

Study of microbiological profile of diabetic foot infections at a tertiary care hospital

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Abstract

Background: Diabetic foot infections (DFIs) are associated with major morbidity, increasing mortality, high costs, increased risk of lower extremity amputation (LEA), and reduced quality of life. The pathophysiology of diabetic foot infections is complex consequences of host-related complications (immunopathy, neuropathy and arteriopathy) and pathogen-related factors (virulence, antibiotic resistance and microbial load). Present study was aimed to microbiological profile of diabetic foot infections at a tertiary care hospital. **Material and Methods:** Present study was single-center, prospective, observational study, conducted in diabetic patients, previously diagnosed or newly diagnosed as diabetics and presenting with lower extremity infection. Results of the culture and antimicrobial sensitivity testing were documented. **Results:** In present study, 98 cases of diabetic foot infections were studied, Majority cases were from 51-60 years age group (41.84 %) followed by 41-50 (28.57 %) & 61-70 (19.39 %) years age group. Mean age of study patients was 52.7 ± 11.3 years. Male (68.37 %) outnumbered female cases (31.63 %). Majority patients had polymicrobial flora (55.1 %) as compared to monomicrobial flora (39%). In present study 21.43 % cases had no growth. Among gram positive organisms majority were staphylococcus aureus (29.59 %), Group B Streptococci (9.18%) & Enterococcus faecalis (2.04 %). While among gram negative organisms pseudomonas aeruginosa (16.33 %), klebsiella pneumoniae (9.18 %) & escherichia coli (7.14 %) were most common organisms. **Conclusion:** Most of specimens were poly microbial infection and predominant bacteria were S. aureus and Pseudomonas sp. All bacterial isolate in our study have sensitivity for Levofloxacin, Pipracillin- Tazobactam, Linezolid, and Meropenem.

Keywords: Diabetic foot; ulcer; infection; microbiology; antibiotic; treatment

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Introduction

Diabetes mellitus is associated with a series of macrovascular and microvascular complications. The complications include diabetic nephropathy, diabetic retinopathy, diabetic foot ulcers, peripheral vascular disease, ischemic heart disease and cerebrovascular disease.¹ It is estimated that 15-25% of diabetic patients have diabetic foot ulcers owing to risk factors such as peripheral vascular disease, impaired immune system and peripheral neuropathy.^{2,3} Diabetic foot infections (DFIs) are associated with major morbidity, increasing mortality, high costs, increased risk of lower extremity amputation (LEA), and reduced quality of life.⁴ Infection is most often as a consequence of foot ulceration, which typically follows trauma to a neuropathic foot. Poorly controlled diabetes is prone to skin infections because elevated

blood sugar reduces the effectiveness of bacteria fighting cells due to reduced resistance and immunocompromised situation.^{5,6}

The pathophysiology of diabetic foot infections is complex consequences of host-related complications (immunopathy, neuropathy and arteriopathy) and pathogen-related factors (virulence, antibiotic resistance and microbial load).⁷ Present study was aimed to microbiological profile of diabetic foot infections at a tertiary care hospital.

Material And Methods

Present study was single-center, prospective, observational study, conducted in Department of Microbiology, Dr Ulhas Patil Medical College & Hospital, Jalgaon, India. Study duration was of 2 years (January 2020 to December 2021). Study approval was obtained from institutional ethical committee.

Inclusion criteria

- Diabetic patients, previously diagnosed or newly diagnosed as diabetics and presenting with lower extremity infection, willing to participate in present study

Exclusion criteria

- Patients with neuropathy other than diabetic neuropathy
- Patients with acute limb ischemia.
- Patients not willing to participate
- Inadequately collected sample.

Study was explained to patients in local language & written consent was taken for participation & study. Patients details such as age, sex, type of diabetes, duration of diabetes mellitus and duration of foot infection were recorded. Various specimens (pus, wound exudates or tissue biopsy) for microbiological study were obtained from ulcer region.

Surface of the ulcer region was rinsed with sterile normal saline and the pus was collected with sterile cotton swab. Sterile cotton swab sticks were moistened with sterile normal saline before collecting the specimens. The swab sticks were extended deeply into the depth of the lesion avoiding touching of surrounding skin area around the wound. The collected samples were immediately transported to the microbiology department. The specimens were cultured on Nutrient agar, MacConkey agar, Blood agar and UTIchrom agar. The plates were then aerobically incubated at 37°C for overnight. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests.

Antibiotic sensitivity test was carried out with following antibiotics such as ampicillin (10 mcg), amikacin (30 mcg), gentamicin (10 mcg), ofloxacin (5 mcg), ciprofloxacin (5 mcg), ceftazidime (30 mcg), imipenem (10 mcg), cefotaxime (30 mcg), ceftriaxone (10 mcg), methicillin (5 mcg), erythromycin (15 mcg), clindamycin (2 mcg), vancomycin (30 mcg) and amoxicillin/clavulanic acid (20/10 mcg) were used to determine antibiotic susceptibility pattern. Isolated colonies were picked up from a fresh isolation plate, inoculated on Trypticase Soya broth medium and incubated for 2 to 6 hrs at 37°C until good visible growth. A lawn of test pathogen was prepared by evenly spreading with the surface of the agar plate. The plates

were allowed to dry before applying antibiotic disc. The antimicrobial discs were placed at equal distance and the discs were pressed gently with forceps. After 16-18 hrs incubation of the plates at 37°C, the zone of inhibition were read with metallic rulers in mm and interpreted using standard zone of inhibition charts.

Results of the culture and antimicrobial sensitivity testing were documented. Collected data was entered in Microsoft excel sheet & analysed. Statistical analysis was done using descriptive statistics.

Results

In present study, 98 cases of diabetic foot infections were studied, Majority cases were from 51-60 years age group (41.84 %) followed by 41-50 (28.57 %) & 61-70 (19.39 %) years age group. Mean age of study patients was 52.7 ± 11.3 years. Male (68.37 %) outnumbered female cases (31.63 %). Common factors likely to be responsible for diabetic foot infections were ill-fitting shoes/ shoe related factors (35.71 %) & Trivial trauma (19.39 %).

Table 1: General characteristics

| Characteristics | No. of patients | Percentage |
|---|-----------------|------------|
| Age groups (in years) | | |
| 21-30 | 0 | 0 |
| 31-40 | 2 | 2.04 |
| 41-50 | 28 | 28.57 |
| 51-60 | 41 | 41.84 |
| 61-70 | 19 | 19.39 |
| 71-80 | 8 | 8.16 |
| Mean age (mean \pm SD) | 52.7 ± 11.3 | |
| Gender | | 0 |
| Male | 67 | 68.37 |
| Female | 31 | 31.63 |
| Factors likely to be responsible | | |
| Ill-fitting shoes/ shoe related factors | 35 | 35.71 |
| Cause not known | 21 | 21.43 |
| Trivial trauma | 19 | 19.39 |
| Barefoot walking | 13 | 13.27 |
| Corn / in-growing nail etc. | 10 | 10.2 |

According to Wagner's classification in majority diabetic foot patient had grade 2 infection (44.9 %), followed by grade 1 & 3 with 29.59 % & 17.35 % patients respectively.

Table 2: Wagner's classification in diabetic foot patients.

| Grade | Clinical signs | No. of patients | Percentage |
|-------|--|-----------------|------------|
| 0 | Intact skin | 0 | 0 |
| 1 | Superficial ulcer of skin/ subcutaneous tissue | 29 | 29.59 |
| 2 | Ulcer extending to tendon/ bone/ capsule | 44 | 44.9 |
| 3 | Deep ulcer with osteomyelitis/ abscess | 17 | 17.35 |
| 4 | Gangrene of toes/ forefoot/ localized gangrene | 6 | 6.12 |
| 5 | Mid foot/ hind foot gangrene | 2 | 2.04 |

Majority patients had polymicrobial flora (55.1 %) as compared to monomicrobial flora (39%). In present study 21.43 % cases had no growth. Among gram positive organisms majority were staphylococcus aureus (29.59 %), Group B Streptococci (9.18%) & Enterococcus faecalis (2.04 %). While among gram negative organisms pseudomonas aeruginosa (16.33 %), klebsiella pneumoniae (9.18 %) & escherichia coli (7.14 %) were most common organisms.

In vitro sensitivity of antimicrobial agents against Gram positive bacteria was done. Linezolid, vancomycin, clindamycin, gentamicin were most effective antimicrobial agents against staphylococcus aureus & streptococci. In vitro sensitivity of antimicrobial agents

against gram negative bacteria Meropenem, piperacillin tazobactam, Levofloxacin & gentamicin were most effective antimicrobial agents against pseudomonas aeruginosa, klebsiella pneumoniae & escherichia coli.

Table 3: Culture reports

| Culture reports | Number | Percentage |
|-------------------------|--------|------------|
| Bacterial flora | | |
| Monomicrobial | 23 | 23.47 |
| Polymicrobial | 54 | 55.1 |
| Organism isolated | | |
| Gram positive organisms | | |
| Staphylococcus aureus | 29 | 29.59 |
| Group B Streptococci | 9 | 9.18 |
| Enterococcus faecalis | 2 | 2.04 |
| Gram negative organisms | | |
| Pseudomonas aeruginosa | 16 | 16.33 |
| Klebsiella pneumoniae | 9 | 9.18 |
| Escherichia coli | 7 | 7.14 |
| Acinetobacter baumannii | 5 | 5.1 |
| Citrobacter sp | 4 | 4.08 |
| Proteus sp | 3 | 3.06 |
| No growth | 21 | 21.43 |

Discussion

Diabetic foot infection (DFI), a complication associated with diabetes mellitus, is a major public health problem and it is the main reason many of the diabetic patient admission.⁸

Ischaemia, neuropathy, and infection are the three cardinal aetiological factors predisposing to diabetic foot ulcers. Diabetic foot infections may be extremely challenging to cure, due to late diagnosis (due to blunted clinical signs), presence of ischaemia, difficult to-treat multidrug-resistant pathogens, and spread of infection to the bones, leading to osteomyelitis. For the treatment of DFI, the combination of debridement and antibiotics, coupled with good nutrition and diabetic control is paramount.⁹

Diabetic foot ulcers are not spontaneous ulcers, but results from the interplay of various factors line neuropathy, autonomic neuropathy, and peripheral vascular disease, superimposed with alterations in the plantar pressure, defective footwear and limited joint mobility. Most of these infections are polymicrobial in nature and mixed organisms are frequently encountered. However, the spectrum of microorganisms depends mainly on microbial flora of lower limb, metabolic factors, foot hygiene and the use of antibiotics.¹⁰ Diabetic foot infections may be extremely challenging to cure, due to late diagnosis (due to blunted clinical signs), presence of ischemia, difficult to-treat multidrug-resistant pathogens, and spread of infection to the bones, leading to osteomyelitis.

Dushyant Singh et al.,¹¹ studied 61 cases of diabetic foot ulcers, non-limb threatening infections were seen in 38 patients (62.29%) and the limb threatening was seen in 23 patients (37.7%). Of these 23 patients with limb threatening infections, 14 (60.87%) had to undergo amputation during one-year follow-up. The organisms isolated from the cultures included Staphylococcus sp in 15 patients (28.84%), Streptococcus sp in 11 (21.15%), Pseudomonas sp in 8 (15.38%), Enterococcus sp in 30 (57.69%) and anaerobes in 27 patients (51.92%). The antibiotics to which they were found to be most sensitive included Amoxy-Clav 49 (94.23%),

Ceftriaxone 48(92.30%), Ceftazidime 41 (78.84%), Cefurexime 46 (88.46%), Chloramphenicol 44(84.61%), Amikacin 46(88.46%) and Polymixin-B 46 (88.46%).

In study by Ankur Kumar et al.,¹² 134 pathogens were isolated from 100 patients, an average of 1.34 organisms per lesion. The most frequently isolated pathogens were Gram-negative bacteria (56.7%), including *Pseudomonas aeruginosa* (22.4%), *Escherichia coli* (17.9%), *Klebsiella pneumonia* (15%) and *Proteus sp.* (1.5%). Gram positive bacteria accounted for 40.3% of all bacterial isolates. *Staphylococcus aureus* was predominant (32.8%) among Gram positive bacteria, followed by streptococci (4.5%) and Coagulase Negative *Staphylococcus* (2.9%). The antimicrobial susceptibility testing, showed that vancomycin and linezolid were the most effective drugs against gram positive organisms and imipenem was the most effective drug against gram negative organisms.

In study by Anand A et al.¹³, mean age of the patients was 52.42 years. The male: female ratio is 3.5:1. The WAGNER grade of III was seen in 48% of the patients, grade I in 16%, grade II in 14%, grade IV in 14% and grade V in 8%. 100% of the patients with grade IV and V underwent amputation, while 8.3% with grade III and 4.16% with grade II had to undergo amputation and none with grade I had to receive amputation. 68 % of these patients had been diagnosed with Diabetes for less than 10 years while the rest have been diagnosed for more than 10 years. 60% of patients were found to have peripheral neuropathy out of which 40% were sensory type and the rest were motor type. *Proteus mirabilis* was isolated in 80% of the patients who underwent amputation, *Streptococcus pyogenes* in 57%, *Pseudomonas aeruginosa* (50%), *Klebsiella pneumonia* (33%), and *Staphylococcus aureus* (12%).

Vasanthan K et al.,¹⁴ studied 253 patients, 169 males and 84 females. 65 patients presented with Grade I ulcer, 175 with Grade II ulcer, and 13 had Grade III ulcer. 12 patients required ICU care and 241 patients were managed in the ward. The mean age was 57.57. Mean fasting and post-prandial sugars were 157.48 and 244.21, respectively. The mean HbA1c was 9.49 with a mean duration of hospital stay of 12.44 days. 40 patients grew *Staphylococcus aureus*, 40 patients grew coagulase-negative *Staphylococcus* (CONS), 28 *Escherichia coli*, 20 *Streptococcus* species, 20 *Enterococcus* species, 10 *Proteus* species, 12 *Klebsiella* species, 25 *Pseudomonas* species, and 6 *Candida* species. Polymicrobial growth was seen in 26 patients. 25 patients had no growth in cultures. A majority of *S. aureus* was sensitive to penicillin and cloxacillin (MRSA was found in two patients), *Streptococcus* to penicillin and clindamycin, CONS to clindamycin and linezolid, and *Enterococcus* was sensitive to linezolid and ampicillin.

Multidrug resistant organisms is a potential risk factor in management of diabetic foot infections which may lead to devastating complications like systemic toxicity, gangrene formation and may herald amputation of lower extremity.^{15,16} Definitive therapy should be based on cultures of infected tissue. Imaging is especially helpful when seeking evidence of underlying osteomyelitis, surgical interventions of various types are often needed and proper wound care is important. Patients with a DFI should be evaluated for an ischemic foot, and employing multidisciplinary foot team improves outcomes.

Conclusion

Most of specimens were poly microbial infection and predominant bacteria were *S. aureus* and *Pseudomonas sp.* All bacterial isolate in our study have sensitivity for Levofloxacin, Piperacillin- Tazobactam, Linezolid, and Meropenem. These wounds may require use of combined antimicrobial therapy for initial management.

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