

A microbiological study of patients with diabetic foot at tertiary care centre

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Abstract

Background: Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients. It is important to know the common pathogens isolated and their antibiogram to select appropriate antibiotics for the proper management of these infections. **Aim:** To determine the common pathogens isolated from diabetic foot in a tertiary hospital and their susceptibility to routinely used antibiotics. **Material and Methods:** This prospective study included a total of 70 patients with diabetic foot infections. The bacterial agents were isolated and their antibiotic susceptibility pattern was determined. Members of Enterobacteriaceae were tested for extended spectrum β -lactamase (ESBL) production and Staphylococcal isolates were tested for MRSA production. **Results:** In 32 (45.7%) patients only one pathogen was isolated, while in 34 (48.6%) patients more than one pathogen was isolated. Gram-negative bacteria accounted for 76 (74.5%), while gram-positive bacteria accounted for 26 (25.5%). Of the 76 gram negative bacteria, majority were *E. coli* 42 (55.3%) followed by *K. pneumoniae* 14 (18.4%) and *Pseudomonas aeruginosa* 12 (15.8%). Majority of isolates of *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to amikacin and imipenem. **Conclusion:** Diabetic foot infections are mostly polymicrobial and caused by gram negative bacteria. There is a need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy.

Key Word: Diabetic foot, polymicrobial, gram negative bacteria, susceptibility pattern

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INTRODUCTION

Diabetes mellitus (DM) is a major global cause of morbidity and mortality. Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration.¹ Diabetic ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes.² Every year more than a million diabetic patients require limb amputation.³ Diabetic foot is a complex and costly complications of DM. It is one of the most feared

complications of diabetes and is the leading cause of hospitalization in diabetic patients. It is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation.^{1,4} Diabetic foot infections are often polymicrobial.^{5,6} It has been shown that *Escherichia coli*, *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Enterococcus* spp. are the most frequent pathogens isolated from diabetic foot infections.^{4,5} Methicillin-resistant *Staphylococcus aureus* (MRSA) has been commonly isolated from 10-40% of the diabetic wounds.^{7,8} It is important to know the common pathogens isolated and their antibiogram to select appropriate antibiotics for the proper management of these infections. So, this study was performed to determine the common pathogens isolated from diabetic foot in a tertiary hospital and their in vitro susceptibility to routinely used antibiotics.

MATERIAL AND METHODS

This prospective study included a total of 70 patients with diabetic foot infections visited tertiary care teaching hospital over a period of two years. Processing of specimens Pus or discharges from the ulcer base and debrided necrotic tissue were obtained. The specimens were taken immediately to the microbiology laboratory and processed without any delay. The specimens were subjected to Gram staining and were simultaneously inoculated on blood agar and MacConkey agar for isolation of aerobic bacteria. After 24 hours incubation at 37°C, the bacterial isolates were identified based on standard bacteriological methods. The isolates were identified based on colony characters on Blood agar and MacConkey agar by standard biochemical tests.⁹ Antibiotic susceptibility testing Antibiotic susceptibility testing was performed by Kirby Bauer's disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.¹⁰ The discs used were ciprofloxacin (5µg), ofloxacin (5µg), amikacin (30 µg), co-trimoxazole (1.25/23.75µg), ceftazidime (30µg), cefepime (30µg), imipenem (10µg), piperacillin+tazobactam combination (100µg+10µg) and colistin (10µg) disc was used. Penicillin, amoxicillin-clavulanic acid, cefoxitin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, linezolid and vancomycin were tested for Staphylococcus species. Staphylococcus aureus isolates were screened for methicillin resistance using cefoxitin according to CLSI guidelines.¹⁰ Combination disc method using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid was performed for detection of extended spectrum β-lactamase (ESBL) among the members of Enterobacteriaceae. Five mm or more increase in zone of inhibition for either cefotaxime-

clavulanic acid or ceftazidime- clavulanic acid disc was taken as confirmatory evidence of ESBL production.

RESULTS

Of the 70 patients with diabetic foot, 52 (74.3%) were male and 18 (25.7%) were female. The age ranged from 32 to 73 years with mean age being 46±8 years. A total of 102 bacteria were isolated from these 70 patients. In 32 (45.7%) patients only one pathogen was isolated, while in 34 (48.6%) patients more than one pathogen was isolated. In 4 (5.71%) samples, no pathogen was isolated. Gram-negative bacteria accounted for 76 (74.5%), while gram-positive bacteria accounted for 26 (25.5%). Of the 76 gram negative bacteria, majority were *E. coli* 42 (55.3%) followed by *K. pneumoniae* 14 (18.4%) and *Pseudomonas aeruginosa* 12 (15.8%).

Majority of isolates of *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to amikacin and imipenem, but resistant to other antibiotics tested. Similarly, most of *Proteus spp.* were susceptible to ciprofloxacin, ofloxacin, amikacin, piperacillin-tazobactam and imipenem, while being less susceptible to trimethoprim-sulfamethoxazole and cefuroxime. *Citrobacter spp.* were susceptible to piperacillin-tazobactam, amikacin and imipenem, but resistant to other antibiotics tested. Colistin resistance was not detected in our study. Most of the *Pseudomonas aeruginosa* were susceptible to piperacillin-tazobactam and imipenem, while they were showing varying susceptibility to ciprofloxacin and amikacin. Similarly, majority of *Acinetobacter spp.* were susceptible to piperacillin-tazobactam, imipenem and trimethoprim-sulfamethoxazole, while being less susceptible to amikacin, ciprofloxacin and ceftazidime.

Table 1: Antibiogram of gram negative bacteria isolated from diabetic foot Bacteria isolated Sensitivity pattern

| Sensitivity pattern | <i>E. Coli</i> (n=42) | <i>K. pneumoniae</i> (n=14) | <i>P. aeruginosa</i> (n=12) | <i>Proteus spp.</i> (n=3) | <i>Acinetobacter</i> <i>spp.</i> (n=3) | <i>Citrobacter</i> <i>spp.</i> (n=2) |
|---------------------|--------------------------|--------------------------------|--------------------------------|------------------------------|---|---|
| CIP | 16 | 06 | 04 | 02 | 00 | 00 |
| OF | 18 | 07 | 06 | 02 | 00 | 00 |
| AK | 29 | 10 | 09 | 02 | 01 | 02 |
| COT | 11 | 03 | NT | 00 | 02 | 00 |
| CAZ | 19 | 05 | 05 | 00 | 00 | 00 |
| CPM | 21 | 07 | 06 | 00 | 00 | 00 |
| PIT | 17 | 07 | 10 | 02 | 02 | 01 |
| IMP | 34 | 12 | 10 | 02 | 03 | 02 |
| CL | 42 | 14 | 12 | 03 | 03 | 02 |

Staphylococcus aureus were most often susceptible to amikacin, linezolid and vancomycin, but were relatively less susceptible to erythromycin, clindamycin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, ciprofloxacin. None of the *Staphylococcus aureus* were susceptible to penicillin G.

Table 2: Antibio gram of gram positive bacteria isolated from diabetic foot Bacteria isolated Sensitivity pattern

| Sensitivity pattern | Staph. aureus (n=18) | CONS (n=08) |
|---------------------|----------------------|-------------|
| CIP | 08 | 04 |
| E | 07 | 04 |
| CD | 08 | 07 |
| AK | 11 | 08 |
| COT | 09 | 03 |
| PnG | 00 | 02 |
| CN | 04 | 08 |
| AMC | 05 | 06 |
| LZ | 18 | 08 |
| VA | 18 | 08 |

Out of the 18 *Staphylococcus aureus* isolates 14 (77.8%) were resistant to cefoxitin and were therefore considered as methicillin resistant *Staphylococcus aureus* (MRSA). ESBL production was detected in 19 of the 56 (33.9%) isolates belonging to Enterobacteriaceae.

DISCUSSION

Diabetic patients are more prone for chronic non-healing foot ulcers due to several underlying factors such as neuropathy, high plantar pressures and peripheral arterial disease.¹¹ A wide range of bacteria can cause infection in these patients. In present study, gram-negative bacteria accounted for 76 (74.5%), while gram-positive bacteria accounted for 26 (25.5%). Of the 76 gram negative bacteria, majority were *E. coli* 42 (55.3%) followed by *K. pneumoniae* 14 (18.4%) and *Pseudomonas aeruginosa* 12 (15.8%). Gadepalli et al and Shankar et al studies documented gram-negative bacteria as the predominant pathogens.^{2,5} Few studies have documented gram-positive bacteria as the predominant organisms associated with diabetic foot infections.^{12,13} This indicates changing bacterial pattern of diabetic foot and therefore clinicians should know the current etiological agents in order to treat such devastating complication of diabetes mellitus. The knowledge of antibiotic susceptibility pattern of the isolates from diabetic foot infections is crucial for appropriate treatment of cases. Majority of isolates of *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to amikacin and imipenem. Most of *Proteus* spp. were susceptible to ciprofloxacin, ofloxacin, amikacin, piperacillin-tazobactam and imipenem. *Citrobacter* spp. were susceptible to piperacillin-tazobactam, amikacin and imipenem. Most of the *Pseudomonas aeruginosa* were susceptible to piperacillin-tazobactam and imipenem. Majority of *Acinetobacter* spp. were susceptible to piperacillin-tazobactam, imipenem and trimethoprim-sulfamethoxazole. In an earlier Indian study, all members of Enterobacteriaceae were found to be uniformly sensitive to gentamicin and ciprofloxacin.⁴ Another study also has reported increasing resistance to these drugs.⁵ Therefore, empirical use of these antibiotics in diabetic foot infections should not be advocated. However, members of Enterobacteriaceae were found to be susceptible to

amikacin, piperacillin-tazobactam and imipenem. *Staphylococcus aureus* were most often susceptible to amikacin, linezolid and vancomycin. In our study, 14 (77.8%) were methicillin resistant *Staphylococcus aureus* (MRSA). Umadevi et al also quoted 65.5% of MRSA isolates in their study. However, most of the studies quoted 10-44% of MRSA isolation from such cases.

CONCLUSION

Diabetic foot infections are mostly polymicrobial and caused by gram negative bacteria. There is a need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy.

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