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Original Research Article

ANTIBIOTIC SENSITIVITY PATTERN IN GRAM POSITIVE MICROORGANISM ISOLATED FROM DIABETIC FOOT ULCERS: A CROSS SECTIONAL STUDY

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ABSTRACT

Background and Objectives: This research was conducted on patients afflicted with diabetic foot ulcer (DFU) in order to evaluate the clinical attributes, range of microbial populations, antibiotic susceptibility, and establish an empirical approach for antimicrobial treatment.

Materials & methods: Clinical information, tissue and and pus collections were gathered from a total of 50 patients diagnosed with diabetic foot ulcer. These samples were handled in accordance with the guidelines set forth by the Clinical and Laboratory Standards Institute. Both the clinical and microbiological data were analyzed.

Results: This study of 50 diabetic foot ulcer patients revealed a male predominance. Most patients were in 50-60 years age group. Among the samples, Gram-positive organisms accounted for 29.48%. Staphylococcus aureus (17.95%) showed sensitivity to amikacin, Linezolid (100%), and Vancomycin (100%).

Conclusion: In DFU, Gram-positive bacteria displayed varying degrees of resistance to commonly utilized antibiotics, while exhibiting sensitivity to aminoglycosides. Based on these results, Amikacin and Gentamicin are potential options for empiric antibiotic treatment in DFU infections.

Key words: Anti-Bacterial Agents, Vancomycin, Linezolid, Gram-Positive Bacteria, Diabetic Foot.

1. INTRODUCTION

Diabetes is emerging as a potential epidemic in India, with a diabetic population exceeding 62 million individuals. The prevalence of diabetes varies across different regions of the country, ranging from 5.3% in central India to 13.6% in Northern India. However, due to diabetes not being a notifiable condition, the true burden of diabetes in India remains unknown, and there is a possibility of underestimating its actual impact and prevalence in the country (1-3).

Foot ulceration represents a prominent complication of diabetes, affecting an estimated 15% of individuals with diabetes over their lifetime. The prevalence of diabetic foot ulcers (DFU)

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varies between 4% and 10%. Among the complications associated with DFU, infections are the primary cause of morbidity and mortality, occurring in 40% to 80% of cases (4).
In the management of DFU infections, empirical antimicrobial therapy is typically initiated. However, the limited development of new antibiotics and the excessive use of existing antibiotics have contributed to the widespread concern of antibiotic resistance within healthcare facilities (5). Furthermore, the presence of diabetic nephropathy, affecting approximately one-third of individuals with diabetes, along with the rising incidence of multi-drug resistant infections in DFU, further complicates the challenges faced by clinicians in effectively treating these patients (6).
The objective of this study was to investigate the clinical and microbiological characteristics of DFU patients, determine the antibiotic susceptibility patterns of Gram-positive microorganisms in DFU, and develop an empirical antibiotic treatment protocol. Given the absence of similar studies in the region that have formulated empiric therapy for diabetic foot infections, understanding the antibiotic sensitivity patterns would assist in establishing an effective empiric antibiotic treatment protocol specific to this region. This would be beneficial for primary care physicians and specialists in initiating more efficient empiric antibiotic therapy, potentially leading to a reduction in antibiotic resistance and treatment costs for patients.

2. MATERIAL & METHODS

This prospective and observational study was conducted at Department of Microbiology, Sri Aurobindo Institute of Medical Sciences, Indore, India, a tertiary care teaching hospital, where 50 patients with DFU attending the diabetic foot clinic were recruited over a period of two years. The study adhered to the guidelines outlined in the Declaration of Helsinki (7) and received approval from the Institutional Ethics Committee of the said indtitute. All study participants provided written informed consent prior to their involvement. As per the criteria set by the International Working Group on the Diabetic Foot (IWGDF), a DFU was defined as a full-thickness wound that extends through the dermis and is situated below the ankle in individuals with diabetes (8).
Inclusion criteria for this study encompassed patients diagnosed with DFU aged 18 years or more who were attending the diabetic foot clinic and provided their consent to participate. On the other hand, children, patients with non-diabetic foot ulcer and repeat isolates from same patient were excluded.
Upon obtaining informed consent, relevant demographic information from all patients was collected and entered into an Excel spreadsheet. The results of blood investigations were also recorded in the same spreadsheet. Fasting blood glucose levels equal to or greater than 126 mg/dL and glycosylated hemoglobin (HbA1c) levels equal to or greater than 7% were considered abnormal, respectively.

The collection of pus and tissue culture specimens was performed in a minor operation room following a specific protocol. The process involved cleansing the wound and vigorously washing it with saline solution. Subsequently, any superficial exudate and slough were debrided to minimize the possibility of picking up superficial colonization flora. Sterile culture bottles containing normal saline were used to collect the specimens after scraping the ulcer base or the deep portion of the wound edge using a sterile curette. Incised tissue samples, approximately 0.5 centimeters in size, were obtained from various sites within the

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wound. This method of collecting deep tissue samples through curetting provides more reliable and representative microbiological cultures compared to swab samples (9).
Standard methods for sample processing, isolation, and identification of aerobic bacteria were employed. The tissue samples were homogenized and inoculated onto Blood agar and MacConkey agar. Incubation of these samples occurred under aerobic conditions at 37 degrees Celsius for 24-48 hours. Subsequently, the obtained colonies were identified, and antibiotic sensitivity was determined using the Kirby-Bauer's disc diffusion technique, as outlined in the Clinical Laboratory Standard Institute guideline of 2012 (10).
The statistical analysis of the data involved both descriptive and inferential statistics. The Chi-square test was utilized for the inferential analysis. The software programs used for the analysis were SPSS (Statistical Product and Service Solutions) version 18.0 and GraphPad Prism version 5.0. A significance level of $P < 0.05$ was considered to determine statistical significance in the results.

3. RESULTS

In this study, the predominant age group of the subjects encompassed individuals between 50 and 60 years old. Among the cohort, a greater proportion consisted of males. The preponderance of cases involved Type II Diabetes, with a duration of 10 to 15 years [Table 1].

Table 1: Demographics and clinical features of study population

Variable	**Number**	**%**
Age (years)		
40-50	11	22
50-60	25	50
60-70	6	12
70-80	7	14
>80	1	2
Gender		
Male	35	70
Female	15	30
Type of Diabetes		
Type I	2	4
Type II	48	96
Duration of Diabetes (years)		
>5	1	2
5-10	15	30
10-15	30	60
>15	4	8
Specimen Collected		
Pus	37	74
Tissue	13	26

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According to Table 2, the composition of organisms isolated from DFU within the study population is provided. A significant portion of the specimens exhibited polymicrobial characteristics, with Gram-positive organisms constituting approximately 30% of the overall isolates.

Table 2: Type of organisms isolated from DFU in study population.

	Number	**%**
Type of Isolate		
Monomicrobial	21	42
Polymicrobial	29	58
Isolated Organism		
Gram Positive		
Enterococcus faecalis	5	6.41
Staphylococcus aureus	14	17.95
Coagulase-Negative Staphylococci	1	1.28
Candida species	3	3.85
Total	23	29.49
Gram Negative	55	70.51

Table 3 displays the antibiotic sensitivity patterns observed among the Gram-positive organisms that were isolated from DFU. The results indicate that these organisms exhibited the highest susceptibility to Vancomycin, Linezolid, and Novobiocin.

Table 3Antibiotic sensitivity among Gram positive organisms isolated from DFU.

	Staph. Aurues (n=15)		**Enterococcus (n=5)**	
Antibiotic	**Sensitive Isolates**	**%**	**Sensitive Isolates**	**%**
Penicillin G	0	0	0	0
Cotrimoxazole	7	47	-	-
Linezolid	15	100	5	100
Vancomycin	15	100	5	100
Rifampin	11	73	-	-
Ampicillin	-	-	5	100
Chloramphenicol	9	60	-	-
Tetracycline	7	47	-	-
Minocycline	7	47	-	-
Cefoxitin	7	47	-	-
Ofloxacin	6	40	-	-
Erythromycin	8	53	-	-
Azithromycin	8	53	-	-
Clindamycin	8	53	-	-
Oxacillin	7	47	-	-
Levofloxacin	5	33	-	-
Gentamicin	5	33	-	-

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Novobiocin	15	100	-	-
Doxycycline	9	60	-	-
Ciprofloxacin	5	33	-	-
High level Streptomycin	-	-	5	5
High Level Gentamicin	-	-	5	5

4. DISCUSSION

Foot ulcer poses a significant burden on individuals with diabetes, often leading to severe consequences. In fact, approximately half of all lower extremity amputations can be attributed to diabetes-related complications. Among the indications for diabetic lower limb amputation, foot infection ranks as the most frequent, surpassed only by gangrene [4]. In the management of diabetic foot infections, initial antibiotic therapy is typically initiated empirically based on the presumed causative organism. Subsequently, treatment is adjusted based on the results of bacterial culture and sensitivity reports. The duration of treatment varies depending on the severity of the infection, ranging from 1 to 2 weeks to over 4 weeks [11]. Clinicians face the added challenge of treating diabetic patients with nephropathy, a condition that affects approximately one-third of individuals with diabetes. Furthermore, the increasing incidence of multidrug-resistant infections in diabetic foot ulcers further compounds the difficulties faced by healthcare professionals in providing effective treatment [6].

The mean age of the patients included in this study was 58.55 years. The majority of the patients fell within the age group of 50-60 years, and the male-to-female ratio was 2.33. These findings are consistent with a study conducted by Yerat et al. [4]. Being situated in a rural area, this center attracted a predominantly rural patient population, with 80% of the patients hailing from rural areas and the remaining 20% from urban areas. This distribution aligns with a study conducted by Shahi et al. [12]. It is worth noting that a significant proportion of the patients with DFU had a diabetes duration of more than 10 years, which corroborates previous findings reported in studies by various researchers [13-15].

Out of the 50 patients enrolled in this study, a total of 101 isolates were obtained, resulting in an average of 2.02 bacteria per lesion. In comparison, Kaur et al. [16] and Bansal et al. [17] reported wound bioburdens of 1.38 and 1.52 bacteria per culture-positive patient, respectively. The isolation rates observed in our study could potentially be attributed to the non-isolation of anaerobic organisms.

In this study, a total of 50 samples were assessed, and among the isolates, 42% exhibited growth of a single organism, while 20% displayed growth of two organisms, and 38% demonstrated polymicrobial growth. Bansal et al. [17], in their evaluation of 103 patients, found that monomicrobial growth was observed in 61.8% of cases, polymicrobial growth in 37.08% of cases, and 7.2% of cases had sterile cultures. Gram-negative bacteria constituted the majority (73.7%) of the total bacterial isolates, whereas Gram-positive bacteria accounted for 27.3%. Similar findings have been reported in other studies by Manikandan et al. [18] and Kaur et al. [16], which align with the higher prevalence of Gram-negative pathogens observed in low-income countries, as reported by Perez-Fevila et al. [19].

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Gram-negative bacilli have been identified as a common cause of diabetic foot infections in India [20]. However, studies from Western countries, such as the research conducted by Mendes et al. [21], have reported a predominance of Gram-positive organisms in diabetic foot ulcers [22]. The underlying reasons for the variation in the prevalence of Gram-negative organisms in the East and Gram-positive organisms in the West remain largely unknown and require further investigation.

In the current study, Staphylococcus aureus exhibited high sensitivity rates to amikacin and gentamicin, followed by ofloxacin and vancomycin. Other Gram-positive organisms also demonstrated 100% sensitivity to amikacin and gentamicin. Vancomycin was found to be 100% effective against Gram positive spp., while Enterococcus spp. showed 100% sensitivity to ofloxacin, amoxicillin-clavulanic acid, erythromycin, clindamycin, and ciprofloxacin. However, it was observed that amoxicillin-clavulanic acid, erythromycin, clindamycin, and ciprofloxacin were relatively less effective against Gram-positive organisms. This reduced effectiveness may be attributed to the common usage of these antibiotics in the community [23].

Rational empiric therapy is considered an essential component in the management of DFU infections. Taking into account the findings of this study and similar investigations conducted in different geographical locations, it was concluded that aminoglycosides demonstrated the highest activity against both Gram-positive and Gram-negative bacteria in DFU infections. These findings can be attributed to the relatively lower usage of aminoglycoside antibiotics within the community [23].

This study has certain limitations that should be taken into consideration. Firstly, the sample size utilized in this study was relatively small, which may limit the generalizability of the findings to a larger population. Additionally, due to logistical and resource constraints, the anaerobic flora was not tested, which may have resulted in an incomplete understanding of the microbial composition in the studied population.

5. CONCLUSION

The findings of this study indicate that DFU are commonly observed in males during their fifth and sixth decades of life. The majority of DFU patients exhibit poor glycemic control. Gram-negative bacteria are the predominant infectious agents in DFU, while Staphylococcus aureus is the most common Gram-positive strain. Notably, aminoglycosides demonstrate sensitivity against both Gram-negative and Gram-positive bacteria, likely due to their limited use in the community setting. Conversely, resistance has developed in the community against commonly prescribed extended-spectrum penicillins and cephalosporins. Based on the study's findings, there is a need to reassess empiric antibiotic therapy for diabetic foot infections.

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