diabetic foot ulcer (DFU) with an incidence of 12-15% [3, 4].

Infected DFUs usually require hospitalization and 20% of them result in leg amputation in Pakistan [5]. Diabetic foot infections (DFIs) usually contain more than one microbe; however, they can be monomicrobial, especially in mild to moderate cases [3, 6, 7]. The literature depicts *Staphylococcus aureus* as being the most common microorganism found in

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DFIs, along with Streptococci of groups A and B [4, 5, 8, 9].

DFIs are complicated infections caused by an array of pathogens that vary geographically. This situation is further complicated by nosocomial infections [10, 11]. The injudicious use of antibiotics has increased antibiotic resistance, so choosing the right antimicrobials is an uphill task [12, 13].

According to UTC, DFUs are classified into four classes (0,1,2,3) based on tissue involvement. They are further subdivided into four groups (A, B, C, D) based on the presence or absence of ischemia or infection or both [14]. Grade 2 and Grade 3 DFUs are limb and life-threatening respectively and almost always infective [15]. The distribution and susceptibility patterns of microbes are needed to set local guidelines to manage DFUs. There is no well-established study that compares microbial distribution and sensitivity pattern among the most common grades of DFUs, locally. Therefore, this study aims to explore the distribution of microorganisms and the difference in their sensitivity patterns in limb and life-threatening grades of DFUs.

2. MATERIALS AND METHODS

This study was conducted at Diabetes and Footcare Clinic (DFC), Mayo hospital, Lahore, Pakistan over a period of 2 years (from January 2019 to December 2020). A total of 293 patients were included in this study. A pre-established proforma, routinely used for keeping patients' registration, was also used to record demographic details, the grade of ulcer, the grade of infection, the type of ulcer, outcomes, follow-up details, and culture and sensitivity profile. The study was approved by the Institutional Review Board of King Edward Medical University, Lahore.

The sample size of 293 was calculated using an estimated prevalence of DFUs as 25%, with a confidence interval of 95% and with 6% absolute precision [16]. Non-probability consecutive sampling method was used as the sampling technique and diabetic patients of all ages and gender were included in the study. However, patients with a prior history of antibiotic use within previous 90 days and non-diabetic patients with ulcers were excluded. Informed consent for participation was taken from all the patients. DFIs were categorized into moderate and severe infections, according to the IWGDF/IDSA guidelines [14].

The grading for DFUs by the University of Texas Classification (UTC) [14] is as follows:

Grade 0: Completely epithelized ulcer

Grade 1: Superficial ulcer not involving the tendon, bone, or capsule

Grade 2: Ulcer reaching tendon and capsule

Grade 3: Ulcer penetrating the bone or joint

The samples were collected using all the aseptic measures, with samples collected after surgical debridement of dead and necrotic tissues and then washing the ulcer with normal saline. Deep-seated tissue samples of about 0.5×0.5 cm were taken from the ulcer bed, collected in a sterile container with normal saline, and immediately sent to the microbiology laboratory for culture and sensitivity (C&S). The antimicrobial susceptibility profile was determined according to Clinical & Laboratory Standards Institute (CLSI) and culture and sensitivity reports were stored in the patient database [17].

Statistical analysis was performed using SPSS 26. Data were presented as Mean ± SD for continuous variables and frequency with percentage for categorical variables.

3. RESULTS

A total of 293 patients with the mean age of 52.85 ± 10.63 participated in the study. Of the total, 197 (67.2%) were male patients and 96 (32.8%) were female patients. Furthermore, the patients were divided into grades according to UTC. Using this criterion, 71 (24.23%) patients were placed in grades 0 and 1, 78 (26.62%) were placed in Grade 2, and 144 (49.14%) were placed in Grade 3. *Staphylococcus aureus* is the most common microorganism, although gram-negative flora remain the most common group of pathogens causing DFIs. There was observed nominal growth of anaerobes. Moreover, the results unveiled 34 fungal isolates as well. Figure 1 and Table 1 depict the frequency and percentage of pathogens present in Grade 2 and Grade 3 DFUs.

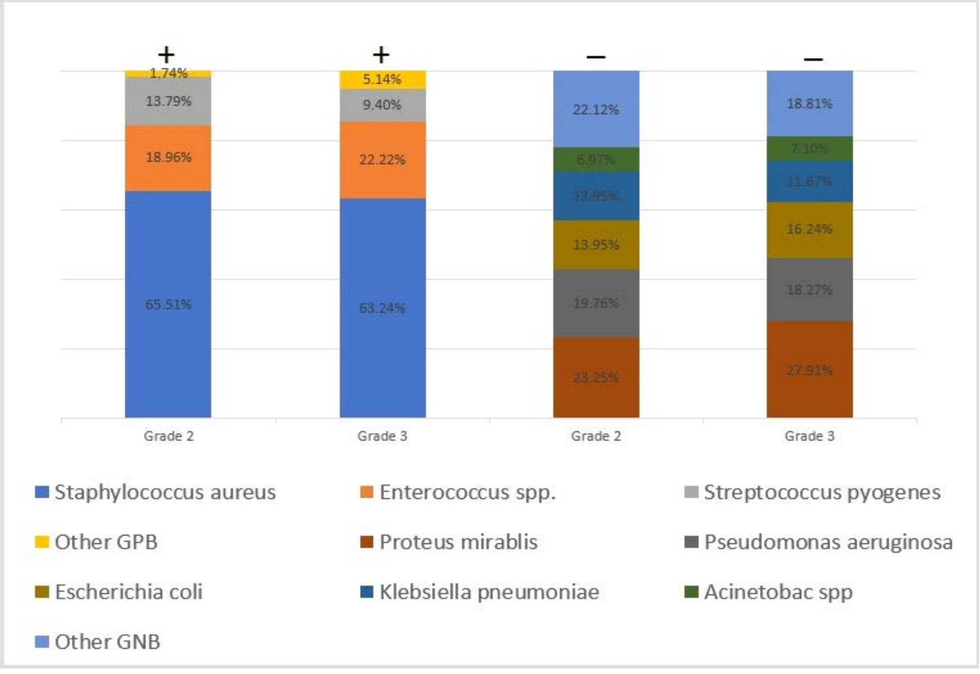

Figure 1. Diagram Showing A Comparison of Microbial Prevalence in 2nd And 3rd Grade of DFU

Table 1. Frequency of Bacterial Pathogens in 2nd and 3rd Grade of DFU (n=222)

Grade 2		Grade 3	
Name of the organism	Frequency of the organism *(n=144)*	Name of the organism	Frequency of the organism *(n=314)*
Gram-Positive organisms	58 (40.27%)	Gram-Positive organisms	117 (37.26%)

Grade 2		Grade 3	
Name of the organism	Frequency of the organism *(n=144)*	Name of the organism	Frequency of the organism *(n=314)*
Staphylococcus aureus	38 (26.38%)	*Staphylococcus aureus*	74 (23.56%)
Enterococcus spp.	11 (7.6%)	*Enterococcus spp.*	26 (8.2%)
Streptococcus pyopgenes	8 (5.5%)	*Streptococcus pyopgenes*	11 (3.5%)
Gram-Negative organisms	86 (59.72%)	Gram-Negative organisms	197 (62.73%)
Proteus mirablis	20 (13.88%)	*Proteus mirablis*	55 (17.51%)
Pseudomonas aeruginosa	17 (11.80%)	*Pseudomonas aeruginosa*	36 (11.46%)
Klebsiella pneumoniae	12 (8.3%)	*Klebsiella pneumoniae*	32 (10.19%)
Escherichia coli	12 (8.3%)	*Escherichia coli*	23 (7.3%)

For UTC Grade 2, out of 78 patients, 39 (50%) had an ulcer at the forefoot, followed by 25 (32.05%) with an ulcer at hindfoot, and 14 (17.94%) with an ulcer at midfoot. Additionally, the patients were divided into those with moderate and severe infection, according to the IWGDF/IDSA guidelines. Among the patients enrolled in the current study, most cases were presented in the 2nd grade (53.84%) and 3rd grade (42.30%), respectively. Many ulcers (75.64%) were neuropathic and three times more common than neuro-ischemic ulcers (24.35%). The current study exhibited a total of 144 isolates, while only 4(5.1%) samples displayed no growth at all. The polymicrobial pattern 47(60.21%) was more prevalent than the monomicrobial pattern 27(34.61%). Among the microbes, 86(59.72%) turned out to be gram-negative, while the rest 58(40.27%) were gram-positive. *Staphylococcus aureus* was the most abundant (38/78) pathogen, although MRSA was also prevalent 68.4% (26/38). Twelve fungal isolates were also reported, with Aspergillus species was the most common of all fungi (50%).

Regarding patients in Grade 3 (144/293), this study laid out the same qualitative spectrum and pattern in relation to the location and type of ulcer, types of microbes, severity of infection, trend of mono/polymicrobial infection/s, frequency of MRSA, and antimicrobial sensitivity profile, as noticed in patients with UTC Grade 2. Quantitatively, 91(63.73%) had an ulcer on their forefoot, 38(26.38%) had an ulcer at their hindfoot, and 15(10.41%) had an ulcer at their midfoot. Furthermore, 63(43.75%) patients were presented with DFI Grade 3. Neuropathic ulcers were observed in 117(81.25%) patients, four times more than neuro-ischemic ulcers 27(18.75%). This group unveiled a total of 314 isolates, notwithstanding 2(1.3%) showed no growth at all. The isolates displayed a polymicrobial pattern in 111(77.08%) tissues against a monomicrobial pattern in 31(21.52%) tissues only. The gram-negative group was the most prevalent group 197(62.73%), whereas the rest of the isolates were gram-positive 117(37.26%). *Staphylococcus aureus* was the most common microbe [23% (74/314)], while MRSA accounted for [66.2% (49/74)]. Eleven fungal isolates were also reported. Aspergillus species was the most common of all fungi (57.89%). The data is presented in Table 2 for DFU 2 and DFU 3 (Table 2).

Table 2. Cumulative Antimicrobial Resistivity Pattern of Gram-Negative and Positive Microbes in 2nd and 3rd Grade of DFU (Antibiograms)

Gram-Negative Organisms								
Antibiotics	*Proteus*		*E. coli*		*Klebsiella*		*Pseudomonas*	
	2nd	3rd	2nd	3rd	2nd	3rd	2nd	3rd
Amoxicillin/Clavulanate	60	49.1	58.3	68.8	50	47.6	-	-
Ampicillin	95	89.1	100	90.3	91.7	100	-	-
Amikacin	10	32.7	8.3	3.2	16.7	9.1	12.5	29.4
Imipenem	26.2	40.3	8.3	3.1	45	10.5	38.5	48.6
Piperacillin/Tazobactam	29.8	7.3	16.7	18.8	18.1	17.4	40	42.4
Ceftriaxone	45	66.7	66.7	62.5	34.38	77.3	-	-
SMX/TMP	85	94.3	58.3	61.3	32.26	82.4	-	-
Cefixime	61.1	75.5	63.6	65.5	30.77	54.5	-	-
Ciprofloxacin	45	67.3	75	59.4	51.61	51.61	47.1	52.8
Meropenem	0	5.5	0	0	0	13.6	35.2	47.2
Colistin	-	-	-	-	0	0	0	0

Gram-Positive organisms						
Antibiotics	*Staph. Aureus*		*Streptococcus*		*Enterococcus*	
	2nd	3rd	2nd	3rd	2nd	3rd
Amikacin	50	68	-	-	10	0
Oxacillin	68.4	66.2	-	-	-	-
SMX/TMP	45.9	31	-	-	-	-
Vancomycin	0	0	12.5	0	0	0
Clindamycin	39.2	43.1	50	27.3	-	-
Fusidic Acid	23.5	28.6	-	-	-	-
Levofloxacin	80	87.1	75.7	0	-	-
Linezolid	0	0	-	-	-	-

Proteus mirabilis (13.88% to 17.51%) and *Escherichia Coli* (8.3% to 10.19%) revealed a rising trend with the advancement of foot ulcers; however, the rest of gram-negative pathogens showed an almost static pattern. Furthermore, gram-positive microbes showed a reciprocal trend with the advancement of foot ulcers.

4. DISCUSSION

This study aimed to determine the types, frequency, and antimicrobial susceptibility patterns found in the most prevalent grades of DFUs. It was meant to suggest an empirical regimen for the prevalent grades. The study was held in a tertiary care center where a large number of patients with Grade 2 and Grade 3 ulcers were present for management. The study had a 2:1 male to female ratio according to another study [18]. It may be due to gender-related differences in lifestyle and socioeconomic responsibilities that make the male population more vulnerable to higher sustained foot pressures. Moreover, this difference can also be due to a poor foot hygiene trend among the male population. The elderly population was found to be more prone to DFUs. This finding is also supported by McGrath et al. [19].

The current study showed the predominance of polymicrobial foot infections over monomicrobial infections in both grades, as reported previously in the literature [3, 7]. However, this pattern was opposite to the trend reported in the survey conducted in Southern China from 2009 to 2014 [20]. There is a study available in the literature that claimed the predominance of monomicrobial growth over polymicrobial flora, when patients were given antibiotics before the collection of tissues for culture and sensitivity [21]. This is against the most commonly reported trend of polymicrobial infections being dominant. This bias was eliminated in the current study by excluding those patients who had already taken antibiotics before sampling. It is most likely due to the inappropriate use, and sometimes abuse, of antibiotics due to poor regulatory policies. However, other studies also corroborate this finding [3, 7].

The current study found that *Staphylococcus aureus* is the most common culprit for DFIs in both grades, separately and cumulatively, and this observation is also corroborated by other studies [4, 5]. The higher the grade of the ulcer, the lower was the incidence of *Staphylococcus aureus*. It could be due to multiple reasons. Firstly, gram-positive organisms tend to flourish in dry milieu, whereas gram-negative pathogens thrive in deep-seated aqueous environments. Secondly, gram-negative pathogens pose greater resistance as compared to gram-positive pathogens. This resistance provides gram-negative microbes ample time to penetrate deeper tissues. Thirdly, gram-negative organisms are not found alone mostly; instead, they are found in group in the form of polymicrobial infections that give them adequate time and resistance to flourish in deeper tissues [8, 21]. On the contrary, *Enterococci* showed an opposite pattern and their prevalence increased with the advancement in the ulcer's grade, an observation supported by literature [22]. Moreover, the results suggested that most gram-positive bacteria, including *Staphylococcus aureus*, showed the least resistance to vancomycin, fusidic acid, clindamycin, and sulphamethoxazole/trimethoprim (SMX/TMP). For *Enterococci*, the quantitative aspect of pathogens followed the same qualitative trend regarding antimicrobial sensitivity, though it differed from *Staphylococcus aureus*. The highest resistance was observed with fluoroquinolones, which differed from a study that revealed the highest susceptibility for gram-positive bacteria against quinolones [23]. In general, the greater was the ulcer's grade, the higher was the antimicrobial resistance profile for a given pathogen, according to the results shared by Xie X et al. [24].

The current study also revealed that MRSA accounted for more than two-thirds (67.3%) of the total isolated floras of *Staphylococcus aureus*. This percentage was one of the highest reported percentages in the literature review carried out by Jouhar et al. [25]. Moreover, the grade of DFU had no influence whatsoever over the prevalence of MRSA. The resistivity profile for antimicrobials against MRSA from Grade 2 to Grade 3 respectively is as follows: vancomycin (grade 2=0%, grade 3=0), fusidic acid (30.76%, 32.65%), SMX/TMP (61.53%,46.93%), clindamycin (50%, 48.97%), tetracycline (57.69%, 67.34%), and levofloxacin (84.61%, 91.83%). In this study, the extraordinarily high incidence of MRSA can be attributed to late referral, inappropriate duration, and inadvisable use of antibiotics, as well as a high grade of ulcer at presentation. Figure 2 gives the resistance percentage of MRSA in Grade 2 and Grade 3.

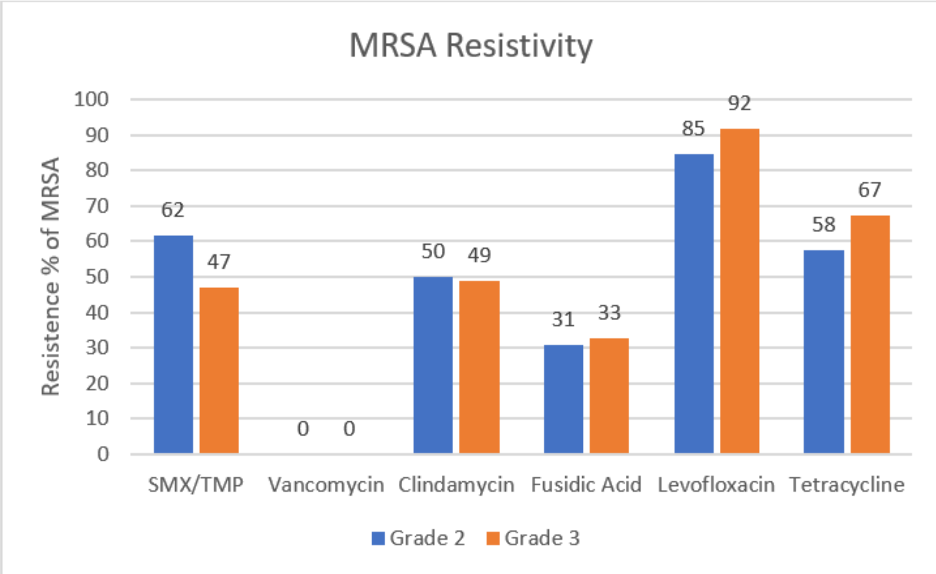

Figure 2. Resistance Percentage of MRSA in 2nd and 3rd grade of DFU

Regarding gram-negative organisms, the most common isolated pathogen was *Proteus mirabilis*, followed *by Pseudomonas aeruginosa*, and Klebsiella. This result was contrary to a previous study in which *Escherichia coli* was claimed to be the most common microbe among gram-negative isolates [7]. Unlike *Staphylococcus aureus*, the prevalence of *Proteus mirabilis* increased with DFU grade advancement, whereas other gram-negative bacteria followed nearly a static course, irrespective of DFU grade. This observation differed from the results seen in a study by Wu et al. [22]. There was a downhill trend taken by *Proteus mirabilis* and an uphill course followed by *Pseudomonas aeruginosa* in relation to the advancement of foot ulcer. Colistin, piperacillin/tazobactam, and carbapenems showed the lowest resistivity in this study; notwithstanding, ampicillin displayed the highest resistance (>90%). Wu M et al. exhibited the same findings [22].

Less than 2% of the total submitted tissue samples showed fungal growth. In this regard, there was found a non-reciprocal fashion of fungal growth with respect to the grade of DFUs. This study identified aspergillus species as the most common isolated species. However, literature depicts a variable trend regarding the types and incidence of fungi among patients with DFIs [26].

4.1. Conclusion

Diabetic foot infections (DFIs) are a leading cause of morbidity and mortality in diabetic patients. These infections are mostly polymicrobial, with the predominance of gram-negative pathogens in temperate climates. Earlier, a systemic combination of antibiotics, modified according to the C/S report, was found to not only help effectively treat limb-threatening localized infections but also to assist in the successful treatment of life-threatening systemic infections. In the backdrop of multiple challenges such as cost-prohibition, rising antimicrobial resistance, lack of local guidelines set with the help of regional data for treatment, and the poor practice to treat DFIs without C/S guided protocols, health policymakers, institutions/facilities, and healthcare professionals should collaborate to design and execute effective antibiotic stewardship strategies to combat these problems, from primary and secondary to the tertiary level.

4.2. Recommendations

Based on Level V evidence derived from the expert experience at our center and clinical guidelines provided by IWGDF, the following regimens of empirical antibiotic use for various grades of DFIs are recommended (Figure 3) [27]. Furthermore, empirical treatment should commence after diagnosis until a definite antimicrobial sensitivity report of tissue isolate is available, following which the dose of antibiotics should be adjusted. The recommendations are color-coded to account for the severity of DFIs and the urgency with which the treatment must be initiated. Definite therapy guided by the C/S report should only be initiated if the clinical response to the empirical regimen is observed as slow, inadequate, and incomplete. Moreover, it should be done as quickly as practical.

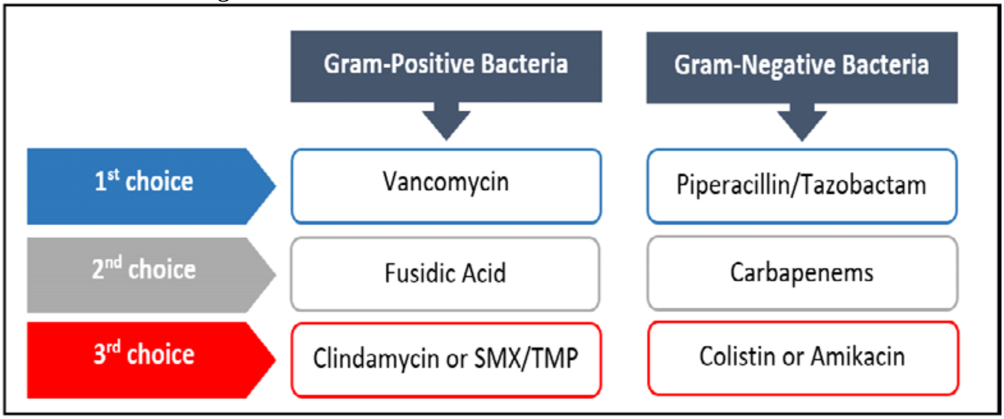

Figure 3. Recommendations for Empirical Antibiotics Use

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