MICROBIOLOGICAL PROFILE OF DIABETIC FOOT ULCER IN A TERTIARY CARE HOSPITAL OF EASTERN ODISHA

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ABSTRACT

INTRODUCTION – Diabetic foot ulcers are a common complication in patients suffering from diabetes. Most of the diabetic foot ulcers get infected which leads to ischemia, gangrene and necrosis of the limb, requiring its amputation. Thus, accurate identification of micro-organism infecting the diabetic foot ulcers is needed for their better management.

Methods – A prospective study was undertaken with 100 diabetic foot ulcer patients who presented to surgery OPD between 2018 and 2020. Specimens were collected from the ulcer sites at the time of admission after thoroughly washing the site with sterile normal saline and debridement.

RESULTS – Overall, the most common organism isolated from the diabetic foot ulcers were Staphylococcus aureus (38%) followed by Pseudomonas spp. (21%) and E. coli (13%). Overall, Piperacillin-Tazobactum (PIT) was found to be the most sensitive (76%) drug towards the organisms isolated followed by Netilmicin (73%), Levofloxacin (68%).

CONCLUSION – Staphylococcus aureus is the most common organism to be isolated from the DFU’s and are mostly sensitive to Linezolid and Vancomycin. Piperacillin-Tazobactum (PIT) showed highest sensitivity of 76% towards all the bacterial isolates which can be used empirically in management of diabetic foot ulcers.

INTRODUCTION
A serious and frequent complication of diabetes mellitus (DM) is diabetic foot ulcer (DFU), that raises treatment costs significantly[1]. Patients with DM have a lifetime risk of as high as 25% to develop DFU and risk of lower leg amputation increases by 15-46 times in patients with DM as compared to patients without it [2]. Due to infections, the morbidity and mortality in DFUs increases and is seen in 40 %-80 % of cases[3].The two major risk factors that cause DFU are diabetic neuropathy and ischemia. Impaired micro-vascular circulation restricts phagocytic cells' access to the infected region, resulting in low antibiotic concentrations in the infected tissue [4-6]. Hence, diabetic foot ulcers are usually infected, which in turn leads to
the formation of microthrombi causing further ischemia, necrosis, and gangrene which is progressive. These types of situations necessitate limb amputation. Staphylococcus aureus, E.coli, Pseudomonas spp. and Enterococcus spp. are the most common bacteria cultured from DFUs [5–7]. In recent times, the incidence of multidrug resistant organisms have increased many folds, leading to increased hospital stay, cost, morbidity and mortality[8]. Thus, accurate identification of the infecting micro-organism and identifying its drug susceptibility is essential for managing such type of cases. The burden of DFU in India is projected to grow to 57 million by 2025[9]. In case of a DFU, Initially an empirical antibiotic is started based on the susceptibility data[10], but selection of correct antibiotics based on the culture and antibiotic susceptible testing is required for controlling the infection in a DFU[11,12]. Hence this study was performed to know the etiological agents of the infected DFUs in a tertiary care hospital of eastern Odisha and their in-vitro antibiotic susceptibility pattern to the commonly prescribed antibiotics.

AIMS AND OBJECTIVES

1. To assess the pattern of pathogens associated with DFU
2. To determine their antimicrobial susceptibility patterns.

MATERIALS AND METHODS

Study design This is a prospective study, conducted over a period of 2 years from June 2018 to June 2020.

Sample size –100 patients fulfilling the inclusion criteria.

Inclusion criteria

All patients aged 18 and above, with a diabetic foot ulcer which were debrided of necrotic tissue and are admitted to surgery wards in IMS and SUM Hospital.

Exclusion criteria

- Patients with other foot ulcers without diabetes.
- Patients with known hypersensitivity to any of the dressing components.
- Patients with conditions that may interfere with wound healing (e.g.- chronic liver or renal disease, connective tissue disorder, immune system disorder, major nutritional deprivation, uncontrolled diabetes mellitus).
- Patients who are not willing to participate in the study.

Method

A total of 100 patients admitted in surgery wards of IMS and SUM Hospital between June 2018 and June 2020, with clinical picture showing Diabetic Foot Ulcer were selected for the study after initial evaluation and screening taking into consideration the above-mentioned inclusion criteria. After explaining the procedure of the study, written informed consent was taken before enrolment into the study.

Specimens were collected from the ulcer sites at the time of admission after thoroughly washing the site with sterile normal saline and debridement. Sterile swab was used to collect
the specimen from the floor of the ulcer or deep portion of the wound edges with a sterile curette. Soft tissue specimens obtained were processed directly for aerobic bacteria.

The specimens were subjected to gram staining and they were inoculated onto Blood agar and Mac Conkey’s agar for the isolation of aerobic bacteria. The plates were then incubated at 37°C for 48 hours. The isolates were identified by the standard biochemical tests. Antibiotic susceptibility testing of the aerobic bacterial isolates was done by standard Kirby-Bauer disc diffusion method as per CLSI guidelines.

Antibiotics used for Gram positive isolates were AMC: Amoxycillin clavulanic acid (30 µg); CX: Cephalexin (30 µg); CTX: Cefotaxime (30 µg); CFS: Cefoperazone Sulbactam (75 µg/30µg); CTR: Ceftriaxone (30 µg); PIT: Piperacillin Tazobactam (100 µg/10µg); LZ: Linezolid (30 µg); OF: Ofloxacin (5 µg); LE: Levofloxacin (5 µg); AK: Amikacin (30 µg); GEN: Gentamicin (10 µg); NET: Netilmicin (30 µg); AZM: Azithromycin (15 µg); V: Vancomycin (30 µg).

Antibiotics used for Gram negative isolates were AMC: Amoxycillin clavulanic acid (30 µg); CTX: Cefotaxime (30 µg); CTR: Ceftriaxone (30 µg); CAC: Ceftazidime clavulanic acid (30 µg/10 µg); CFS: Cefoperazone Sulbactam (75 µg/30µg); PIT: Piperacillin Tazobactam (100 µg/10µg); OF: Ofloxacin (5 µg); LE: Levofloxacin (5 µg); AK: Amikacin (30 µg); GEN: Gentamicin (10 µg); NET: Netilmicin (30 µg); IPM: Imipenem (10 µg).

Dressing was done with isotonic sodium chloride and liquid povidone iodine moistened gauze were applied over the DFU and covered with gauze bandage and tapes. The dressings were changed every alternate day or earlier if the dressing was soaked till the wound heals or till it’s conducive to go for skin grafting. Strict glycaemic control was maintained with regular post prandial blood glucose monitoring and appropriate sub-cutaneous insulin dosage.

RESULTS
A total of 100 diabetic patients with DFU were included in this study. A male predominance was noted, as out of the 100 patients with DFU, 72 patients (72 %) were male and 28 patients (28%) were female. Most patients, 56 (56%) belonged to the age group of 51 – 65 years followed by 27 patients (27 %) to age group of more than 65 years and 17 patients (17%) were less than 50 years old. (Table 1)

In this study all patients (100%) showed culture positivity. Most common organism isolated in the wound swab culture was found to be *Staphylococcus aureus* (38%) followed by *Pseudomonas aeruginosa* (21%) and *Escherichia coli* (13%). (Figure 1)

Among the gram-positive isolates, *Staphylococcus aureus* were mostly sensitive to Vancomycin (100%) and Linezolid (100%). (Figure 2)

Among the Gram-negative isolates belonging to Enterobacteriaceae family, *Escherichia coli* were sensitive to Imipenam (84.6%) and Netilmicin (84.6%) have higher sensitivity followed by Piperacillin-Tazobactum (76.9%) while *Klebsiella* spp. were found to sensitive to Imipenam (85.7%), Netilmicin (85.7%) and Levofloxacin (85.7%). (Figure 3)
However, among Gram negative non fermenter isolates, *Pseudomonas aeruginosa* were sensitive to Piperacillin-Tazobactum (81%) followed by Imipenem (76.2%) while *Acinetobacter spp.* were sensitive to Imipenam (75%) followed by Piperacillin-Tazobactum (50%), (Figure 4)

Overall, Piperacillin-Tazobactum (PIT) was found to be the most sensitive (76%) drug towards the organisms isolated followed by Netilmicin (73%), Levofloxacin (68%).

**DISCUSSION**

Ulcers of the foot are a major complication of DM and can lead to lower limb amputation \[^{2,5}\]. A wide range of bacteria can cause infection in these patients. Although DFUs are initially treated with empirical antibiotics, treatment targeted at the identified causative microorganism may help us to get better results \[^{7,10,13}\]. This study presents a detailed microbiological survey of infected DFUs and their drug sensitivity patterns of the patients admitted to our hospital.

Foot ulcers make up 4% of all DM related hospitalizations \[^{14}\]. Neuropathy, impaired glycaemic control, ischaemia and inflammation are the most important risk factors for DFU in a patient of DM.

As seen in several other studies, DFU had a male predominance in our study. Out of the 100 patients with DFU, 72% were male while 28% were females. These findings are similar to the results of Jeffcoate WJ et al, who reported a higher incidence of DFU in males with 67% in his study report \[^{15}\] and K.M. Mohanasoundaram’s study, which showed a prevalence of 65% in males \[^{16}\]. This could be due to more outdoor activities performed by males, having more chances of getting trivial injuries leading to chronic non-healing ulcers.

In our study, culture positivity was found to be 100% which was comparable to the report of Khare et al of 90.32% from a Tertiary care centre in South India \[^{17}\]. A culture negative in certain studies can be attributed to antibiotic therapy prior to collection of tissue or pus for microbiological processing.

In our study, Gram negative isolates from the DFUs were more common than gram positive isolates, which is similar to the findings of Viswanathan V et al’s study \[^{7}\]. The differences in the study region, age group, study settings might be the reason for differences in the prevalence of pathogens in a DFUs.

DFU bacterial colonization may advance to become an active infection which will delay the healing of the wound. Regular monitoring of the bacterial profile and its antibiotic susceptibility should therefore also be part of the overall DFU management strategy to direct effective antibiotic therapy while the dressings do their part \[^{18}\]. In this study, wound swab culture was sent at the time of admission, at the end of 2\(^{nd}\) and 4\(^{th}\) week of treatment or when specifically indicated. The most common organism isolated in the first wound swab culture in the present study was found to be Staphylococcus aureus (38%) followed by *Pseudomonas spp.* (21%) and *E. coli* (13%).These findings were similar to that of Chincholikar et al\[^{19}\] and Tahawy’s\[^{12}\] studies. The drug sensitivity patterns varied according to the isolated organism, of which, Piperacillin-Tazobactum (PIT) was found to be the most sensitive (76%) drug towards the organisms isolated followed by Netilmicin (73%), Levofloxacin (68%). This
information may help us to start Piperacillin-Tazobactum (PIT) empirically in patients of DFU whose wound swab culture is still awaited. For S.aureus isolates, Vancomycin (92.1%) and Linezolid (89.5%) were found to be most sensitive.

Limitations of the Present Study - Anaerobic cultures were not done due to limited resources.

**CONCLUSION**

Appropriate management of DFUs is of paramount importance as it can prevent limb amputation and even sometimes potentially life-threatening limb complications. Isolation of microbial agent and determination of the sensitivity / resistance of different anti-microbial drugs are vital for the in-hospital management of these patients. Development of multi-drug resistance can be prevented by institution of appropriate antibiotic regimen. Prompt initiation of antibiotic therapy, as well as surgical debridement of necrotic or de-vascularised tissue along with strict glycaemic control is essential for control of infection in DFU patients who are often found to be battling with various complications of DM involving multiple organ systems.

Present study highlights and suggests that prospective multicentre studies with higher sample size are required to assess the appropriate antibiotic regimen in diabetic foot ulcers and proper management of antibiotics must be implemented to decrease the incidence and development of multi drug resistant organisms.

**Tables and Figures**

**Table 1**

<table>
<thead>
<tr>
<th>Age Group (in Years)</th>
<th>Total (n=100)</th>
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<tbody>
<tr>
<td>≤ 50</td>
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<tr>
<td>51-65</td>
<td>56 (56%)</td>
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<tr>
<td>&gt; 65</td>
<td>27 (27%)</td>
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<table>
<thead>
<tr>
<th>SEX</th>
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<tbody>
<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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</tbody>
</table>

**Figure 1**

![Organisms Isolated Chart](image-url)
### Figure 2

**Susceptibility Pattern of Gram-Positive Isolates**

- **S. aureus** (n=38)
- **Enterococcus spp.** (n=3)

### Figure 3

**Susceptibility of Gram-Negative Isolates Belonging to Enterobacteriaceae Family**

- **E. coli** (n=13)
- **Klebsiella spp.** (n=7)
- **Enterobacter spp.** (n=4)
- **Citrobacter spp.** (n=3)
- **Proteus spp.** (n=6)
REFERENCES


11. Sharma VK, Khakda PB, Joshi A SR. The common pathogens which were isolated from diabetic foot infections in Bir hospital. Kathmandu Univ Med J. 2006;4(3):295–


