**Original Research Article** 

# Assessment of major microorganisms involved in diabetic foot infection which delays wound healing

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### Abstract

**Background:** India has the largest diabetic population of 50.8 million that could reach an epidemic proportion by 2030. Diabetic foot infection is one of the dreaded complications of diabetes. Only a few studies that focus on patterns of diabetic foot infection in our region, where diabetic foot care is inadequate. This study evaluated microbial and clinical characteristics of diabetic foot infections that will be helpful in taking appropriate measures for their management.

Aim and objectives: This study was undertaken to evaluate the clinical and the microbial characteristic of diabetic foot infection in patients. The ulcers were graded and swab samples were collected under aseptic condition.

**Materials and methods:** In this prospective study conducted during 2016-2017, Totally 100 diabetic foot patients underwent detailed history, clinical examination, and laboratory investigations including parameters of systemic infections. Microbial culture and sensitivity were performed at the time of presentation.

**Results:** Among 100 cases, 27 (43.5%) had mono-microbial infection, 22 (35.5%) had poly-microbial infection, and 13 (21%) had sterile culture. Altogether 82 bacteria were isolated from 49 cases. Among 82 bacterial isolates, 56 (68.3%) were Gram negative while 26 (31.7%) were Gram-positive bacteria. Escherichia coli was the most common pathogen isolated followed by Staphylococcus aureus.

**Conclusion:** Gram-negative bacteria were most prevalent in diabetic foot infection. It is not uncommon to have culture reports negative despite clinical evidence of infection. This study suggests that piperacillin/ tazobactam should be the treatment of choice on an empirical basis prior to a definitive bacteriological study and in cases with negative culture reports.

### Key words

Antimicrobial susceptibility, Bacterial profile, Diabetic foot ulcer, Staphylococcus aureus, Pseudomonas aeruginosa.

### Introduction

Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalisation in diabetic patients [1]. Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration Diabetic ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes. Every year more than a million diabetic patients require limb amputation [2]. The impaired microvascular circulation in patients with diabetic foot limits the access of phagocytes favouring development of infection Escherichia coli, Proteus spp. Pseudomonas spp., Staphylococcus aureus and Enterococcus spp. are the most frequent pathogens contributing to progressive and widespread tissue destruction [3]. Diabetic foot infections are often polymicrobial. Methicillin -resistant Staphylococcus aureus (MRSA) has been commonly isolated from 10-40% of the diabetic wounds. The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation [4].

### Materials and methods

### **Collection and Processing of Sample**

This prospective study was conducted involving diabetic foot infection patients of both sexes, clinically suspected of having microbial infections in their foot presenting at outpatient unit of a private hospital, in cuddalore district from 2016-2017 for a period of one year. The institutional ethical clearance was obtained. The clinical history of the patients such as age, sex, types of diabetes, duration of diabetes, size of ulcer and duration of ulcer were recorded on a proforma. The ulcers were graded according to the Wagner's grade classification. A total of hundred swabs were collected and processed for bacteriological investigations. To eliminate the

possibility of isolating colonizing bacteria, superficial ulcers of Wagner's grade 1 were excluded from the study. After rinsing the wound area with saline and debriding the dead tissue, swab/tissue samples were collected aseptically from the wound site using a sterilized punch biopsy needle (6 mm) under local anaesthesia and placed in a sterile vial containing phosphate buffered saline. Photographs of the wound area were also taken to document depth, ischemic changes, and characteristics of the diabetic foot wound. All the above tests were performed on the day of enrolment.

## Microbiology and antibiotic susceptibility tests

Culture materials from all the wounds were obtained; either by washing the wound with sterile physiological saline and then making a puncture-aspiration from the base of the wound or by applying a sterile cotton swab to the wound .Specimens were sent to the laboratory and processed for aerobic bacteria. To minimize bias, laboratory technicians were kept blind to the clinical data. Anaerobic cultures were disregarded because of the lack of technical and logistical support [5, 6].

### Statistical methods

Quantitative variables were expressed as means  $\pm$ SD while qualitative variables were expressed as percentages. Comparison of mean values was performed using the Student's t test.

### Results

Among 100 diabetic foot cases 55 males and 45 females were included in this study. The mean age of cases was 52.4 ( $\pm$  11.6) years. The duration of diabetes ranged from less than a year to 15 years with a mean duration of 8.9 ( $\pm$  5.5) years. The duration of diabetic foot ulcer varied from five days to one year and the cases enrolled were of Wagner's grade 2 to 4. Among 100

cases, 27 (43.5%) had mono-microbial infection, 22 (35.5%) had poly-microbial infection, and 13 (21%) had sterile culture. Altogether 82 bacteria were isolated from 49 cases. Among 82 bacterial isolates, 56 (68.3%) were Gram negative while (31.7%) were Gram-positive bacteria. 26 Escherichia coli was the most common pathogen isolated followed by Staphylococcus aureus. Other commonly isolated bacteria were Pseudomonas aeruginosa, Streptococci, Proteus mirabilis, Citrobacter sp., Proteus vulgaris, Klebsiella pneumoniae, Bacillus sp., Morganella sp., Acinetobacter sp., Enterococcus faecalis, Klebsiella oxytoca, Enterobacter aerogenes, Coagulase -ve Staph, Pneumococcus, and Enterococci. Co-infection with Candida spp. was also found in one case with Gram- negative infection (E. coli). In another case with a sterile culture report, the wound was foul smelling and full of maggots. Gram-negative infection was common (74%) in mono-microbial most infections, whereas both Gram-positive and Gram- negative were high (63.6%) in cases with poly- microbial infection. Isolated bacteria showed differential sensitivity patterns against commonly used antibiotics. The majority of the isolates were resistant to several antibiotics that are usually prescribed on an empirical basis. Antibiotic sensitivity of the isolated microbes showed highest sensitivity for piperacillin/ tazobactum, followed by amikacin, gentamycin, levofloxacin, and azithromycin.

### Discussion

Diabetic foot ulcers are common and serious complications of chronic DM. In parallel with increased prevalence of DM, the prevalence of foot infection are increasing In the patients included in this study the duration of DM and foot infection were found to be more than a year (92.3%) and less than a month (59%)respectively [7]. The level of their mean glycosylated haemoglobin was high. Therefore it was confirmed that the complications of DM were seen in the patients who had irregular glucose levels. Hyperglycemias and other derangements metabolic cause impaired immunological (especially neutrophil) function and wound healing and excess collagen crosslinking [8]. But earlier studies have documented gram-positive bacteria as the predominant organisms associated with diabetic foot infections Therefore, there seems to be a changing trend in the organisms causing diabetic foot infections, with gram-negative bacteria replacing gram-positive bacteria as commonest agents. Polymicrobial infection was observed in 52% patients, which is similar to other studies. In cases of chronic and deep foot ulcers with tissue necrosis and gangrene or in patients with recent antibiotic treatment has failed infection usually occurs with 3-5 different species of bacteria [9]. Anaerobic bacteria are almost always isolated with aerobes from diabetic foot infections. Gramnegative anaerobes were recovered from 12 (8.1%) patients and in mixed infections. In our study the ulcers were mostly on the distal phalanges. Infections of the lower extremities in diabetic patients commonly occur on the plantar surface of the forefoot, in particular the toes and metatarsal heads. Enterococci are considered commensals with low virulence except in compromised patients, such as diabetics, in whom they can act as opportunistic pathogens. All the Enterococcus spp. was susceptible to vancomycin, though they showed varying susceptibility to other antibiotics. Similarly, in another study all enterococcal isolates were noted to be uniformly susceptible to vancomycin. Hence, vancomycin can be considered as an important drug in the empirical regimen for treatment of diabetic foot infections especially in settings with high resistance to other antibiotics. Anaerobic bacteria are almost always isolated with aerobes from diabetic foot infections. Gramnegative anaerobes were recovered from 12 (8.1%) patients and immixed infections [10].

### Conclusion

From our study, we conclude that prevalence of Gram-negative infection was higher in diabetic foot patients from our region. In cases of polymicrobial infection, coexistence of Gramnegative and Gram-positive microorganisms was

more common. Piperacillin- tazobactum showed the highest sensitivity and it may be started empirically based on the clinical characteristics of infection, and can be changed subsequent to learning the results from а definitive bacteriological study. 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 [11, 12].

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