Original Research Article

A clinical study of commonly isolated organism and its antibiotic sensitivity in diabetic foot ulcers in Sri Manakula Vinayagar Medical College and Hospital, Pondicherry

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ABSTRACT

Background: Diabetic foot ulcer is one of the major surgical problem leading to hospital admission. Diabetic foot ulcer patients with uncontrolled diabetes may end up in forefoot amputation. Early aggressive debridement, control of blood sugar and empirical antibiotic therapy would reduce the morbidities in patients with diabetic foot ulcer. Further the knowledge of commonly isolated microbes and their antibiotic sensitivity pattern would be helpful to start empirical therapy. The purpose of this study was to determine the microbiological profile of diabetic foot infections (DFIs) and assess the antibiotic susceptibility of the causative agents.

Methods: This cross-sectional study was conducted in 115 patients admitted with diabetic foot ulcer over a period of 9 months from October 2015 to June 2016 at the department of general surgery, Sri Manakula Vinayagar Medical College and Hospital, Pondicherry. Tissue scrapping samples were collected and processed as per standard guidelines.

Results: 167 organisms were isolated from 115 patients. 52% of culture showed polymicrobial growth. There was increased prevalence of gram-negative organisms 53% compared to gram positive organisms 47%. When comes to individual isolate, Staphylococcus aureus was the most common organism isolated 24.6% followed by Pseudomonas aeruginosa 21%. All gram-positive aerobes were sensitive to vancomycin and gram-negative isolates were sensitive to amikacin, piperacillin-tazobactum, gentamycin and cefotaxime.

Conclusions: Staphylococcus aureus and Pseudomonas were the common pathogens isolated. This study recommends use of vancomycin along with piperacillin-tazobactum as an empirical therapy along with adequate blood sugar control and early debridement of devitalized tissues in patients with diabetic foot infections.

Keywords: Diabetic foot ulcer, Amputation, Polymicrobial infection, Bacterial isolates, Antibiotic susceptibility pattern

INTRODUCTION

One of the major causes of morbidity and mortality is diabetes mellitus (DM). In India approximately 62 million people currently diagnosed with diabetes mellitus and around 70000 new cases are diagnosed each year.1-3 Complications of diabetes mellitus like pedal ulcers, gangrene, peripheral neuropathy, vasculopathy and retinopathy can occur in patients with uncontrolled diabetes mellitus.4 15% of patients with diabetic mellitus will develop foot ulcers with or without osteomyelitis.5,6 Diabetic foot ulcer accounts for the majority of diabetes related hospital admission and also for forefoot amputation.7,8
Various studies have found the organisms causing infection in diabetic foot ulcer are usually polymicrobial, predominantly consisting of aerobic Gram positive cocci, Gram negative bacilli and anaerobes.\textsuperscript{9,11}

Early identification, initiation of appropriate antibiotic therapy, early surgical debridement of necrotic tissue and bone would reduce infection related morbidities, hospital stay and incidence of foot ulcer amputations.\textsuperscript{12} Initiation of appropriate antibiotics based on culture and sensitivity is very important so as to improve chances of limb salvage.\textsuperscript{13}

Hence knowledge of common microbes causing infection in diabetic foot ulcers and their sensitivity is helpful in determining proper antibiotic therapy.\textsuperscript{3} The current study was undertaken to identify most common pathogen and its antibiotic sensitivity in diabetic foot ulcers in Sri Manakula Vinayagar Medical College and Hospital (SMVMCH), Pondicherry. This hospital is a tertiary care center with separate diabetic clinic.

**METHODS**

This prospective study was focused on 115 patients with diabetic foot ulcer, admitted in surgery ward at Sri Manakula Vinayagar Medical College and Hospital, Pondicherry.

**Study settings**

This study was a cross sectional study, conducted over a period of 9 months from October 2015 to June 2016.

**Inclusion criteria**

Inclusion criteria were age >25 years, patient and/or his/her legal representative has read and signed the informed consent form before treatment and circulation status: with palpable pulses.

**Exclusion criteria**

Exclusion criteria were patients with diabetic ketoacidosis, active malignancy, being treated with immunosuppressant drugs and patients who had received systemic antibiotic therapy for more than 24 hours within the previous 72 hours of admission.

All patients were clinically assessed their foot ulcer were classified according to the diabetic foot infection severity classification system of the infectious diseases society of America (IDSA) as mild, moderate and severe infections.\textsuperscript{12}

**Ethical committee approval**

The study was approval by the ethical committee was obtained (12/184/IEC25/2015).

**Procedure**

**Sample collection**

For all patient’s tissue scrapping from the ulcer was taken rather than swabbing as proposed by international working group on diabetic foot (IWGDF).\textsuperscript{14,15} Swabs may be contaminated with normal skin flora and if swabbing is done, it may fail to identify deep tissue pathogens.\textsuperscript{16}

**Isolation and identification**

These samples were subjected to gram stain and inoculated on blood agar, MacConkey agar and nutrient agar. isolates were identified and confirmed by biochemical reaction.

**Antibiotic susceptibility testing**

As per CLSI guidelines, Kirby Bauer disk diffusion method was used for antibiotic susceptibility testing.\textsuperscript{17} Gram positive organisms were tested for penicillin, amoxicillin, Linezolid, vancomycin, tetracycline, gentamicin, Co-trimoxazole, erythromycin and clindamycin. Gram negative isolates were tested for amikacin, amoxicillin clavulanic acid, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin, gentamycin, piperacillin tazobactum, imipenem and co-trimoxazole.

Statistical analysis of this study will be by standard ‘t’ test, chi-square method.

**RESULTS**

Out of 115 patients taken for the study, 71.3% (82/115) were males and 28.7% (33/115) were females. The mean (SD) age of the subjects was 56.4 (6.2) years. The mean (SD) duration of diabetes and hospital stay was 4.3 (3.5) years and 14.3 (6.4) days respectively. Table 1 shows the baseline characteristics.

| Parameters                  | Values (%) |
|-----------------------------|------------|
| Mean (SD) age               | 56.4 (6.2) |
| Sex                         |            |
| Male                        | 82 (71.3)  |
| Female                      | 33 (28.7)  |
| Duration of diabetes (years)| 4.3±3.5    |
| Duration of hospital stay (days)| 14.3±6.4 |

A variety of microorganisms were isolated in patients with diabetic foot ulcer. Total of 167 pathogens were isolated from 115 patients. Aerobic Gram-negative bacteria (53%, 89/167) were found to be more than gram positive organisms (47%, 78/167) however the most common individual isolate was *Staphylococcus aureus* (24.6%, 41/167) followed by *Pseudomonas aeruginosa* (21%, 35/167). Other Gram-negative pathogens isolated...
were *E. coli* (16/89), *Klebsiella pneumonia* (16/89), *Proteus* species (14/89) and *Acinetobacter* (8/89).

**Table 2: Pathogens isolated.**

| Bacteria                          | Total number | Percentage |
|----------------------------------|--------------|------------|
| **Gram positive bacteria**       |              |            |
| *Staphylococcus aureus*          | 41           | 24.6%      |
| Methicillin resistant *S. aureus*| 07           | 4.2%       |
| *Enterococcus*                   | 20           | 12%        |
| Beta haemolytic *Streptococcus*  | 10           | 6%         |
| **Gram negative bacteria**       |              |            |
| *Pseudomonas aeruginosa*         | 35           | 21%        |
| *E. coli*                        | 16           | 9.6%       |
| *Klebsiella pneumonia*           | 16           | 9.6%       |
| *Proteus mirabilis*              | 8            | 4.7%       |
| *Proteus vulgaris*               | 6            | 3.6%       |
| *Acinetobacter*                  | 8            | 4.7%       |
| **Total**                        | 167          | 100%       |

Apart from *Staphylococcus aureus* other gram-positive pathogens isolated were beta hemolytic *Streptococcus* (10/78), *Enterococcus species* (20/78), MRSA (7/78). Table 2 shows pathogens isolated in 115 diabetic foot ulcer. 60 out of 115 (52%) patients had polymicrobial infections and 55 patients 48% had monomicrobial infections.

The antibiotic susceptibility pattern of the gram-positive pathogens is shown in (Table 3). It was observed that all 100% *Staphylococcus aureus*, *Enterococcus* group B *Streptococcus* and MRSA were sensitive to vancomycin. Apart from vancomycin, *Staphylococcus aureus* was sensitive to sulphonamides trimethoprim (TMP/SMX), gentamycin and clindamycin, *Enterococcus was sensitive to tetracyclines and gentamycin*, group B *Streptococcus was sensitive to penicillin and clindamycin*. All MRSA was sensitive to both vancomycin and sulphonamides trimethoprim. The antibiotic susceptibility pattern of the gram-negative pathogens is shown in (Table 4). It was observed most of the gram negatives were sensitive to amikacin, gentamycin, imipenem, cefotaxime and piperacillin-tazobactam.

**Table 3: Antimicrobial sensitivity pattern of gram-positive organisms.**

| Agent          | *S. aureus* (n=4) N (%) | *Enterococcus* (n=20) N (%) | Beta haemolytic *Streptococcus* (n=10) N (%) | MRSA (n=7) N (%) |
|----------------|-------------------------|------------------------------|---------------------------------------------|-----------------|
| Ampicillin     | 20 (50)                 | -                            | 10 (100)                                   | 0               |
| Clindamycin    | 29 (70)                 | -                            | 8 (80)                                     | 0               |
| Co-amoxiclav   | 25 (60)                 | -                            | 9 (90)                                     | 0               |
| Erythromycin   | 25 (60)                 | 2 (10)                       | 8 (80)                                     | 0               |
| Gentamycin     | 29 (70)                 | 19 (95)                      | 2 (20)                                     | 0               |
| Penicillin     | 2 (5)                   | 14 (70)                      | 10 (100)                                   | 0               |
| TMP/SMX        | 33 (80)                 | -                            | 9 (90)                                     | 7 (100)         |
| Tetracycline   | 10 (25)                 | 20 (100)                     | 8 (80)                                     | 0               |
| Vancomycin     | 41 (100)                | 20 (100)                     | 10 (100)                                   | 7 (100)         |

**Table 4: Antimicrobial sensitivity pattern of gram-negative organisms.**

| Agent          | *P. aeruginosa* (n=35) N (%) | *Proteus* (n=14) N (%) | *Klebsiella* (n=16) N (%) | *E. coli* (n=16) N (%) | *Acinetobacter* (n=8) N (%) |
|----------------|-----------------------------|------------------------|---------------------------|------------------------|---------------------------|
| Amikacin       | 31 (90)                     | 14 (100)               | 16 (100)                  | 15 (95)                | 0                         |
| Amoxiclav      | -                           | 9 (65)                 | 11 (70)                   | 8 (50)                 | 0                         |
| Cefotaxime     | -                           | 14 (100)               | 14 (90)                   | 14 (90)                | 0                         |
| Ceftazidime    | 33 (95)                     | -                      | -                         | -                      | 0                         |
| Cefuroxime     | -                           | 11 (80)                | 11 (70)                   | 10 (65)                | 0                         |
| Ciprofloxacin  | 28 (80)                     | 13 (90)                | 13 (80)                   | 4 (25)                 | 0                         |
| Gentamycin     | 29 (85)                     | 11 (80)                | 14 (90)                   | 13 (80)                | 0                         |
| Imipenem       | 31 (90)                     | 14 (100)               | 16 (100)                  | 15 (95)                | 0                         |
| Piperacillin tazobactam | 33 (95)          | 14 (100)               | 14 (90)                   | 14 (90)                | 0                         |
| TMP/SMX        | -                           | 8 (60)                 | -                         | -                      | 0                         |
DISCUSSION

In our study, we have conducted a cross sectional study in Department of General Surgery, SMVMCH, Pondicherry from October 2015 to June 2016 among 115 patients admitted with diabetic foot ulcer and have analysed the baseline characteristics, common pathogen isolated and its antibiotic sensitivity.

According to the observation based on Table 1, the peak age incidence of diabetic foot ulcer is 56.4±6.2 years. A total of 82 males and 33 females were evaluated in this study and the male-to-female ratio in this study is 2.5:1 showing male predominance. Various studies have shown increased incidence of diabetic foot ulcer in male population.6,9 The mean duration of diabetes in these patients who developed diabetic foot ulcer was 4.3±3.5 years. The mean hospital stay in our study was 14.3±6.4 days.

In our study tissue scrapping was done and sent for culture and sensitivity. 167 pathogens were isolated from 115 patients and in 52% it was polymicrobial. Studies conducted by Bansal et al, Lipsky et al and Frykberg et al also reported polymicrobial growth in diabetic foot ulcer.9,11

In our study we observed that the prevalence gram negative microbes 53% were more than gram positive pathogens.47 However based on individual pathogen isolated, staphylococcus aureus 24.6% was the most common pathogen followed by Pseudomonas aeruginosa 21%. These results were in concordance with studies conducted by Gadepalli et al and Bansal et al.6,9 Studies conducted by Abdulrazak et al and Goldstein et al reported high prevalence of staphylococcus aureus in diabetic foot infections.18,19

In our study, vancomycin and trimethoprim sulphonamide were most effective against Gram positive pathogens. This is in accordance with study conducted by Benwan et al.20 It was observed that staphylococcus aureus was sensitive to vancomycin, sulphonamides trimethoprím, gentamycin and clindamycin.

It was observed that amikacin, gentamycin, imipenem, cefotaxime and piperacillin-tazobactam were effective against gram negative pathogens. Similar sensitivity pattern was reported by a study conducted by Benwan et al.20

In this study we found Acinetobacter was totally resistant to all common antibiotics tested which is in accordance to the study conducted by Bansal et al.9

CONCLUSION

This study observed increased incidence of polymicrobial etiology in diabetic foot ulcer of which Staphylococcus aureus and Pseudomonas aeruginosa were the most common single agents isolated. As per our antibiotic susceptibility data, piperacillin tazobactam and vancomycin may be considered for empirical coverage in patients with diabetic foot ulcer. This study also concludes that adequate blood sugar control, early aggressive debridement of devitalised tissues along with empirical antibiotics are essential to reduce morbidity and for better outcome.

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