

# Bacteriological Profile and Antimicrobial Resistance of Bacteria Isolated from Pus/Wound Swabs of Diabetic Foot Patients Treated at a Tertiary Care Hospital in Bengaluru

Soundarya Yamakanamardi, V H Ashwath Venkataramana, Thammegowda Kemparaj

Department of General Surgery, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India

## Abstract

**Objectives of the Study:** The objectives of the study were to study the pattern of bacteriological prevalence from sample isolated by swab/pus from diabetic foot ulcerated wounds and to study the antibiotic resistance of the prevailing organisms.

**Materials and Methods:** Prospective study, between July 2020 and November 2020, outpatient and inpatient admitted to Bowring and Lady Curzon and Hospitals, Bengaluru. Sample collection done using commercially available sterile cotton swabs. Gram staining was done, followed by inoculation on the respective culture plates. Results of gram staining were typically obtained within 2 days and the culture and sensitivity reports were obtained within 5 days.

**Results:** Seventy-two patients, 42 were males and 30 were females. Age varied between 40 and 77 years, peak – 70 years. Twenty-two patients suffered from peripheral arterial diseases. Thirty-two patients (44%) had peripheral neuropathy. Most common organism isolated by culture was *Escherichia coli*, followed by *Staphylococcus aureus*. Majority of the *S. aureus* were sensitive to methicillin 2 out of the 72 harbored Methicillin-resistant *S. aureus*. Four patients suffered from *Klebsiella Pneumonia*, and one patient revealed *Citrobacter freundii*. Almost all were uniformly resistant to penicillin, followed by tetracycline, ampicillin, and ceftriaxone. Resistance to higher antibiotics such as colistin, piperacillin, and meropenem was also present.

**Conclusion:** The study determines the regional bacterial prevalence and its antibiotic resistance profile, in patients suffering from diabetic foot. The increase in third-generation cephalosporin resistance is an alarming concern. Strict protocols need to be developed at region levels with respect to antibiotic usage.

**Key words:** Antibiotic resistance, Culture and sensitivity, Diabetic foot, Wound

## INTRODUCTION

A wound is defined as a breakdown in the protective function of the skin associated with the loss of continuity of epithelium, with or without loss of underlying connective tissue.<sup>[1]</sup> Wounds can be accidental, pathological, or post-operative. This breach in continuity followed by a super added bacterial colonization constitutes wound infection. Wound infection is characterized by the presence of pus in a lesion, associated

necrosis of the tissue, along with systemic or local features of sepsis such as pyrexia, pain, and induration.

It is estimated that approximately 15% of diabetes worldwide will develop diabetic foot ulceration (DFU).<sup>[2]</sup>

The prevalence of DFU was found to be 18.1% in one cohort. Duration of diabetes was significantly associated with DFU. Living with diabetes for more than 10 years is associated with an increase in the diabetic foot probability by 3.16 folds.<sup>[3]</sup> Diabetic foot ulceration is well known for its notoriously non healing nature, adding significantly to the cost of treatment on the patient along with the adverse impact on the quality of life.

One of the main reasons for the chronicity or the non-healing nature is the presence of super added infection. A multitude of factors can be seen to be responsible for the etiology of

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**Corresponding Author:** Dr. Soundarya Yamakanamardi, Department of General Surgery, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India. E-mail: soundaryavy@gmail.com

diabetic foot ulceration and its chronicity, but at the root level they can be broken down to three main factors, that is, vascular insufficiency, neuropathy, and infections.<sup>[4]</sup>

Several studies on wound infection have predominantly focused on surgical site infections. However, in developing and resource-poor countries, wounds such as diabetic foot and other infected non-healing wounds are important causes of morbidity and mortality. It is of paramount importance to be aware of the regional and local variations of the causative micro-organisms and its antimicrobial sensitivity for the optimal management of and appropriate antimicrobial administration.<sup>[5]</sup>

Molecular studies provide evidence that chronic wounds, including DFUs, have a polymicrobial nature that largely exceeds the identification capabilities of traditional culture methods.<sup>[5]</sup> However, molecular methods are not routinely used in clinical settings and become obsolete in developing countries. Pus/swab culture is still reliably practiced in most parts of the world and provides accurate results.<sup>[6]</sup>

In this study, we explore the various organisms isolated from the wounds of patients suffering from diabetic foot, at a regional level in a tertiary center in Bengaluru, India. The antimicrobial sensitivity of the isolated cultures is also studied. Hence, this clinical study gains importance at a regional level to know the pattern of microorganism prevalence and aid in instituting an appropriate antibiotic therapy.

### Objectives of the Study

The objectives of the study are as follows:

1. To study the pattern of bacteriological prevalence from sample isolated by swab/pus from diabetic foot ulcerated wounds.
2. To study the antibiotic resistance of the prevailing organisms.

## MATERIALS AND METHODS

### Source of Data

Out patients and in patients of the Department of General Surgery in Bowring and Lady Curzon Hospitals, a tertiary center attached to BMCRI.

### Methods of Collection of Data

- A. Study design: This was a prospective Study
- B. Study period: The study period from July 2020 to Nov 2020

Place of study: The study is planned to be conducted on both outpatient and inpatient basis at Bowring and Lady Curzon and Hospitals attached to Bangalore Medical College and Research Institution, Bengaluru.

- C. Sample size: A total of 73 patients were included in the study.

### Inclusion Criteria

Patients willing to give a written and informed consent, either sex of any age group presenting with wound or callus were included in the study.

### Exclusion Criteria

Patients suffering from malignancy, end stage renal disease, HIV, Hep B and Hep C positivity, previous lower extremity amputation, psychiatry disorders and patients not willing to give written informed consent were excluded from the study.

After obtaining institutional ethics committee clearance and written informed consent, thorough history and examination findings are obtained.

Sample collection was conducted by medical officers in the outpatient clinic and in the wards using commercially available sterile cotton swabs and following existing departmental guidelines. Swab was collected after carefully cleaning the wound with sterile water and transported to the microbiology department within 1 h of collection.

Gram staining was done for all samples followed by inoculation on the respective culture plates. Results of Gram staining were typically obtained within 2 days and the culture and sensitivity reports were obtained within 5 days.

All patients were subjected to an arterial duplex scan and the cutaneous sensations were tested using a Semmes - Weinstein monofilament.

The results were extrapolated into data sheets and analysis was performed with Microsoft Excel version 16.0.

## RESULTS

The results of this study are tabulated in terms of the variables in relation to diabetic foot [Figure 1]. Of the 72 patients, 42 (58%) were males and (41%) were females. The cases varied between the age group of 40 and 77, the peak being more than 70 years age group. The mean age was 54 years [Table 1 and Figure 2]. All the patients suffered from lower limb diabetic infections. Swab was taken on first presentation, before the administration of antibiotics or wound debridement, under sterile conditions. Most of the patients belonged to the lower strata of the society and were manual laborers or performed agriculture related occupations, before the onset of the disability.

Doppler investigation showed that 22 patients, that is, 30% of the patients suffered from peripheral arterial

diseases. Monophasic flow was present in 18 out of the 22 and bi phasic flow was present in the rest that is 4. However, there were no signs of frank gangrene or sepsis [Table 2].

Thirty-two patients (44%) had loss of sensations when tested with a monofilament suggesting that peripheral neuropathy was prevalent in this population.

Twenty-eight patients (38%) patients had diabetes in control and the other 44 (61%) had uncontrolled diabetes. Ten (13.8%) had an abnormal renal function test signifying renal damage. Forty-five (62.5%) were found to be anemic.

The first culture taken from patients showed the presence of a variety of organisms including *Pseudomonas*, *E. coli*, *Klebsiella*, and *Staphylococcus aureus* were present, the percentage of distribution of which is described in Table 3.

As it is evident that the most common organism isolated by culture was *E. coli*, followed by *S. aureus*. Majority of the *S. aureus* were sensitive to methicillin two out of the 72 harbored Methicillin-resistant *S. aureus*. These patients were isolated in our diabetic ward and were treated with intravenous antibiotics. Four patients suffered from *Klebsiella pneumonia*, and one patient revealed *Citrobacter Freundii* [Figure 3].

The antibiotic profile of the organisms cultures was obtained. Only the commonly used antibiotics and those available in the hospital, free of cost were of significant in our setup and hence were analyzed. Table 4 describes the antibiotic resistance pattern. Almost all were uniformly resistant to penicillin, followed by tetracycline, ampicillin, and ceftriaxone.

**Table 1: Age distribution of the patient**

Age	Number of patients
40–50	16
50–60	17
60–70	13
>70	26
Total	72

**Table 2: Associated comorbidities of the patient population**

Comorbidities	Number of patients
Peripheral vascular disease	22
Monophasic	18
Bi phasic	4
Peripheral neuropathy	32
Abnormal renal function	10
Anemia	45

Resistance to higher antibiotics such as colistin, piperacillin, and meropenem was present too, which is an alarming concern.

## DISCUSSION

Foot ulceration is a morbid and incapacitating complication of diabetes. Diabetic foot ulceration (DFU) is a result of uncontrolled diabetes and incomplete health self-care.<sup>[6]</sup> This study investigates clinical and microbiological findings of DFU in patients. Of the 72 patients, 42 (58%) were males and (41%) were females. The cases varied between the age group of 40 and 77, the peak being more than 70 years age group. The majority of the patients with DFU were male and older than 40 years, consistent with other reported studies.<sup>[7]</sup>

A significant number of patients suffered from vascular and peripheral neuropathy, which was also found in other similar studies.<sup>[8]</sup>

In our study, we found that Gram-negative organisms were most commonly isolated in the culture. However, other studies report Gram-positive and polymicrobial infections too. A number of studies report Gram-positive, *Staphylococcal aureus* was the most frequently found organism.<sup>[9]</sup> Gadepalli et al. found that Gram-negative bacteria (*Proteus* species, *E. coli*, and *Pseudomonas aeruginosa*) were predominant strains.<sup>[10]</sup> *Enterococcus* spp.

**Table 3: Bacterial profile of the wound**

Organism	Number
<i>E coli</i>	43
<i>Pseudomonas aerogenosa</i>	8
<i>Methicillin sensitive staph aureus</i>	14
<i>Klebsiella pneumonia</i>	4
<i>Citrobacter Freundii</i> =1	1
MRSA	2
TOTAL	72

**Table 4: Antibiotic resistance profile of the isolated organisms**

Antibiotic resistance	Number	Percentage
Ampicillin	46	63
Cefoxitin	6	8
Penicillin	68	94.4
Cefuroxime	20	27
Tetracycline	60	83
Cefepime	12	16
Amikacin	20	27
Ceftriaxone	43	59
Colistin	2	2
Piperacillin	4	5
Meropenem	4	5
Ciprofloxacin	18	25

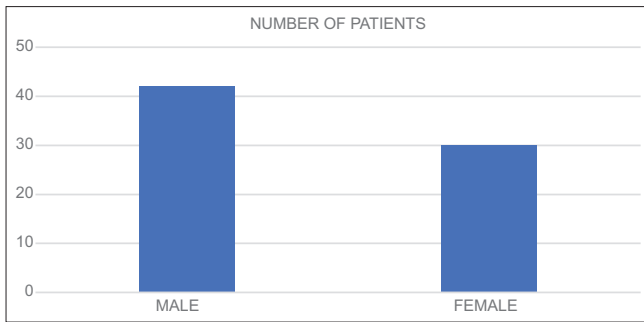


Figure 1: Gender distribution of the patient population

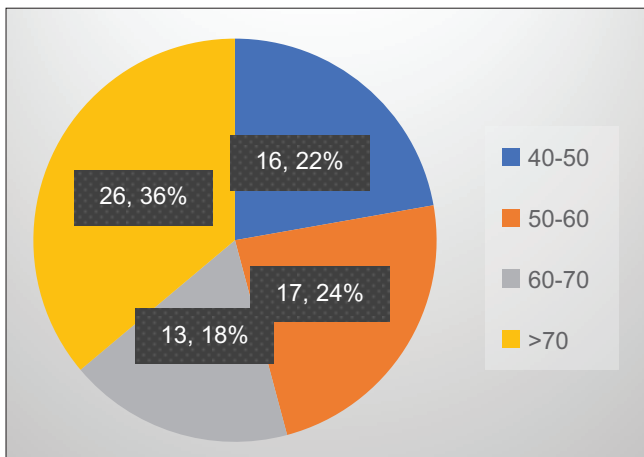


Figure 2: Age distribution of the patient population

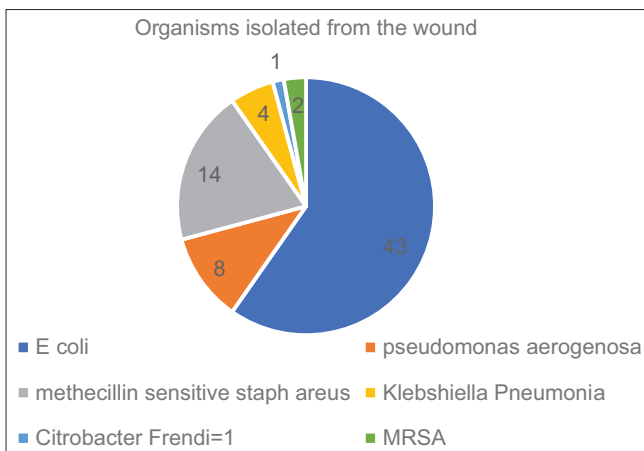


Figure 3: Microbiological profile of the wound

were the most frequent microorganisms which reflect the previous usage of antibiotics that the prevalence of *E. coli* is a public concern and are commonly detected in immune compromised patients.

A study by Mojtaba *et al.* states that the high rates of antibiotic resistance shown in the present study may be due to such factors including hospitalization, recent

use of broad-spectrum antibiotics, irrational use of antibiotics, and the transfer of resistance genes. It is apparent that the same factors could be a possibly responsible for the high rates of antibiotic resistance seen in our study.<sup>[11]</sup>

The presence of MRSA is of concern. The infected patients were shifted to isolated diabetic wards and were treated with intravenous higher antibiotics such as colistin or imipenem. The treatment continued until the cultures turned negative. Ward sterilization procedures were followed after discharging the patients. Inappropriate usage of antibiotics has led to devastating implications.

Significant number of patients was also resistant to third-generation cephalosporins. The reason being that these are the most commonly available antibiotics in the government setup in Karnataka, India, and most of the patients are empirically treated with ceftriaxone for inappropriate periods.

Significant cultures were also resistant to tetracyclines. However, the usage of tetracycline in our setup is limited.

## CONCLUSION

Thus, the study determines the regional bacterial prevalence and its antibiotic resistance profile, in patients suffering from diabetic foot. The increase in third-generation cephalosporin resistance is an alarming concern as it leaves the treating surgeon with limited options of antibiotics for use. Strict protocols need to be developed at region levels with respect to antibiotic usage.

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