

PREVALENCE OF BACTERIAL INFECTION IN DIABETIC FOOT ULCER

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Abstract

Background: DFU, which is a prevalent and severe complication of DM, substantially escalates the expenses associated with treatment. In the United States, DM presently impacts around 8.3% of the population, with over 79 million individuals having prediabetes. Additionally, 12% to 25% of people with diabetes (PWD) are at risk of developing a foot ulcer at some point in their lives. **Material & Method:** Total population of study was 130. This study was conducted in Department of Microbiology, Heritage Institute of Medical Sciences, Varanasi. The duration of study was over a period of two years. **Result:** Total 127 pathogenic bacteria were isolated from samples taken from 130 patients with a diabetic foot ulcer. Sixty-eight percent had growth of multiple microorganisms. Two-thirds (66.7%) of the isolates were gram-negative bacteria. The predominant bacterial species were *S. aureus* 25.19% (32/127), *Pseudomonas* species 18.89% (24/127), and *Escherichia coli* 16.53% (21/127). Overall, 92.9% (118/127) of the isolates were identified as multi-drug resistant. Gram-positive isolates were susceptible to chloramphenicol, clindamycin, and amikacin. Gram-negative isolates were also sensitive to chloramphenicol, aztreonam, and amikacin. **Conclusion:** This study concludes that Diabetic foot ulcers can be infected with a wide variety of pathogens and a large number of multi-drug resistant bacteria. The results showed an overall increase in the resistance of bacteria to antimicrobial agents and emphasize the importance of microbiological analysis and antimicrobial susceptibility testing before initiating antibiotics treatment for diabetic foot ulcer infections.

INTRODUCTION

DFU, which is a prevalent and severe complication of DM, substantially escalates the expenses associated with treatment.^[1] In the United States, DM presently impacts around 8.3% of the population, with over 79 million individuals having prediabetes.^[2] Additionally, 12% to 25% of people with diabetes (PWD) are at risk of developing a foot ulcer at some point in their lives.^[3,5] In DFU, infections are the primary cause of morbidity and mortality, occurring in approximately 40% to 80% of cases.^[6] Diabetic neuropathy and micro or macro ischemia are the main risk factors responsible for the development of DFU.^[7] Due to impaired microvascular circulation, phagocytic cells have limited access to the infected area, resulting in inadequate antibiotic concentration within the infected tissue. This contributes to the poor treatment outcomes in infected tissue.^[8]

Consequently, diabetic foot wounds frequently become infected, leading to the development of microthrombi that further exacerbate ischemia, necrosis, and progressive gangrene. In severe cases, limb amputation becomes necessary. Therefore, precise identification of the causative organism is crucial for effective management of such cases. It is anticipated that the prevalence of people with diabetes (PWD) in India will rise to 57 million by the year 2025, adding to the burden of this condition.^[4] Individuals with diabetes have a significantly higher likelihood, about 10 times greater, of being hospitalized due to soft tissue and bone infections compared to those without diabetes.^[9] DFUs are characterized by their chronic nature, often requiring multiple hospitalizations for treatment. People with diabetes are frequently exposed to various antibiotics, which increases their vulnerability to developing infections that are resistant to multiple drugs. The majority of diabetic

foot infections (DFIs) are caused by a combination of bacterial species, necessitating appropriate antibiotic selection based on culture and antimicrobial susceptibility testing results for effective management.^[11]

According to recent studies, bacterial infection is recognized as a key factor contributing to the chronic nature of DFUs.^[12] Typically, DFUs become infected through bacteria present on the skin surface, which subsequently form colonies consisting of diverse bacterial polycultures. While the skin surface serves as a common source of bacterial entry in DFUs, the environment created by initial invaders eventually allows for the presence of non-native bacteria that are obligate pathogens.^[13-14]

Hence, the objective of this study was to identify the specific bacteria responsible for diabetic foot ulcers and assess the antimicrobial sensitivity patterns of these bacterial isolates

MATERIALS AND METHODS

Study Population: Total population of study was 130.

Study Area: This study was conducted in Department of Microbiology, Heritage Institute of Medical Sciences, Varanasi.

Study Duration: The duration of study was over a period of two years.

Data Collection

All adult diabetic patients with diabetic foot ulcers, whose ulcers were greater than or equal to the Wagner first degree grading system, who visited the HIMS, Varanasi during the study period, and gave informed consent were included in the study. Samples were taken from the deepest part of the ulcer using two sterile swabs, soaked in sterile glucose broth. The samples were taken using a firm circular motion with the swab. One swab was used for Gram staining and the other was used for culture. Semi-structured questionnaires were used to collect sociodemographic and other clinical data.

Data analysis: Data were analyzed by using Microsoft Excel.

RESULTS

130 cases were included in this study. Among all 89 were male rest were female. Most of the cases were belonged to 50-75 year age. A total of 127 bacterial isolates were identified from 130 patients who had diabetic foot ulcers. Out of these, 32% (41 isolates) were Gram-positive, while 68% (86 isolates) were Gram-negative. The presence of multiple bacterial species was observed in 68% (82 out of 120) of the samples. Gram-negative bacteria accounted for a

larger proportion, with 68% (86 isolates), compared to Gram-positive bacteria, which accounted for 32% (41 isolates). The most prevalent bacteria among the isolates were *Staphylococcus aureus*, constituting 25.19% (32 out of 127), followed by *Pseudomonas* species at 18.89% (24 out of 127), and *Escherichia coli* at 16.53% (21 out of 127). Other isolated bacteria included *Acinetobacter* species (9.4%), *Klebsiella pneumoniae* (7.9%), *Serratia* species (4.7%), *Enterococcus* species (3.1%), *Proteus vulgaris* (3.1%), and *Proteus mirabilis* (3.1%). Out of the 127 bacterial isolates, 32.2% (41 out of 127) were Gram-positive bacteria. All *Staphylococcus aureus* and *Enterococcus* species among the Gram-positive isolates demonstrated resistance to oxacillin, penicillin, cefoxitin, and bacitracin. However, a lower susceptibility rate of 18.7% (6 out of 32) was observed for oxacillin among the *S. aureus* isolates. A high level of resistance was also noted among the majority of *S. aureus* isolates and all *Enterococcus* species, as they were resistant to gentamycin, doxycycline, erythromycin, and cotrimoxazole. Conversely, all *Streptococcus pyogenes* and *Viridans streptococcus* species exhibited sensitivity to the majority of antimicrobial agents. Regarding *S. aureus* isolates, the majority showed sensitivity to amikacin at a rate of 81.25% (26 out of 32), ciprofloxacin at a rate of 50% (16 out of 32), clindamycin at a rate of 62.5% (20 out of 32), and vancomycin at a rate of 62.5% (20 out of 32). All isolated *S. aureus* and *Enterococcus* species were sensitive to chloramphenicol, with a sensitivity rate of 100%. Among the *Enterococcus* species, 50% (2 out of 4) were resistant to vancomycin. Overall, 56.4% of *S. aureus* isolates, 78.8% of *Enterococcus* species isolates, and 72% of *Viridans streptococcus* species showed resistance to the antibiotics tested in this study. All the Gram-negative isolates exhibited resistance to cefoxitin, ampicillin-sulbactam, tobramycin, polymyxin B, cefepime, and augmentin. Among these, *Acinetobacter* and *Pseudomonas* species displayed the highest resistance to various antibacterial drugs, including amikacin, chloramphenicol, aztreonam, ceftriaxone, ceftazidime, imipenem, and meropenem. More than half of the Gram-negative bacterial isolates demonstrated resistance to doxycycline, trimethoprim, piperacillin, tazobactam, ceftriaxone, cefotaxime, imipenem, and meropenem. On the other hand, the majority of *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia*, *Proteus vulgaris*, *Proteus mirabilis*, *Citrobacter* species, and *Klebsiella oxytoca* were found to be sensitive to chloramphenicol, amikacin, and ceftazidime. Among these, amikacin was identified as the most effective drug for treating *Citrobacter* species.

Table: 1 Distribution according to Gender

Gender	No.
Male	89
Female	41
Total	130

Table 2: Distribution according to Age group

Age group	No.	
<40	4	3
41-50	36	27.6
51-60	42	32.3
61-70	30	23
71-80	17	13
>80	1	0.7
Total	130	

Table 3: Distribution according to diabetes

Type of diabetes	No.	
Type I	67	51.5
Type II	63	48.5
Total	130	

Table 4: Distribution according to other parameters

Other parameters		
Hypertensions	YES	70
	NO	60
CKD	YES	24
	NO	106
PVD	YES	43
	NO	87
Leg skin texture	Dry skin	40
	Moist skin	53
	Cracked skin	37
Wagner's classification system	Grade 1	3
	Grade 2	45
	Grade 3	62
	Grade 4	19
	Grade 5	1

Table 5: Distribution according to Bacterial isolates

Bacterial isolates	No.
S. aureus	32
Enterococcus species	4
Viridans streptococcus spp	3
Streptococcus pyogenes	2
Total	41
Pseudomonas species	24
Escherichia coli	21
Acinetobacter species	12
Klebsiella pneumonia	10
Serratia	6
Proteus vulgaris	4
Proteus mirabilis	4
Citrobacter species	4
Klebsiella oxytoca	1
Total	86
Fungal isolates	3

Table 6: Antibiotic susceptibility pattern of Gram positive bacteria

Antibiotics	S. aureus(32)		Enterococcus(4)		S. pyogenes(2)		Viridian streptococcus(3)	
	R	S	R	S	S		R	S
Penicillin	32	0	4	0	2		1	2
Cephameycin	26	6	4	0	2		1	2
Gentamycin	25	7	4	0	2		2	1
Amikacin	6	26	2	2	2		0	3
Tetracycline	26	6	4	0	-		2	1
Quinolones	16	16	3	1	-		1	2
Sulfonamides	21	3	3	1	2		3	0
Chloramphenicol	0	32	0	4	2		0	3
Macrolides	22	10	4	0	2		0	3
Vancomycin	12	20	2	2	2		0	3

Table 7: Antibiotic susceptibility pattern of Gram negative bacteria

Antibiotics	Acinetobacter spp (12)		Pseudomonas spp(24)		K.P(10)		E.coli(21)		Serratia (6)		P. mirabilis(4)		P. vulgaris (4)		Citrobacter spp (4)		Klebsiella oxytoca (1)	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Amikacin	10	2	11	13	5	5	5	16	2	4	2	2	2	2	0	4	1	0
Tobramycin	12	0	21	3	7	3	19	2	5	1	4	0	3	1	3	1	1	0
Tetracycline	11	1	23	1	10	0	18	3	6	0	4	0	3	1	3	1	1	0
Quinolones	8	4	13	11	4	6	8	13	5	1	2	2	2	2	2	2	1	0
Sulfonamides	10	2	21	3	9	1	19	2	5	1	2	2	4	0	4	0	0	1
Chloramphenicol	8	4	16	8	3	7	2	19	6	0	2	2	0	4	2	2	0	1
Monobactam	7	5	13	11	2	8	5	16	2	4	2	2	3	1	0	4	0	1
Cephalosporins	11	1	24	0	10	0	20	1	6	0	4	0	4	0	4	0	0	1
Beta-lactam	6	6	16	8	4	6	7	14	2	4	2	2	0	4	2	2	0	1
AMC	12	0	24	0	10	0	21	0	6	0	4	0	4	0	4	0	1	0
Imepenem	6	6	11	13	6	4	5	16	4	2	1	3	0	4	0	4	0	1
Meropenem	7	5	9	15	4	6	6	15	3	3	1	3	1	3	0	4	1	0

DISCUSSION

Diabetic foot ulcers represent a significant complication of Diabetes mellitus, and if not properly addressed, can lead to subsequent complications including infection, gangrene, osteomyelitis, and the need for amputation. The management of such infections typically involves a combination of surgical intervention and antibiotic therapy. The purpose of this study was to identify the primary pathogenic bacterial infections associated with diabetic foot ulcers and evaluate their susceptibility to commonly used antibiotics at the designated research sites. It is worth noting that this study observed a male predominance among the participants, consistent with previous research conducted in Indonesia and India.^[15-16] The higher proportion of men in the study, as seen in previous research conducted in India and Indonesia.^[15-16], may be attributed to their increased involvement in outdoor activities, which can lead to injuries and a higher risk of developing ulcers. Furthermore, the majority of participants with DFU infections fell within the age range of 51-60 years, aligning with findings from similar studies in India and Indonesia.^[15,17] In this study, the classification of ulcers was performed using the Wagner Diabetic Foot Ulcer Classification System. The most frequently observed grade was grade 3, accounting for 47.69% (62/130) of cases, followed by grade 2 at 34.61% (45/130). These findings are consistent with research conducted in Egypt, where grade 3 ulcers were present in 50% (60/120) of participants, followed by grade 2 in 25% (30/120).^[18] In contrast to the findings mentioned earlier, a study conducted in India demonstrated a higher prevalence of grade 2 ulcers (69.2%) compared to grade 3 ulcers (5.1%). Furthermore, this current study revealed a high growth rate of bacteria at 92.3%, which is consistent with a previous study conducted in Ethiopia where the growth rate was reported as 77.3% (92/119), with no growth observed in 22.7% (27/1190) of

cases. Another recent study reported a growth rate of 81.7% (98/120) and no growth in 22% (18.34%) of cases.^[18] In general, gram-negative bacteria were the predominant isolates, accounting for 71.6% (86/120), compared to gram-positive isolates at 34.16%. This finding aligns with a previous study conducted at the same study site, Tikur Anbessa Specialized Hospital, one of the three included in the current study. The earlier study reported the isolation of gram-negative bacteria in 88.55% (54/61) of cases, whereas gram-positive bacteria accounted for 7% (11.47%). Similarly, a study from Egypt reported gram-negative bacteria at 56% and gram-positive bacteria at 27.7%, while in northeast India, 79% of isolates were gram-positive and 21% were gram-negative.^[18,19] The rate of bacterial isolation and the type of bacteria isolated from the ulcers showed an increasing trend as the severity of the ulcer progressed. The influence of microorganisms on the healing process of DFUs is well supported by several published papers from various regions, including Nigeria, China,^[20] and India.^[16,21] In the current study, *Staphylococcus aureus* was the predominant isolate, accounting for 25.19% (32/127) of cases. This differs from a previous study conducted in Ethiopia, which reported *Klebsiella* species as the predominant bacteria in 23.9% (22/92) of cases, followed by *Proteus* species in 18.47% (17/92). In Egypt, *Pseudomonas mirabilis* was the most common isolate, accounting for 16.8% of cases.^[19] Similarly, in Saudi Arabia, *Pseudomonas* species were found in 15.6% (n=134) of cases.^[22], and in South America, *Pseudomonas* species constituted the most common isolate at 18.8% (reference 28). Similarly, findings from studies conducted in Kenya (17.5% prevalence),^[23,24] Nigeria (32.9% prevalence),^[25] India (24.42% prevalence),^[26] China (65.2% prevalence) [20], and Iran (28% prevalence).^[27] are consistent with the results of this study. These findings indicate that the predominant bacteria causing DFU infections can vary across different settings. In the current study, the most commonly

isolated gram-negative bacteria were *Pseudomonas* species (18.89%), followed by *Escherichia coli*, which is comparable to findings from studies conducted in Libya (17.5% prevalence).^[28], India (23.2% and 23.6% prevalence).^[21] However, a previous study in Ethiopia reported no isolation of *Pseudomonas* species from the 92 samples cultured, while *E. coli* was isolated in 5.43% of cases. Similarly, in Pakistan, *E. coli* was identified as the most common gram-negative bacterium at a prevalence of 15.72%.^[29] These variations could be attributed to differences in sample sizes among the studies and unique characteristics of each study site. Notably, a very high rate of multidrug resistance (92.9%) was observed in the present study, consistent with findings from studies conducted in India.^[21] and Nigeria.^[30] In this study, a majority of the isolated *Staphylococcus aureus* strains exhibited resistance to gentamicin, doxycycline, erythromycin, and trimethoprim. However, they were found to be sensitive to amikacin, oxacillin, ciprofloxacin, clindamycin, and vancomycin. Similarly, all enterococci were resistant to gentamicin, doxycycline, erythromycin, and trimethoprim, while they remained susceptible to chloramphenicol (100%).^[31] The high level of resistance observed could be attributed to several factors. These may include inappropriate use of antibiotics, self-medication practices, repeated courses of antibiotics due to the chronic nature of DFUs, and the potential exposure of patients to the hospital environment during frequent follow-up visits.

CONCLUSION

Diabetic foot ulcers can be infected with a wide variety of pathogens and a large number of multi-drug resistant bacteria. In this study, *Staphylococcus aureus* was the dominant isolate followed by other gram-negative bacteria. In the current study, a high level of resistance to commonly used antibiotics was found highlighting the need for cautious care in the use of antibiotics for the treatment of infections. Some isolates in the current study were more sensitive to chloramphenicol, aztreonam, amikacin, clindamycin, and vancomycin, which can be used as first-line treatment for these infections. The results showed an overall increase in the resistance of bacteria to antimicrobial agents and emphasize the importance of microbiological analysis and antimicrobial susceptibility testing before initiating antibiotics treatment for diabetic foot ulcer infections.

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