

## **Bacteriological Profile and Spectrum of Susceptibility Pattern in Diabetic Foot Ulcer Patients at Tertiary Care Hospital, Puducherry.**

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### **ABSTRACT**

Diabetic foot infection is a global concern and develops in regions with improper foot care and increases the lifetime risk for developing foot ulceration. This wound infection begins superficially, but with delay in treatment and impaired body defense mechanisms, can spread to the other subcutaneous tissues and to deeper structures ultimately leading to dreaded complications such as gangrene and amputations. These infections are polymicrobial in nature. The aim of the present study was to isolate the bacterial pathogens in patients with diabetic foot ulcers and their susceptibility pattern. In the present study 124 samples from patients with foot ulcers were obtained during the period of June 2020 to August 2020. 26 samples taken from patients with ulcers who were non-diabetic were taken as control group. Samples were processed as per CLSI guidelines. Out of 124 isolates, 74 isolates (59%) were polymicrobial in nature and 50 isolates (40.5%) were Monomicrobial in nature. In our study among Gram negative pathogens *Pseudomonas aeruginosa* was the most common (48) (38.70%), followed by *Escherichia coli*, *Klebsiella species*, *Proteus species*, *Acinetobacter species*. Among 85 Gram positive isolates *Staphylococcus aureus* was the most common isolate (38)(30.64%), followed by CONS and Streptococcus. In the present study, all the aerobic Gram-positive organisms were 100% sensitive to Vancomycin, followed by Amoxicillin/Clavulanic Acid, ciprofloxacin and they were highly resistant to Ampicillin, Cotrimoxazole, and Gentamicin. 6 MRSA strains were isolated. Gram-negative organisms were sensitive to Gentamycin, Imipenem, Amikacin and Ciprofloxacin. 6 strains were resistant to all antibiotics tested. This study directs us that proper management of diabetic foot ulcers with appropriate antibiotics for treating this infection and there by preventing mortality rate.

**Key words:** Diabetic foot ulcers, Polymicrobial infection, Diabetes, Antibiotic Resistant.

### **INTRODUCTION**

Diabetic foot ulcer is defined as Infection, Ulceration and destruction of deep tissues associated with neurological abnormalities and various degrees of Peripheral Vascular Disease in the lower limb.

Diabetes mellitus is one of the major endocrine disorder of global concern. It is a serious health problem expanding worldwide where foot ulcers and lower extremity amputations are among the most serious complications associated with it [1]. Foot ulceration and infection in diabetic patients is one of the major causes of morbidity, hospitalization and foot amputation. Hence there arises the need to evaluate these infecting microorganisms on a routine basis in addition to administering regular glycemic control, wound care, surgical debridement, pressure-off loading and maintaining adequate blood supply[2]

Once the protective layer of skin is broken, the deep tissues are exposed to bacterial colonization. Infections are facilitated by immunological deficits (especially in neutrophils), which are related to DM, and they rapidly progress to the deep tissues. Patients with DM frequently require minor or major amputations of the lower limbs (15–27%), and in more than 50% of cases, infection is the preponderant factor[3]. The life time risk to a person with diabetes for developing foot ulcer could be as high as 25%[4].

Infection may be caused by pathogenic bacteria originating from external environment as well as by bacteria forming physiological microflora of skin. The presence of infection depends mainly on the number of microorganisms residing in the wound, where as the healing process depends on the type of bacterial strains and their pathogenicity[5].

These infections are polymicrobial in nature. *Escherichia coli*, *Proteus spp.*, *Pseudomonas spp.*, *Staphylococcus aureus* and *Enterococcus spp.*, are reported as frequent organism isolated from cases of diabetic foot infections [6]. The presence of MRSA and ESBL strains further worsen the prognosis and increase the risk of amputation [7]. Providing effective antimicrobial therapy plays a major role in treating these infections.

## **AIMS AND OBJECTIVES:**

To evaluate the bacteriological Profile and spectrum of susceptibility pattern in Diabetic Foot ulcer patients to minimize the diabetes associated mortality & morbidity.

## **MATERIALS & METHODS:**

The present study was conducted in the Department of Microbiology at Sri Lakshmi Narayana institute of medical sciences, Pondicherry during the period of June 2020 to August 2020.

### **Sample collection:**

A total of 124 pus samples were collected from patients having foot ulcer infections, 26 samples from patients with ulcers who were non-diabetic formed control group admitted at surgery ward in Sri Lakshmi Narayana institute of medical sciences, Pondicherry.

After obtaining proper patient informed consent samples collected from the patients. All swabs were collected before applying an antiseptic dressing to the wound and before starting treatment. Infected area should be decontaminated with 70% ethyl/ isopropyl alcohol.

Wearing a sterile, disposable gloves samples (purulent drainage or curetted material) were collected from the deeper portion of the ulcers (ulcer base) by using 2 sterile swabs. The samples were collected by making a firm, rotatory movement with the swabs. Cultures are best taken from the ulcer base. Care was taken not to touch the adjacent skin margins to avoid contamination with the skin commensals/.

One swab was used for Gram staining and the other was used for culture. Complete history of the patients were recorded which includes Age, Sex, Socio-economic status, Duration of ulceration, Treatment (if any) for Diabetes mellitus and duration of Diabetes mellitus. The ulcers were graded according to the Wagner's grade classification.

### **Wagner Grading System**

1. Grade 1: Superficial Diabetic Ulcer
2. Grade 2: Ulcer extension

Involves ligament, tendon, joint capsule or fascia No abscess or [Osteomyelitis](#)

3. Grade 3: Deep ulcer with abscess or Osteomyelitis
4. Grade 4: Gangrene to portion of forefoot
5. Grade 5: Extensive gangrene of foot

After collection samples were transported to the Microbiology department. Samples were processed as per standard CLSI guidelines.

### **Isolation and Identification**

Samples were subjected to Gram stain to screen for presence of bacterial pathogen. Samples were inoculated on Blood agar, Macconkey agar and Nutrient agar. Isolates were identified and confirmed by biochemical reaction.

### **ANTIBIOTIC SUSCEPTIBILITY TESTING**

The turbidity of the inoculated broth is compared with 0.5McFarland standard. Lawn culture of the broth suspension was made over the surface of the media and the antibiotic discs were placed and the plates were incubated at 37c for 24 hrs .The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method, as per the CLSI guidelines.

### **GRAM NEGATIVE BACILLI**

The antimicrobial discs which were used for GNB are Ampicillin (20µg), Aztreonam (30µg), Gentamicin (10µg), Amikacin (30µg), Cefazolin (30 µg), Cefuroxime (30µg) Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Cefoperazone/sulbactam (75/10µg), Piperacillin/tazobactam(100/10µg), Imipenem (10µg), Meropenem (10 µg), Polymyxin B (300 units) and Colistin (10µg).

### **GRAM POSITIVE COCCI**

Penicillin, Ampicillin, Azithromycin (15µg), Cefoxitin (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Clindamycin (2µg), Erythromycin (15µg), Oxacillin (1µg), Vancomycin (30µg), Teicoplanin (30µg)), Ciprofloxacin, Oxacilin (5µg), Linezolid (30µg) and Tetracycline (30µg) were used to study the susceptibility patterns of the Gram positive cocci.

### **RESULTS**

In the present study 124 samples from patients with foot ulcers were obtained following standard procedures for identification of bacteria. 26 samples from patients with ulcers who were non-diabetic were taken as control group.

**Table 1: Sex wise distribution of samples**

sex	Total Number of Samples
Males	76
Females	48

Out of 124 patients 76 (61%) were male and 48 (39%) were females infected. Male were more infected when compared to females in table 1.

**Table 2: Age wise distribution of diabetic ulcers in the study**

Age	Diabetic ulcers patients
15-25	1
26-35	3
36-45	19
46-55	41
56-65	50
66-75	8
76-85	2
Total	124

The ulcers occurred from a wide range from 15yrs to 85 yrs .50 patients (40.3%) who had the ulcers occurred in the age group of 56 to 65 years followed by 46-55years,36-45years shown in table 2.

**Table 3: Wagner's grading**

Wagner's ulcer grade	Total number of Samples
0	0
1	6
2	54

3	16
4,5	48

In the present study the diabetic ulcers were graded according to wagner's classification which is the most widely accepted . Patients under Grade 2 were mostly infected followed by others in Table 3/

**Table 4: Infection type:**

Type of infection	Total no of Isolates
Monomicrobial	50
Polymicrobial	74

Out of 124 samples with ulcers 74(59%) had POLYMICROBIAL (2 and more than 2 types of bacteria) The infection in 50(40.5%) patients were Monomicrobial shown in Table 4. Based on Wagner's grading of diabetic ulcers .That shows superficial diabetic ulcers with infection (Grade 0,1,2) are mostly monomicrobial in nature. Deeper ulcers with infection grade 2,3,4 and 5 are Polymicrobial in nature.

**Table 5: Bacterial Isolates in The Present Study**

Name of the organism	No. of Organisms	%
<i>Staphylococcus aureus</i>	38	30.64%
<i>CONS</i>	45	36.29%
<i>Pseudomonas species</i>	48	38.70%
<i>E.coli</i>	23	18.54%
<i>Klebsiella species</i>	19	15.32%
<i>Proteus vulgaris</i>	6	8.06%
<i>Proteus mirabalis</i>	4	
<i>Streptococci</i>	2	1.61%
<i>Diptheroids</i>	14	11.2%

<i>Acinetobacter</i>	2	1.61%
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The most common isolate is *Staphylococcus species* (66.39%) out of which 38 (30.64%) were *Coagulase positive staphylococcus* and 45(36.29%) is *CONS* followed by other bacterial isolates in table 5.

In the present study, all the aerobic Gram-positive organisms were 100% sensitive to Vancomycin, followed by Amoxicillin/Clavulanic acid , Ciprofloxacin ,Amikacin and they were highly resistant to Ampicillin , Cotrimoxazole , and Gentamicin .6strains were resistant to oxacilin.

Gram-negative organisms were mostly sensitive to Gentamycin ,Imipenem ,Amikacin (57.37%), and Ciprofloxacin . 6 strains were resistant to all antibiotics tested .

## CONTROL GROUP

A total of 26 patients with ulcers but who were non-diabetic were included in the study.

1. All the infected ulcers were Monomicrobial in nature.
2. The bacterial isolates were mainly *Staphylococcus species* (9),*Pseudomonas species*(7),*Klebsiella*(5),*Escherichia coli*(3) and *proteus*(2).
3. *Staphylococcus* was sensitive to Amikacin . *Enterobacteriaceae* were sensitive to third generation Cephalosporins ,Amikacin And Gentamycin.
4. *Pseudomonas* was sensitive to Amikacin.

## DISCUSSION

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients. With the rise in the prevalence of diabetes mellitus there is increasing problem of infections among diabetic patients especially the diabetic foot infection which according to some studies accounts for 20% of hospital admissions[8]

Various factors like age, sex, type of diabetes, smoking, immunocompromised status, duration of diabetes, injury to the foot, duration of ulcer, neuropathy, peripheral vascular disease

and resistance to ongoing treatment are responsible for aggravation of diabetic foot ulcer. Proper treatment of diabetes, Proper care of foot, and rigorous adherence to the principles of asepsis is the foundation of ulceration site infection prevention.

In our study males are more common infected than females correlates with Mohammad Zubair et al study the prevalence of diabetic foot ulcers among male subjects was found to be infected than female i.e. a ratio of 3.5:1, which may be due to higher level of outdoor activity among males compared to females [9]

In our study the ulcers occurred in the age group 56 to 65 years followed by 46-55years. In a similar study by Anandhi et al the mean age of diabetic ulcers was 43years. The complication of diabetes set in 15-20 years after the onset of diabetes which makes them susceptible to ulcerations and infections. This emphasizes the fact that diabetic ulcers occur in an older age group above 45 years [10]

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In our study also, we found polymicrobial etiology in 74(59%) and monomicrobial in 50(40.5%) patients which is quite higher than the previous [11].

In the present study Gram negative aerobic bacteria were most frequently isolated, which is correlating with study of Logerfo FW et al., 1984. [12].

A bacteriological evaluation of diabetic foot ulcer infections showed that the prevalence of gram-negative organisms were found to be more than gram-positive organisms which is in accordance with the previous findings [13]

In our study the commonest isolate was *Pseudomonas spp* (38.7%), followed by *Staphylococcus aureus* (30.64%) *Escherichia coli* (27.63%) and *Staphylococcus aureus* (25%). *Escherichia coli*, *Klebsiella* etc. *Pseudomonas aeruginosa* were predominant among the monobacterial isolates. These findings correlated well with those of Pappu K et al., 2011 [14], who reported that 76% of the organisms which were isolated were gram negative bacilli, *Pseudomonas* being the predominant pathogen (23%), followed by *Staphylococcus aureus* (21%) *Escherichia coli* (26.6%) and *Pseudomonas aeruginosa* (10.6 %) as the predominant gram negative isolates [15] Zubair M et al., 2010. In the study of Benwan et



al.,2012[16] which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than gram-positive pathogens (32.3%) or anaerobes (15.3%).

Viswanathan J et al .,2005[17] ,have isolated *Streptococcus* species 16.8% of the ulcers but in the present study it was 1.61% .This low isolation of *Streptococcus* may be because of the prior usage of antibiotics in the patients referred to the tertiary centers.

In the present study, all the aerobic Gram-positive organisms were 100% sensitive to Vancomycin, Followed By Amoxicillin/Clavulanic Acid ,Ciprofloxacin ,Amikacin and they were highly resistant to Ampicillin , Cotrimoxazole , and Gentamicin .6strains were resistant to oxacillin. Gram-negative organisms were sensitive to Gentamycin ,Imipenem ,Amikacin (57.37%), and Ciprofloxacin .6 strains were resistant to all antibiotics tested. This results was similar to the one reported by Tiwari et al.,2012.[18]

## CONCLUSION

This study assesses the microbial isolates of patients with diabetic foot infections and their antibiotic susceptibility pattern. Proper control of diabetes and avoiding risk factors for diabetic foot ulcers and appropriate usage of antibiotics based on local antibiogram pattern can certainly help the clinician in reducing the burden of diabetic foot infections, which ultimately reduces the rate of amputations.

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