Bacterial Profile and Antibiotic Resistance in Patients with Diabetic Foot Infections in A Tertiary Care Hospital Palakkad

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ABSTRACT

BACKGROUND: Foot infections are a major complication of diabetes mellitus and eventually lead to development of gangrene and lower extremity amputation. Many studies reported the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory. AIM & OBJECTIVES: To determine the bacterial profiles of Diabetic foot Infections and the antibiotic Resistance pattern of the isolated organism. Methods: The study is designed as a prospective study. Predesigned data collection forms were obtained. The data collection is planned to carried out for duration of five months. (November 2015 to March 2016). RESULTS: During this period, A total of 42 cases were Analysed. Among these 13% was Staphylococcus Aureus, following 10% E.coli, and 5% Klebisella spp.. As for Linezolid (53.8%) was the most effective antibiotic against Staphylococcus Aureus Isolates and Levofloxacin (7.69%) was the least effective antimicrobial and more resistant to Co-trimoxazole (38.4%). As for E.coli, Amikacin is more sensitive (60%) than Co-trimoxazole and Gentamycin. Ciprofloxacin and Amoxicillin (50%) were the most resistant Antibiotics. CONCLUSION: Among Gram positive and Gram Negative organism, Staphylococcus Aureus and E.coli were the most frequent organism isolated Respectively.

Keywords: Diabetic foot ulcer, bacterial profile, antibiotic sensitivity, staphylococcus aureus, klebisella spp, E. coli

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1. INTRODUCTION

A diabetic foot is one of the most feared complications of diabetes and it is the leading cause of the hospitalization among diabetic patients. [1] It is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to the development of gangrene and which even necessitates limb amputation. [2] The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes. [3] The impaired micro-vascular circulation in patients with a diabetic foot limits the access of phagocytes, thus favoring the development of an infection. The local injuries and the improper foot wear further compromise the blood supply in the lower extremities. While the foot infections in persons with diabetes are initially treated empirically, a therapy which is directed at the known causative organisms may improve the outcome. [4]

Many studies have reported the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory. These discrepancies could partly have been due to the differences in the causative organisms, which had occurred over time geographical variations, or the type and the severity of the infection, as were reported in the studies. Mostly, the diabetic foot infections are mixed bacterial infections and the proper management of these infections requires an appropriate antibiotic selection, based on the culture and the antimicrobial susceptibility testing results. This study was undertaken with the aim of determining the bacterial profile of infected diabetic foot ulcers and
the antibiotic resistance pattern of the bacteria isolates. [5]

**SENSITIVITY TESTING**

It is a microbiological test to determine the susceptibility pattern of isolated organism to antibiotics. It is otherwise known as antibiotic susceptibility testing. It is usually carried out to determine which antibiotics are most successful in treating the infection.

2 Types of antibiotic sensitivity testing includes

- Disk diffusion or Kirby–Bauer antibiotic testing
- Tube dilution method

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**Figure 1: Disk diffusion or Kirby–Bauer antibiotic testing**

It is a test which uses antibiotic-impregnated wafers to test whether bacteria are affected by antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition. The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth. [6]

The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. [7] The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. [8] If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion is used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for those bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection. [9] It is another standard antibiotic sensitivity testing. In this test, serial dilutions of antibiotics are made in liquid agar medium which is inoculated with a standardized number of organism and incubated for a prescribed period (usually 24-48 hours). The lowest concentration (highest dilution) of appearance of turbidity is considered as the minimal inhibitory concentration (MIC). [10] Additionally, the minimal bactericidal concentration (MBC) can be determined by sub culturing the contents of tube on to antibiotic–free solid medium. Although the tube dilution test is fairly precise, the test is laborious because serial dilutions of antibiotic must be made and only one isolate can be tested in each series. [11]
Figure 2: Tube dilution

TREATMENT

❖ Wound care

• Cleaning

Cleaning can be accomplished using a number of different solutions, including tap water and solution. Infection rates may be lower with the use of tap water in regions where water quality is high. [12]

• Closure

If a person presents to a healthcare center within 6 hours of wound it should be closed immediately after the cleaning in order to prevent microbial contamination. Closure of wound is usually done using the following bandages, a cyanoacrylate glue, staples, and sutures. Absorbable sutures have the benefit over non absorbable sutures of not requiring removal. They are often preferred in children. Buffering Adhesive glue and sutures have comparable outcomes for minor lacerations <5 cm in adults and children. [13] The use of adhesive glue involves considerably less time for the doctor and less pain for the person with the cut. The risk for infections (1.1%) is the same for both. Adhesive glue should not be used in areas of high tension or repetitive movements, such as joints or the posterior trunk. [14]

• Dressings

The effectiveness of dressings and creams containing silver to prevent infection or improve healing is not currently supported by evidence. [15]

❖ ANTIBIOTICS

Prophylactic antibiotic therapy is preferred for wound infection. The length of antibiotic therapy may vary, but will be for at least one week. The pus from wound may be tested to isolate the organism in order to determine the sensitivity pattern. [16] First patient should be treated with empirical antibiotic therapy then patient should specific Antibiogram according to culture and sensitivity testing. Some wounds are infected with methicillin-resistant Staphylococcus aureus (MRSA), which is resistant to commonly used antibiotics. A MRSA infection will need a specific antibiotic to treat it.

OTHER MEASURES

Invasive procedure for the treatment of wound especially in diabetic foot ulcer, septic arthritis e.t.c is preferred. [17]

MATERIALS AND METHODS

42 diabetic patients with foot ulcers were included in this study, which was conducted for a period of 5 months at the Karuna medical college palakkad. A clinical history was elicited with regards to the duration of diabetes, the type of treatment that the patient received and the presence of other systemic illnesses. The samples were collected after obtaining informed consents from the patients. Samples were collected from the deeper portion of the ulcers by using 2 sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and the other was...
used for culture. A direct Gram stained smear of the specimen was examined. The specimens were inoculated onto blood agar, chocolate agar, Chapman agar and eosine methylene blue agar. The inoculated plates were incubated at 37°C overnight and the plates were examined for growth, the next day. The further processing was done according to the nature of the isolate, as was determined by Gram staining and the colony morphology. The organisms were identified on the basis of their Gram staining properties and their biochemical reactions. The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method.

2. OBSERVATION AND RESULTS
42 cases were reported from both Male & Female surgery ward during the study period and were analysed on the basis of evaluation parameters.

Gender wise distribution of patients:
(Table 1 and Fig. 1) shows the gender wise prevalence of patients with Foot infection among the population in the hospital. Out of 42 patients 24 (57.1%) were males and 18 (42.8%) were females.

![Figure 1: Gender wise Distribution](image)

<table>
<thead>
<tr>
<th>Gender</th>
<th>No of Cases (n=42)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>24</td>
<td>57.1</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>42.8</td>
</tr>
</tbody>
</table>

Age wise distribution of patients:
(Table 2 and Fig. 2) show the age wise prevalence of patients with foot infection among the population in hospital. Out of 42 patients age group ranging from 50-59 (n = 13) shows more predominant with foot infections followed by 60-69 & 20-29 age groups (n = 9).

![Figure 2: Age wise Distribution](image)

<table>
<thead>
<tr>
<th>Age</th>
<th>No of Cases (n=42)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>9</td>
<td>21.4</td>
</tr>
<tr>
<td>30-39</td>
<td>6</td>
<td>14.2</td>
</tr>
<tr>
<td>40-49</td>
<td>5</td>
<td>11.9</td>
</tr>
<tr>
<td>50-59</td>
<td>13</td>
<td>30.9</td>
</tr>
<tr>
<td>60-69</td>
<td>9</td>
<td>21.4</td>
</tr>
</tbody>
</table>

Distribution of isolated organism
(Table 3 and Fig. 3) show distribution of isolated organism in foot infection. The most predominant organism isolated was S. aureus, which contribute 30.9% (n=13) cases. E. coli was found to be the second most predominant organism isolated, 10% (n=23.8).

![Figure 3: Distribution of Gram Positive & Gram Negative Organism](image)

<table>
<thead>
<tr>
<th>Organism Isolated</th>
<th>No of Cases (n=42)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Aureus</td>
<td>13</td>
<td>30.9</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>23.8</td>
</tr>
<tr>
<td>Klebisella spp</td>
<td>5</td>
<td>11.9</td>
</tr>
</tbody>
</table>
SENSITIVITY PATTERN OF S. AUREUS (Fig. 4 & Table 4) show the sensitivity pattern of staphylococcus Aureus.

![Figure 4: Sensitivity Pattern of S. Aureus](image)

Table 4: Sensitivity Pattern of S. Aureus

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity %</th>
<th>Resistance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>53.8</td>
<td>0</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>7.69</td>
<td>0</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>0</td>
<td>38.4</td>
</tr>
</tbody>
</table>

Figure 5: Sensitivity Pattern of E. Coli.

![Figure 5: Sensitivity Pattern of E. Coli.](image)

Table 5: Sensitivity Pattern of E. Coli

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity %</th>
<th>Resistance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

3. DISCUSSION

Our study showed a predominance of male gender which is contrary to the literatures and 50-59 yrs age group shown more prevalence to infectious wounds. [18] Diabetes Mellitus is found to be the common risk factor for infectious wounds, and diabetic foot ulcers were more common. [19-43] Out of the total number of cases, Gram negative organisms (15%) are more prevalent than Gram positive (13%) Among the Gram negative organisms E.coli (23.8%) was the most predominant followed by Klebisella spp (11.90%) respectively.[44] Among Gram Positive Organism S.aureus (30.9%) was predominant.As for Linezolid (53.8%) was the most effective antibiotic against Staphylococcus Aureus Isolates and Levofoxacin (7.69%) was the least effective antimicrobial and more resistant to Co-trimoxazol (38.4%). [45] As for E.coli, Amikacin is more sensitive (60%) than Co-trimoxazole and Gentamycin. Ciprofloxacin and Amoxicillin (50%) were the most resistant Antibiotics. [46] Knowledge of the antibiotic susceptibility pattern of the
isolates from diabetic foot infections is crucial for planning the appropriate treatment of these cases prior to getting the susceptibility reports from the laboratory. Periodic surveillance of bacteria and antibiotic susceptibility is important to prevent further emergence and spread of resistant bacteria pathogens. [47]

4. CONCLUSION

- Wound infectious are one of the most hospital acquired infection and are important cause of morbidity and mortality.
- Most prevalent organism was found to be S. aureus followed by E. coli, Klebsiella spp.
- Gram negative organism was found more predominant
- The Gram positive organism was found to be more sensitive to Linezolid followed by Levofloxacin.
- Most of the gram negative organisms was found to be sensitive to Amikacin followed Ciprofloxacin.

REFERENCES


40. S. V. Upasani, P. V. Ingle, P. H. Patil, R. Y. Nandedkar, V. S. Shah, S. J. Surana Traditional


