Original Research Article

Study on bacteriological profile and antibiotic susceptibility in diabetic foot infection in a teaching hospital, Telangana

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ARTICLE INFO

Article history:
Received 25-11-2020
Accepted 13-01-2021
Available online 25-08-2021

Keywords:
Bacteriological profile
Diabetic foot infection

ABSTRACT

Background: Diabetic foot is one of the most significant complications of diabetes. Chronic infections are caused by Enterococci, various Enterobacteriaceae obligate anaerobes, Pseudomonas aeruginosa.

Aim of the study: To study bacteriological profile and antibiotic susceptibility in diabetic foot infection.

Materials and Methods: Cross sectional study was done in 110 diabetic foot infection cases in the Department of Microbiology, Prathima Institute of Medical Sciences, Nagunuru, Karimnagar, Telangana. The grading of diabetic foot ulcers was done according to Wagner’s Classification system. The samples were collected from the exudates and ulcers. Gram stain was done and standard protocol for culture and sensitivity was followed for all the cases.

Results: A total of 110 cases were studied. The patient age ranged from 35 to 75 years and the male to female ratio was 2:1. Out of 110 isolates, 72.7% isolates were Gram negative bacilli with P. aeruginosa 31.8% being the predominant followed by E.coli 27.2%, K.pneumoniae 10%, P.mirabilis 4.5%, S.aureus 12.7% was the predominant isolate followed by Enterococci spp 4.5% and Coagulase negative staphylococci (CONS) 4.5% and S. pyogenes were 5.4%.

Conclusion: Both Gram positive cocci and Gram negative bacilli can cause diabetic foot infections and this study showed a preponderance of Gram negative bacilli. Early culture and sensitivity test of the bacterial isolates helps in guiding the treatment plan.

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1. Introduction

Diabetic foot infection (DFI) is one of the most significant and devastating complications of diabetes, and is defined as a foot affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with diabetes. The prevalence of diabetic foot ulceration in the diabetic population is 4–10%; the condition is more frequent in older patients. Two types of diabetes mellitus exist: type 1 and type 2 (formerly known as insulin and non-insulin diabetes mellitus). Type 2 diabetes(T2DM) is the most common type of diabetes (about 90 percent of diabetics). It was historically referred to as diabetes non-insulin-dependent or adult-onset. T2DM signs are either less apparent or absent. Therefore, for many years, the illness could not be detected until the complications have already arisen.¹–³

The infections usually occur in the site of skin trauma or ulceration. Major predisposing factors for DFI are neuropathy, vasculopathy, and immunopathy.⁴

Aerobic gram-positive cocci such as Staphylococcus aureus and the Beta-hemolytic Streptococci are the most common pathogens that are found in acute infection.⁵

Serious infections in hospitalized patients are caused by aerobes and anaerobes.⁶
2.1. Inclusion criteria

Informed consent was obtained from all the participants included in the study. Nagunuru, Karimnagar, Telangana over a period of 21 months from June 2018 to February 2020.

This was a cross-sectional study done in the department of Microbiology, at Prathima Institute of Medical Sciences, which included in diabetic foot infections.

2.2. Exclusion criteria

1. Patient not willing to participate in the study
2. Age less than 35 years and more than 75 years.
3. Patients on antibiotic therapy.
4. Other than diabetic foot ulcer.

2.3. Methodology

Demographic characteristics were noted in detail including age, gender, history of present illness, past history regarding the duration of diabetes, history of any drug intake, allergies.

The patients were examined clinically, and the grading of diabetic foot ulcers was done according to Wagner’s Classification and the University of Texas Wound Classification System.

2.4. Wagner’s classification of diabetic foot ulcers

Grade 0: no ulcer in a high-risk foot.
Grade 1: superficial ulcer involving the full skin thickness but not underlying tissues.
Grade 2: deep ulcer, penetrating down to ligaments and muscles, but no bone involvement or abscess formation.
Grade 3: deep ulcer with cellulitis or abscess formation, often with osteomyelitis.
Grade 4: localized gangrene.
Grade 5: extensive gangrene involving the whole foot.

Under all aseptic precautions samples were collected from the affected site using sterile cotton swabs. The samples were obtained from the deeper portion of the ulcers using two sterile swabs.

One swab was used for Gram-staining, and another was used for inoculation of culture and sensitivity. The samples after collection were transported within 2 hours to the microbiology laboratory.

All the Gram-stained smears were examined for Gram-positive and Gram-negative bacteria. Standard protocol for culture and sensitivity was followed by inoculating the swab onto different culture plates such as blood agar, chocolate agar, and McConkey’s agar medium. Overnight incubation at 37°C of the inoculated plates was done and examined for the growth next day. Based on Gram-staining and colony morphology, bacterial isolates were identified, and biochemical reactions were performed for confirmation.

The antibiotic susceptibility testing was done by Kirby–Bauer disk diffusion method as per CLSI guidelines. According to recommendations of Clinical Laboratory Standard Institute (CLSI) for antibiotic sensitivity testing and based on the size of inhibition zone around the disc, three forms of Sensitive (S), Intermediate (I) and Resistant (R) pattern were interpreted.

Gram-negative isolates were tested using antimicrobial discs of cefoperazone/sublactam, piperacillin/tazobactam, ceftiraxone, ceftazidime amox-clavulnate, levofloxacin, imipenem, meropenem, and amikacin. Susceptibility pattern
of Gram-positive isolates was tested using piperacillin + tazobactum, cefotaxime, linezolid, teicoplanin, vancomycin, azithromycin, amoxclav antimicrobial discs.

All the data collected was entered into the master chart and excel sheet and subjected to further analysis.

Microsoft Excel applications performed sufficient data entry and mathematical analysis. Categorical variables were expressed as frequencies and percentages. The comparison of normally distributed continuous variables between the groups was performed using Student’s t test. For all statistical tests, a p value less than 0.05 was taken to indicate a significant difference. Microsoft word and Excel have been used to generate graphs, tables.

3. Observations and Results

Total of 110 patients with diabetic foot infection were studied.

Table 1: Age distribution

| Age distribution (in years) | No of cases | Percentage (%) |
|----------------------------|-------------|----------------|
| 35-45                      | 12          | 10.9%          |
| 46-55                      | 36          | 32.7%          |
| 56-65                      | 43          | 39%            |
| 66-75                      | 19          | 17.2%          |
| Total                      | 110         | 100%           |

Gender

| Gender | No of cases | Percentage (%) |
|--------|-------------|----------------|
| Males  | 74          | 67.2%          |
| Females| 36          | 32.7%          |

Duration of diabetes

| Duration of diabetes | No of cases | Percentage (%) |
|----------------------|-------------|----------------|
| 2-6 years            | 27          | 24.5%          |
| 7-11 years           | 73          | 66.3%          |
| 12-16 years          | 10          | 9%             |

In the present study age distribution ranged from 35 years to 75 years. Majority of the patients with diabetic foot infection were among 55-65 years constituting 39% (43/110).Table 1

3.1. Gender distribution

There were 74 (67.2%) male patients and 36 (32.7%) female patients and the male to female ratio was 2:1.

3.2. Type of diabetes in cases with diabetic foot ulcers

There were 15 (13.6%) cases of Type 1 diabetes mellitus and 95 (86.3%) cases of Type 2 diabetes mellitus.

3.3. Duration of diabetes mellitus and diabetic foot ulcer frequency

In the present study, 27 (24.5%) cases had history of diabetes since 2-6 years, 73 (66.3%) cases had diabetes for 7-11 years and 10 (9%) cases had diabetes since 12-16 years.

Table 2: Wagner’s system of grading of ulcers

| Grading | Number of patients | percentages |
|---------|--------------------|-------------|
| Grade 1 | 10                 | 9           |
| Grade 2 | 46                 | 41.8        |
| Grade 3 | 24                 | 21.8        |
| Grade 4 | 25                 | 22.7        |
| Grade 5 | 5                  | 4.5         |

There were 10 (9%) cases in Grade 1, 46 (41.8%) cases in Grade 2, 24 (21.8%) in Grade 3, 25 (22.7%) in Grade 4 and 5 (4.5%) cases in Grade 5. Grade II ulcers were the most predominant. Table 2

3.4. Risk factors associated with diabetic foot ulcers

Some of the risk factors observed were as follows: Hypertension was present in 45 (40.9%) cases, history of smoking was seen in 26 (23.6%) cases, history of trauma was present in 7 (6.3%) cases, alcohol intake was seen in 32 (29%) cases.

3.5. Clinical presentations associated with diabetic foot ulcers

In the present study, vasculopathy was the commonest clinical presentation associated with foot ulcers and was seen in 41 (37.2%) cases followed by neuropathy seen in 30 (27.2%) cases. History of steroid therapy was present in 10 (9%) cases, HIV positivity was seen in 19 (17.2%) cases and 10 (9%) cases were immunocompromised.

3.6. Treatment history for diabetes mellitus

In the present study, 54.5% (60/110) of diabetic foot ulcer patients were on oral hypoglycemic drugs, 27.2% (30/110) patients were not on any medications and 18.1% (20/110) patients were on insulin injections.

3.7. Number of organisms isolated from diabetic foot ulcer

In the present study, 75 (68.1%) cases showed single organism growth and 35 (31.8%) cases showed growth of multiple organisms.

3.8. Bacterial isolates

In the present study, Gram-negative growth was seen in 80 (72.7%) cases and Gram-positive bacterial isolates were present in 30 (27.2%) cases.

The gram negative bacilli in diabetic foot ulcers were E. coli in 30 (27.2%) cases, Pseudomonas aeruginosa in 35 (31.8%) cases, K. pneumoniae in 10 (10%) cases and Proteus mirabilis in 5 (4.5%) cases.
3.9. Bacterial isolates of Gram positive cocci in diabetic foot ulcers

Out of 110 isolates, 30 (27.1%) were Gram positive organisms. S. aureus accounted for 14 (12.7%) cases, followed by Enterococcus spp. 5 (4.5%) and Coagulase negative staphylococci (CONS) 5 (4.5%) cases each and S. pyogenes accounted for 6 (5.4%) cases.

4. Discussion

4.1. Comparative studies related to Age distribution

In the present study, the patients age ranged from 35-75 years. Majority of the patients with diabetic foot were among 55-65 years constituting 39% and next common age group was among 46-55 years ie, about 32.7%. Our findings were compared with other studies. Shashanka R et al in their study observed maximum number of patients (54%) in the age group of 56-65 years and the second most common age group was between 45 and 55 years (22%). Otta S et al noted most of their patients (45.9%) among 51–60 years. In the study by Hefni AH et al diabetic foot infections were highest among the age group of 51–60 years, followed by 41–50 years age group. In the study by Shanmugam P et al maximum number of patients (20%) was in the age group of 60 to 65 years followed by 50 to 55 years (18%).

4.2. Comparative studies related to gender wise distribution

In the present study, males (67.2%) were commonly affected by diabetic foot ulcers when compared to females (32.7%). Similar findings were observed in Otta S et al study where, among l 148 diabetic patients presenting with ulcers, 106 (71.6%) were males and 42 (28.4%) were females. Whereas, a study conducted by Hefni AH et al in 2011, it was showed that P. aeruginosa strains were 83.3% sensitive to cefotaxime. Our findings did not compare well with these results. Otta S et al observed Pseudomonas spp. were usually sensitive to piperacillin-tazobactam (86.6%) and ceftazidime-clavulanic acid (71.4%), whereas Acinetobacter spp. was mostly sensitive to netilmicin (60%). Highest degree of production of extended spectrum beta lactamase (ESBL) and metallo-beta lactamase (MBL) was shown by Klebsiella spp. and Acinetobacter species respectively.

In our study, all the strains of staphylococci which were isolated were 100% sensitive to teicoplanin, linezolid, 95% to vancomycin and 80% to Piperacillin +tazobactum. CONS showed 100% sensitivity to vancomycin and to Piperacillin + tazobactum. Table 5

4.3. Comparative studies related to Antibiotic sensitivity

In the present study, Pseudomonas aeruginosa, which was the most commonly isolated bacteria showed 100% sensitivity to meropenem and 93% to imipenem and 80% sensitivity to piperacillin-tazobactam, 90% to Cefaperazone + sulbactum. In a study conducted by Shashanka R et al also, Pseudomonas aeruginosa, was the most prevalent bacteria and showed 100% sensitivity to imipenem and meropenem and 75% sensitivity to piperacillin-tazobactam. In a study done, in 2011, it was showed that P. aeruginosa strains were 83.3% sensitive to cefotaxime. Our findings did not compare well with these results. Otta S et al observed Pseudomonas spp. were usually sensitive to piperacillin-tazobactam (86.6%) and ceftazidime-clavulanic acid (71.4%), whereas Acinetobacter spp. was mostly sensitive to netilmicin (60%). Highest degree of production of extended spectrum beta lactamase (ESBL) and metallo-beta lactamase (MBL) was shown by Klebsiella spp. and Acinetobacter species respectively.

In our study, all the strains of staphylococci which were isolated were 100% sensitive to teicoplanin, linezolid. They were 95% sensitive to vancomycin and 80% to Piperacillin + tazobactum. CONS showed 100% sensitivity to vancomycin and Piperacillin + tazobactum.
Table 3: Antibiogram of gram negative isolates

| Antibiotics                  | E.coli | P.aeruginosa | K.pneumonia | P.mirabilis |
|-----------------------------|--------|--------------|-------------|-------------|
| Piperacillin +tazobactum     | 70%    | 80%          | 80%         | 100%        |
| Cefepirazone + sulbactum     | 70%    | 90%          | 75%         | -           |
| Ceftriaxone                  | 50%    | 40%          | 30%         | -           |
| Ceftazidime                  | 50%    | 40%          | 35%         | -           |
| Amox-Clavulinate             | 34%    | 30%          | 65%         | 100%        |
| Levofoxacin                  | 50%    | 75%          | 50%         | -           |
| Meropenam                    | 65%    | 100%         | 80%         | -           |
| Imipenem                     | 100%   | 93%          | 100%        | 100%        |
| Amikacin                     | 100%   | 80%          | 75%         | 65%         |

Table 4: Antibiogram of Gram positive isolates

| Antibiotics | S aureus | CONS | Streptococci |
|-------------|----------|------|--------------|
| Cefotaxime  | 80%      | 60%  | 100%         |
| linezolid   | 100%     | -    | -            |
| Teicoplanin | 100%     | -    | -            |
| Vancomycin  | 95%      | 100% | 100%         |
| Azithromycin| 75%      | 60%  | -            |
| Amoxclav    | 76%      | 60%  | -            |
| Piperacillin +tazobactum     | 80%    | 100% | 60%          |

CONS: Coagulase negative Staphylococci

Table 5: Wagner’s system of grading of ulcers

| Wagner’s system | Hefni AH et al17 | Present study |
|-----------------|------------------|---------------|
| Grade 1         | 10 (13.3 %)      | 10 (9.09%)    |
| Grade 2         | 20 (26.7%)       | 46 (41.8%)    |
| Grade 3         | 18 (24%)         | 24 (21.8%)    |
| Grade 4         | 16 (21.3%)       | 25 (22.7%)    |
| Grade 5         | 11 (4.3%)        | 5 (4.5%)      |

Table 6: Comparative studies related to Bacterial isolates

| Bacterial isolates        | Shashanka R et al15 | Shammugam al18 | Mehta VJ et al19 | Present study |
|---------------------------|----------------------|----------------|------------------|---------------|
| Pseudomonas aerogenosa    | 26.2%                | 16%            | 27%              | 31.8%         |
| Escherichia coli          | 18.4%                | 14.6%          | 17%              | 27.2%         |
| Staphylococcus aureus     | 16.8%                | 13.3%          | 7%               | 12.7%         |
| Streptococcus pyogenes    | 9.6%                 | 10.6%          | 5.4%             | 5.4%          |
| Klebsiella species        | 8.4%                 | 8%             | 22%              | 10%           |
| Acinetobacter species     | 7.6%                 | 8%             | 2%               | -             |
| Proteus mirabilis         | 4.8%                 | 6.6%           | 3%               | 4.5%          |
| Enterobacter species      | 2.9%                 | 1.3%           | -                | -             |
| Enterococcus species      | -                    | -              | 2%               | 4.5%          |
| CONS                      | -                    | 2.6%           | 1%               | 4.5%          |

CONS: Coagulase negative Staphylococci

In the study by Shashanka R et al15 all the strains of staphylococci, were 100% sensitive to teicoplanin, linezolid, and netilmicin in contrast to a study where S. aureus isolates were resistant to nearly all antibiotics, except for ciprofloxacin (91%) and amikacin (80%) sensitivity. In Otta S et al16 study, most of the S. aureus were sensitive to vancomycin (91.5%), teicoplanin (91.1%), and linezolid (90%). Among the aminoglycosides, netilmicin was the most sensitive drug (81.8%). They showed 87.5% and 71.8% sensitivity to levofloxacin and piperacillin-tazobactam, respectively. Nearly 77.8% of S. aureus were methicillin-resistant S. aureus (MRSA). Coagulase-negative Staphylococcus was more susceptible to the antibiotics than S. aureus and showed highest sensitivity to vancomycin and cefoperazone-sulbactam.
5. Conclusion

Both Gram positive cocci and Gram negative bacilli can cause diabetic foot infections and this study showed a preponderance of Gram negative bacilli. Early culture and sensitivity test of the bacterial isolates helps in guiding the treatment plan by instituting appropriate antibiotics. Knowledge on 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. These observations are important, especially for patient management and development of empirical antibiotic guidelines.

6. Limitations

The limitation for this study is that the validated diabetic foot infection tool classification was not used as it is not been practiced routinely in our centre. Despite that, there is no difference in the management of our diabetic foot infections in compare to the practice that has been published elsewhere.

7. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

8. Source of Funding

None.

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Cite this article: Kiran BU, Pothati D. Study on bacteriological profile and antibiotic susceptibility in diabetic foot infection in a teaching hospital, Telangana. Panacea J Med Sci 2021;11(2):315-320.