# Study on Bacteriological Profile and Antibiotic Susceptibility in Diabetic Foot Infection in a Teaching Hospital, Telangana

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**ABSTRACT**

**BACKGROUND**

Diabetic foot is one of the most significant complications of diabetes. Chronic infections are caused by Enterococci, various Enterobacteriaceae obligate anaerobes, and *Pseudomonas aeruginosa*. We wanted to study bacteriological profile and antibiotic susceptibility in diabetic foot infection.

**METHODS**

A 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 staining 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 %.

**CONCLUSIONS**

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.

**KEYWORDS**

Bacteriological Profile, Diabetic Foot Infection

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*DOI: 10.18410/jebmh/2021/291*

*How to Cite This Article:*
*Vaish R. Study on bacteriological profile and antibiotic susceptibility in Diabetic Foot Infection in a teaching hospital, Telangana. J Evid Based Med Healthc 2021;8(20):1538-1543. DOI: 10.18410/jebmh/2021/291*

*Submission 29-10-2020,*
*Peer Review 09-11-2020,*
*Acceptance 30-03-2021,*
*Published 17-05-2021.*

## BACKGROUND

Diabetic foot infection (DFI) is one of the devastating complications of diabetes, and is outlined as a foot suffering from ulceration that's related to pathology and / or peripheral blood vessel disease of the lower limb in a patient with diabetes. The prevalence of diabetic foot ulceration within the diabetic population is 4 – 10 %; the condition is more common 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] Chronic infections are caused by Enterococci, various Enterobacteriaceae, obligate anaerobes, *Pseudomonas aeruginosa*, and sometimes, other non-fermentative gram-negative rods.[7] Gram-negative bacilli, mainly of Enterobacteriaceae, are found in patients with chronic or previously treated infections. pseudomonas is usually occurs in soaked or treated wounds. Enterococci originate from previously-treated patient's Anaerobic species are found in tissues that have ischemia. Antibiotic-resistant organisms, especially methicillin resistant *S. aureus*, are found from patients who have previously received antibiotic therapy; they are often acquired during the previous hospitalization.[8] The risk factors for diabetic foot infection (DFI) are presence of wounds that have penetrated to the bone, wounds with a duration of > 30 days, recurrent wounds, wounds with traumatic aetiology and the presence of peripheral arterial disease. Neuropathy and history of previous amputation are significant risk factors for infection. The risk of infection is more in walking barefoot. Positive history of hospitalization and amputation are greater in patients with DFI compared to those without.[9] Regular foot examination, patient education, simple hygienic practices, provision of appropriate footwear, and prompt treatment of minor injuries can decrease ulcer occurrence by 50 % and eliminate the need for major amputation in nonischemic limbs.[10,11]

We wanted to study bacteriological profile and antibiotic susceptibility in diabetic foot infections.

## METHODS

This was a cross-sectional study done in the Department of Microbiology, at Prathima Institute of Medical Sciences, Nagunuru, Karimnagar, Telangana over a period of 21 months from June 2018 to February 2020. There were no ethical issues involved in the study. Informed consent was obtained from all the participants included in the study.

### Inclusion Criteria

- Patients willing to participate in the study
- Age distribution range from 35 - 75 years
- Both genders
- Diabetic patients with all grade foot lesions (Wagner grading system for diabetic foot lesions).
- Purulent exudates in diabetic foot ulcer patients.

### Exclusion Criteria

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

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.[12]

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.

Gram-positive and Gram-negative bacteria were included in both of the Gram-stained smears. Inoculating the swab onto different culture plates such as blood agar, chocolate agar, and McConkey's agar medium followed standard protocol for culture and sensitivity.

The inoculated plates were incubated overnight at 37°C and the development was observed the next day. Bacterial isolates were classified using Gram staining and colony morphology, and biochemical reactions were used to validate the findings.

According to Clinical laboratory standard institute (CLSI) guidance, antibiotic susceptibility research was performed using the Kirby–Bauer disc diffusion process.[13,14] Three types of Sensitive (S), Intermediate (I), and Resistant (R) patterns were interpreted based on the size of the inhibition zone around the disc, as recommended by the CLSI for antibiotic sensitivity testing.

Gram-negative isolates were tested using antimicrobial discs of cefoperazone / sulbactam, piperacillin / tazobactam, ceftriaxone, 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.

### Statistical Analysis

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.

## RESULTS

A total of 110 patients with diabetic foot infection were studied. 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).

| | | No of Cases | Percentage (%) |
|---|---|---|---|
| Age Distribution (in years) | 35 - 45 | 12 | 10.9 % |
| | 46 -55 | 36 | 32.7 % |
| | 56 - 65 | 43 | 39 % |
| | 66 - 75 | 19 | 17.2 % |
| | **Total** | **110** | **100 %** |
| Gender | Males | 74 | 67.2 % |
| | Females | 36 | 32.7 % |
| Duration of diabetes | 2 - 6 years | 27 | 24.5 % |
| | 7 - 11 years | 73 | 66.3 % |
| | 12 - 16 years | 10 | 9 % |

***Table 1. Age Distribution***

### Gender Distribution

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

### 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.

### 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. 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.

### 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.

| 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 |

***Table 2. Wagner's System of Grading of Ulcers***

### 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.

### 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.

### 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.

### 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.

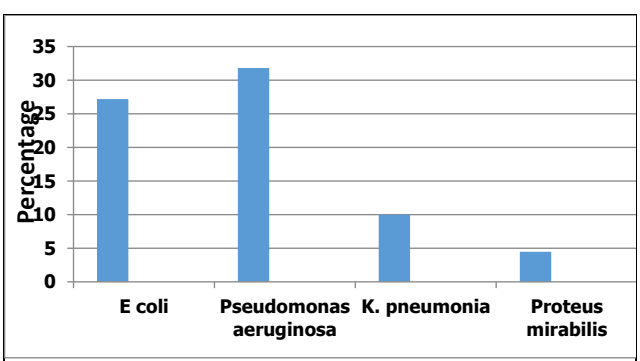


***Figure 1. Gram Negative Bacterial Isolates***

### 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.

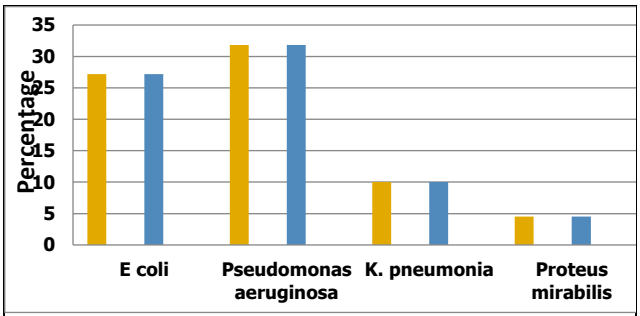


***Figure 2. Gram Positive Bacterial Isolates***

| Antibiotics | *E.coli* | *P.aeruginosa* | *K.pneumonia* | *P.mirabilis* |
|---|---|---|---|---|
| Piperacillin + tazobactum | 70 % | 80 % | 80 % | 100 % |
| cefaperazone + sulbactum | 70 % | 90 % | 75 % | - |
| ceftriaxone | 50 % | 40 % | 30 % | - |
| ceftazidime | 50 % | 40 % | 35 % | - |
| amox-clavulnate | 34 % | 30 % | 65 % | 100 % |
| levofloxacin | 50 % | 75 % | 50 % | - |
| meropenam | 65 % | 100 % | 80 % | |
| imipenam | 100 % | 93 % | 100 % | 100 % |
| amikacin | 100 % | 80 % | 75 % | 65 % |

***Table 3. Antibiogram of Gram Negative Isolates***

In the present study, out of 80 Gram negative bacterial isolates, *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.

| 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 % |

***Table 4. Antibiogram of Gram Positive Isolates***

CONS: Coagulase negative Staphylococci

All the strains of staphylococci which were isolated, were 100 % sensitive to teicoplanin, linezolid, 95 % to vancomyicin and 80 % to piperacillin + tazobactum. CONS showed 100 % sensitivity to vancomyicin and to piperacillin + tazobactum.

## DISCUSSION

### 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.[15] 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.[16] noted most of their patients (45.9 %) among 51 – 60 years. In the study by Hefni AH et al.[17] 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.[18] maximum number of patients (20 %) were in the age group of 60 to 65 years followed by 50 to 55 years (18 %).

### 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[16] where, among 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.[17] 37 were males and 38 were females with an almost equal male to female ratio.

| Wagner's System | Hefni AH et al.[17] | 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 5. Wagner's System of Grading of Ulcers***

In the present study, Grade 2 ulcers were the most predominant constituting 41.8 % cases. Our findings were similar to those reported by Hefni AH et al.[17] where they also noted Grade II ulcers as the most predominant type of ulcers.

| Bacterial Isolates | Shashanka R et al.[15] | Shanmugam P et al.[18] | Mehta VJ et al.[19] | 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 % |

***Table 6. Comparative Studies Related to Bacterial Isolates***

CONS: Coagulase negative Staphylococci

### 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.[15] 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.[17] Otta S, et al.[16] 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 vancomyicin and 80 % to piperacillin + tazobactum. CONS showed 100 % sensitivity to vancomyicin and piperacillin + tazobactum. In the study by Shashanka R et al.[15] 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 al.[16] 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.

## CONCLUSIONS

Both Gram positive cocci and Gram negative bacilli can cause diabetic foot infections and this study showed a preponderance of Gram negative bacilli. Early administration of antibiotics helps in guiding the treatment of bacterial culture and sensitivity tests. Knowing the antibiotic resistance patterns for these situations until receiving laboratory reports is important as these findings are critical for developing empiric antibiotic guidelines, and treatment guidelines.

### Limitations

In our institution, this research method was limited by the validated diabetic foot infection scale, which has not been in use. Even though this approach was published elsewhere, there was no significant difference in our methods for diabetic foot infections.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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