

Original research article**A microbiological study of patients with diabetic foot****¹Lahiri Somsubhra, ²Narmadha E, ³Reddy Spoorti Channa**^{1,2}Assistant Professor, Department of Microbiology, Anna Medical College, Mauritius³Junior Resident, Department of Medicine, Dr. S.S. Tantia Medical College, Hospital and Research Centre, Sri Ganganagar, Rajasthan, India**Corresponding Author:**

Dr. Sudheer Kumar Reddy K

Abstract

One of the most feared complications of diabetes is diabetic foot, which is also the main cause of hospitalisation in diabetic individuals. In order to pick the appropriate antibiotics for the effective management of these illnesses, it is essential to have a solid understanding of the prevalent microorganisms that have been isolated and their respective antibiograms.

Keywords: Diabetic foot, polymicrobial, gram negative bacteria, susceptibility pattern

Introduction

Diabetes mellitus (DM) is a significant contributor to mortality and morbidity on a global scale. Patients with diabetes have a risk of foot ulcers that can reach as high as 25% throughout the course of their lives [1]. Ulcers on the foot caused by diabetes have a 15 to 46 times higher risk of amputation than ulcers on the foot caused by other causes [2]. Amputation of a leg is necessary for more than one million diabetic patients each and every year [3]. One of the more difficult and expensive effects of diabetes is diabetic foot. It is one of the problems of diabetes that causes the most anxiety, and it is also the primary reason that diabetic patients end up in the hospital. It is distinguished by a number of clinical consequences, including neuropathy, peripheral vascular disease, foot ulcers, and infection with or without osteomyelitis, which can lead to the development of gangrene and may even be necessary to amputate a limb. Infections of the diabetic foot are frequently caused by several microorganisms [5, 6]. It has been demonstrated that the most common pathogens identified from diabetic foot infections are *Escherichia coli*, *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus*, and *Enterococcus* spp. [4, 5] Methicillin-resistant *Staphylococcus aureus* (MRSA) has been routinely isolated from 10-40% of diabetic lesions in recent years [7, 8]. It is essential to have an understanding of the prevalent pathogens that have been isolated as well as their anti-biograms in order to choose the most appropriate medicines for the effective therapy of these illnesses. Therefore, the purpose of this study was to identify the prevalent bacteria that were isolated from diabetic foot infections at a tertiary hospital and to evaluate their in vitro susceptibility to commonly prescribed antibiotics.

Materials and Methods

For the purpose of this retrospective study, of patients suffering from diabetic foot infections who had been seen at a tertiary care teaching hospital over the course of two years were included. All the records were maintained in the MRD. In the processing of the specimens, they had obtained pus or discharges from the ulcer base as well as necrotic tissue that had been debrided. The samples were analysed without any lag in time because they were brought to the microbiology laboratory as soon as possible. Gramme staining was performed on the specimens, and they were simultaneously inoculated on blood agar and MacConkey agar for the purpose of isolating aerobic bacteria. Standard bacteriological techniques were used to determine the identities of the bacterial isolates following an incubation period of 24 hours at 37 degrees Celsius. Standard biochemical tests were used to determine the identities of the isolates based on the colony characteristics observed on Blood agar and MacConkey agar [9]. Evaluation of the patient's resistance to antibiotics The Kirby Bauer disc diffusion method was utilised for the antibiotic susceptibility testing that was carried out in accordance with the recommendations provided by the Clinical Laboratory Standards Institute (CLSI).10 Ciprofloxacin (5 micrograms), ofloxacin (5 micrograms), amikacin (30 micrograms), co-trimoxazole (1.25/23.75 micrograms), ceftazidime (30 micrograms), cefepime (30 micrograms), imipenem (10 micrograms), piperacillin + tazobactam combination (100 micrograms plus 10 micrograms) and colistin (10 micrograms) disc was utilised. In order to identify *Staphylococcus* species, a number of antibiotics, including penicillin, amoxicillin-clavulanic acid, cefoxitin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, linezolid, and vancomycin, were put through their paces. Isolates of *Staphylococcus aureus* were tested with cefoxitin to determine whether or not they were resistant to methicillin, as recommended by CLSI. The members of the Enterobacteriaceae family were tested using a combination disc approach that

included both cefotaxime and ceftazidime, both on their own and in combination with clavulanic acid. This was done in order to identify the presence of extended spectrum beta-lactamase (ESBL). Confirmatory evidence of ESBL generation was determined to exist when there was an expansion of at least five millimetres in the zone of inhibition for either the cefotaxime- clavulanic acid or the ceftazidime-clavulanic acid disc.

Results

Table 1: Age and sex distribution of CSOM

| Age | Males 97(43.7%) | Females 125(56.3%) |
|----------|-----------------|--------------------|
| 0-25yrs | 55(56.7%) | 62(49.6%) |
| 26-50yrs | 32(32.9%) | 45(36%) |
| 51-75yrs | 10(10.3%) | 18(14.4%) |

Table 2: Distribution of various isolates in CSOM

| Microbes | No. of isolates |
|-------------------------|-----------------|
| S. aureus | 85(37.9%) |
| <i>Pseudomonas</i> spp. | 62(27.6%) |
| <i>Proteus</i> spp. | 29(12.9%) |
| <i>Klebsiella</i> spp. | 20(8.9%) |
| E Coli | 15(6.6%) |
| <i>Citrobacter</i> spp. | 11(4.9%) |

Table 3: Sensitivity pattern of *S. aureus*

| Antibiotic | Sensitivity % (totalno. of <i>S. aureus</i> = 85) |
|----------------|--|
| Ciprofloxacin | 56.4%(48) |
| Erythromycin | 51.7%(44) |
| Clindamycin | 47%(40) |
| Gentamicin | 87%(74) |
| Amikacin | 96.4%(82) |
| Amoxyclav | 70.5%(60) |
| Co trimoxazole | 52.9%(45) |
| Cefixime | 58.8%(50) |

Table 4: Antibiotic sensitivity pattern of Gram negative bacilli

| Antibiotic | Sensitivity % (total no. of GNB=137) |
|-------------------------|--------------------------------------|
| Amoxyclav | 47.4%(65) |
| Amikacin | 91.9%(126) |
| Ceftriaxone | 53.2%(73) |
| Cefoperazone-salbactum | 71.5%(98) |
| Cefixime | 67.8%(93) |
| Cotrimoxazole | 61.3%(84) |
| Aztreonam | 56.9%(78) |
| Gentamicin | 55.4%(76) |
| Meropenem | 100%(137) |
| Ciprofloxacin | 72.9%(100) |
| Piperacillin-tazobactum | 94.8%(130) |

Discussion

Patients with diabetes have an increased risk of developing chronic foot ulcers that do not heal completely due to a number of underlying causes, including neuropathy, elevated plantar pressures, and peripheral artery disease [11]. These people are susceptible to infection from a diverse array of bacterial species. In the current investigation, gram-negative bacteria made up 76 (74.5%), while gram-positive bacteria made up just 26 (25.5%). The bulk of the 76 gram-negative bacteria were E. coli, which accounted for 55.3% of the total, followed by K. pneumoniae, which accounted for 18.4% and Pseudomonas aeruginosa, which accounted for 15.8%. Studies conducted by Gadepalli *et al.* and Shankar *et al.* both found that gram-negative bacteria were the most common type of pathogen [2, 5]. Gram-positive bacteria have been identified as the primary organisms associated with diabetic foot infections, but only a small number of studies have done so [12, 13]. This suggests that the bacterial pattern of diabetic foot is shifting, and as a result, doctors need to be aware of the most recent etiological agents in order to manage this life-threatening consequence of diabetes mellitus. In order to provide effective therapy for cases, it is essential to have knowledge of the antibiotic susceptibility pattern of the isolates obtained from diabetic foot infections. The antibiotics amikacin and imipenem were effective against the vast

majority of *Escherichia coli* and *Klebsiella pneumoniae* strains isolated. The majority of the *Proteus* species tested positive for susceptibility to the antibiotics ciprofloxacin, ofloxacin, amikacin, piperacillin-tazobactam, and imipenem. Piperacillin-tazobactam, amikacin and imipenem were effective antimicrobial agents against *Citrobacter* spp. The majority of the *Pseudomonas aeruginosa* strains tested positive for susceptibility to piperacillin-tazobactam and imipenem. The piperacillin-tazobactam, imipenem and trimethoprim-sulfamethoxazole antibiotic combinations were able to kill the majority of the *Acinetobacter* species. An earlier study conducted in India indicated that all members of the Enterobacteriaceae family had the same level of sensitivity to gentamicin and ciprofloxacin ^[4]. A different study has also found evidence of growing drug resistance to these medications ^[5]. Because of this, the use of these antibiotics in diabetic foot infections on a trial-and-error basis should not be encouraged. However, it was shown that members of the Enterobacteriaceae family are vulnerable to amikacin, piperacillin-tazobactam and imipenem are the antibiotics that were used. Amikacin, linezolid, and vancomycin were the antibiotics that *Staphylococcus aureus* was most likely to respond favourably to. In our research, there were 14 cases of methicillin-resistant *Staphylococcus aureus* (MRSA), accounting for 77.8 percent. In their research, Umadevi et al. also cited 65.5% of MRSA samples as their source. On the other hand, the majority of the investigations cited a rate of MRSA isolation from such cases ranging from 10-44%.

Conclusion

Infections of the diabetic foot are almost always multimicrobial and are brought on by gram-negative bacteria. Continuous surveillance of drug-resistant microorganisms is required to establish a foundation for empirical treatment, as this is currently lacking.

References

1. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005;293:217-28.
2. Shankar EM, Mohan V, Premlatha G, Srinivasan RS, Usha AR: Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med* 2005; 16: 567–570
3. Khanolkar MP, Bain SC, Stephens JW. The diabetic foot. *QJM* 2008; 101: 685-95.
4. Anand C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M, et al. Bacteriology of diabetic foot lesions. *Indian J Med Microbiol* 2004; 22: 175-8.
5. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico- microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care* 2006; 29: 1727-32.
6. Frykberg RG. An evidence-based approach to diabetic foot infections. *Am J Surg* 2003; 186: 44S-54S.
7. Tentolouris N, Jude EB, Smirnof I, Knowles EA, Boulton AJ. Methicillin-resistant *Staphylococcus aureus*: an increasing problem in a diabetic foot clinic. *Diabet Med* 1999; 16:767-71.
8. Goldstein EJ, Citron DM, Nesbit CA. Diabetic foot infections. Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. *Diabetes Care* 1996; 19: 638-41.
9. Mackie TJ, McCartney JE. Practical medical microbiology. 14th ed. New York: Churchill Livingstone; 1996.
10. Clinical Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 6th ed. CLSI document M7-A6. Wayne, PA: CLSI; 2018.
11. Frykberg RG, Armstrong DG, Giurini J, Edwards A, Kravette M, Kravitz S, et al. Diabetic foot disorders: a clinical practice guideline. American College of Foot and Ankle Surgeons. *J Foot Ankle Surg* 2000; 39: S1-60.
12. Mantey I, Hill RL, Foster AV, Wilson S, Wade JJ, Edmonds ME. Infection of foot ulcers.