

**Research Article****Clinical and Susceptibility Profile from Diabetic Foot Patients in Tertiary Care Hospital****Narinder Kaur<sup>1</sup>, Amandeep Kaur<sup>2</sup>, Rajiv Kumar<sup>\*3</sup>, Amarjit Kaur Gill<sup>4</sup>**<sup>1,2,3</sup>Assitant Professor, Department of Microbiology, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda, Punjab, India<sup>4</sup>Professor and Head, Department of Microbiology, Adesh Institute of Medical Sciences and Research (AIMSR), Bathinda, Punjab, India**\*Corresponding author**

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**Abstract:** Diabetic foot ulcer is an important complication of diabetes mellitus and improper use of topical antibiotics can lead to non healing ulcer. This study was conducted to know the clinical and sensitivity profile of diabetic foot patients. A record based study was conducted for a period of one year in patients attending the diabetic clinic of tertiary care hospital. 106 diabetic patients presenting with lower extremity infection were included in the study. Various specimens were collected for bacteriological study processed using standard microbiological procedures. The antimicrobial susceptibility pattern was studied by Kirby- Bauer disc diffusion method. A total of 136 organisms were isolated, averaging 1.36 isolate per culture positive patient. *Proteus* spp (18.3%) and *S.aureus* (18.3%) were the predominant pathogens. They were followed by *E. coli* (16.1%), *Klebsiella* spp (13.9%), *Pseudomonas* spp (11.7%). Polymyxin- B, meropenem, imipenem and piperacillin/tazobactam were most effective against gram negative organisms while vancomycin, linezolid and amikacin were most effective against gram positive organisms. Appropriate antibiotic therapy is an essential part of diabetic foot management and the prevalence of MDROs was alarmingly high and patient should be given organism targeted therapy rather than empirical therapy.

**Keywords:** Diabetes mellitus, Foot ulcer, Infection, Culture sensitivity pattern

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**INTRODUCTION**

Diabetes Mellitus (DM) is a serious public health problem Worldwide [1]. Magnitude of diabetes mellitus is increasing globally at an alarming rate. About 150-170 million populations are suffering from this diseases worldwide and the prevalence of diabetes will be double by 2025 as per WHO reports [2]. Diabetes mellitus hinders the life of nearly 40 million people in India and of equivalent magnitude in other developing countries. Diabetic Foot infections are seen in up to 20% of these patients and hence are the most commonly faced surgical problem. Unless treated appropriately, it leads to amputation or disarticulation of varying levels, at least ones in such patient's lifetime [3]. There is strong evidence that Indian population have a greater susceptibility to diabetes mellitus. The prevalence of Diabetes in Indian adult is 2.4% in rural and 4-11.6% in urban area. But the prevalence of impaired glucose tolerance is 3.6 - 9.1 which indicate that prevalence may be much higher [2]. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and target organ damage. Main complications associated with diabetes mellitus are cardiovascular disease, retinopathy, nephropathy,

neurological, peripheral vascular diseases and infections. Sensory neuropathy, atherosclerotic vascular disease and uncontrolled hyperglycemia are the favoring factors for development of skin and soft tissue infections. Infection can affect any part of the body. But the most common area affected in skin is feet. Diabetes mellitus affect micro vessels which limits the blood supply to superficial and deep structures. Local injuries and improper foot wear further compromise the blood supply in the lower extremities. Non-healing foot ulcer is common in clinical practice. It will increase the hospitalization and amputation which in turn result in long term economic, physical, social and mental disability to the patient [4]. Infection is a frequent complication of diabetic foot ulcers Infection occurs following the traumatic injury with introduction of bacteria [5]. *Escherichia coli* (*E.coli*), *Proteus* spp, *Pseudomonas* spp, *Staphylococcus aureus* (*S.aureus*), and *Enterococcus* spp are the most frequent pathogens which are cultured from diabetic foot ulcers. The infections in the diabetic foot are usually polymicrobial due to aerobic bacteria, anaerobes and *Candida* spp [6]. In superficial wounds, aerobic bacteria are predominant pathogens. Anaerobic organisms are found in deeper

wound [7]. The severe infections usually yield polymicrobial isolates, whereas the milder infections are generally monomicrobial [5]. More recently an increase in the incidence of multi-drug resistant organisms (MDROs), namely methicillin-resistant *S. aureus* (MRSA) and extended-spectrum  $\beta$ -lactamase (ESBL)-producing gram negative bacteria, is threatening the outcome of anti-infectious therapy in the community and in hospitalized patients [8]. Therefore, this study was undertaken to determine the common aetiological agents of the diabetic foot infections in a tertiary care hospital and their in vitro susceptibility pattern to the routinely used antibiotics.

#### MATERIAL AND METHODS

This prospective study was done on 106 diabetic patients, previously diagnosed or newly diagnosed as diabetics and presenting with lower extremity infection and were attending the diabetic clinic of a tertiary care hospital.

The study was conducted over a period of 1 year. Patients with foot infection due to any other causes such as non diabetics – post traumatic, arterial disorder alone, venous disorder alone, non diabetic peripheral neuropathy and secondary to implant infection were excluded.

Patients included were briefed about the study and details regarding age, sex, type of diabetes, duration of diabetes mellitus, duration of foot infection, treatment and associated co morbidities like neuropathy, nephropathy, retinopathy, urinary incontinence, osteomyelitis were recorded. Various specimens (pus, wound exudates or tissue biopsy) for microbiological study were obtained from ulcer region. Surface of the ulcer region was rinsed with sterile normal saline and the pus was collected with sterile cotton swab. Any fluid discharged from the wound was aspirated with needle and syringe aseptically. Specimens were sent to the laboratory and were processed for aerobic bacteria.

The specimens were cultured on blood agar and MacConkey agar and incubated at 37° C for 24-48 hrs. Isolates obtained were identified using standard microbiological procedures (9). Antimicrobial susceptibility testing was performed as per institutional antibiotic policy for first line and second line antibiotics (Hi Media laboratories, India) by Kirby Bauer disc diffusion method as recommended by clinical and laboratory standard institute (CLSI) (10). Ampicillin (10 mcg), amoxicillin/clavulanic acid (20/10 mcg), ampicillin/sulbactam (10/10 mcg), amikacin (30

mcg), gentamicin (10 mcg), gentamicin (120 mcg), ofloxacin (5 mcg), ciprofloxacin (5 mcg), cotrimoxazole (25 mcg), ceftazidime (30 mcg), chloramphenicol (30 mcg), imipenem (10 mcg), meropenem (10 mcg), piperacillin/tazobactam (100/10 mcg), polymyxin-B (300U), cefoperazone (75 mcg), cefotaxime (30 mcg), netilmicin (30 mcg), methicillin (5 mcg), ceftiofur (30 mcg), erythromycin (15 mcg), cephalexin (30 mcg), cefuroxime (30 mcg), clindamycin (2 mcg), vancomycin (30 mcg), linezolid (30 mcg) were used. Multiple drug resistance was defined as resistance to three or more groups of drugs.

#### RESULTS

In the study 106 patients were included. 82(77.3%) were males and 24(22.6%) were female. Average age of the patients was from 40-60 yrs. 102(96.2%) patients were of type II diabetes mellitus. 48(45.2%) patients had diabetes for more than ten year and 58(54.7%) patients were having foot infection for more than one month. 38(35.8%) were on oral antidiabetics and 40(37.7%) were on insulin while 20(18.8%) were on both insulin and oral anti diabetic drugs. 65(61.3%) had neuropathy, 20(18.8%) had nephropathy, 15(14%) had retinopathy. 35(33%) had urinary incontinence while 38(35.8%) patients suffered from osteomyelitis as shown in Table 1.

A total of 106 specimens were cultured and 98(92.4%) were culture positive. 136 organisms were isolated, averaging 1.38 isolates per culture positive patient. In 8(7.6%) patients, no growth was obtained. 70(71.4%) patients showed growth of one organism and 2 organisms were isolated from 18(18.36%) patients while 10(10.2%) showed growth of three or more organisms. Gram negative bacteria accounted for 67.6% (n=92), gram positive bacteria accounted for 28.6% (n=39) while yeast accounted for 3.67% (n=5) of the isolates as shown in Table 2.

The most frequent organisms among gram negative bacteria were *Proteus* spp (n=27) and *E. coli* (n=22) while *S. aureus* (n=25) was the most frequent isolate among gram positive bacteria as shown in Table 3.

Polymyxin B, meropenem, imipenem and piperacillin/tazobactam were most effective against gram negative organisms while vancomycin, linezolid and amikacin were most effective against gram positive organisms. The antimicrobial sensitivity to gram negative and gram positive organisms are shown in table 4 and table 5 respectively.

**Table 1: General characteristics of study objects**

Sl. No.	Characteristics	No. of patients (n=106)	Percentage
1.	Age (yrs)- <40	8	7.5%
	40-60	49	46.2%
	60-80	44	41.5%
	>80	5	4.7%
2.	Gender- Male	82	77.3%
	Female	24	22.6%
3.	Type of diabetes mellitus- Type 1	4	3.8%
	Type 2	102	96.2%
4.	Duration of illness- Newly detected	8	7.5%
	< 1 yr	4	3.7%
	1-10 yrs	46	43.3%
	>10 yrs	48	45.2%
5.	Duration of foot infection- <1 month	48	45.2%
	>1 month	58	54.7%
6.	Diabetic medication- Oral antidiabetics	38	35.8%
	Insulin	40	37.7%
	Oral antidiabetics and insulin	20	18.8%
	None	8	7.5%
7.	Comorbid conditions – Neuropathy	65	61.3%
	Nephropathy	20	18.8%
	Retinopathy	15	14%
	Urinary incontinence	35	33%
	Osteomyelitis	38	35.8%

**Table 2: Characteristics of diabetic foot specimens**

No. of patients	106
No. of patients with positive culture	98(92.4%)
No. of isolates	136
Average no. of isolates per culture positive patients	1.28
Samples with one organism	70(71.4%)
Samples with two organisms	18(18.36%)
Samples with three or more organisms	10(10.2%)
Gram negative bacteria	92(67.6%)
Gram positive bacteria	39(28.6%)
Candida albicans	5(3.67%)

**Table 3: Bacteria isolated from diabetic foot specimens**

Gram Negative Bacteria (n=92)	Gram Positive Bacteria (n=39)
<i>Proteus</i> spp- 25(18.3%)	<i>S. aureus</i> - 25(18.3%)
<i>E. coli</i> - 22(16%)	<i>Enterococcus</i> spp- 6(4.4%)
<i>Klebsiella</i> spp- 19(13.9%)	CONS- 4(2.9%)
<i>Pseudomonas</i> spp-16(11.7%)	<i>Streptococcus pyogenes</i> - 4(2.9%)
<i>Acinetobacter</i> spp- 6(4.4%)	
<i>Citrobacter</i> spp- 4(2.9%)	
<b>Mixture of Two Organisms</b>	<b>No. of Patients (N= 18)</b>
<i>S. aureus</i> + <i>Pseudomonas</i> spp	3
<i>E. coli</i> + <i>Enterococcus</i> spp	2
<i>Citrobacter</i> spp + CONS	2
<i>Proteus</i> spp + <i>Enterococcus</i> spp	2
<i>Proteus</i> spp + <i>S. aureus</i>	2
<i>Streptococcus pyogenes</i> + <i>Klebsiella</i> spp	2
<i>Proteus</i> spp + <i>Pseudomonas</i> spp	2
<i>Acinetobacter</i> spp + <i>Enterococcus</i> spp	1
<i>E. coli</i> + <i>Candida albicans</i>	1
<i>Klebsiella</i> spp + <i>Candida albicans</i>	1

Mixture of Three Organisms	No. of Patients (N=10)
<i>E. coli</i> + <i>Pseudomonas</i> spp + <i>S.aureus</i>	3
<i>S. aureus</i> + <i>Proteus</i> spp + <i>Acinetobacter</i> spp	2
<i>Streptococcus pyogenes</i> + <i>Pseudomonas</i> spp + <i>Candida albicans</i>	2
CONS + <i>Klebsiella</i> spp + <i>Proteus</i> spp	2
<i>S .aureus</i> + <i>Candida albicans</i> + <i>Acinetobacter</i> spp	1

**Table 4: Antibiotic sensitivity profile of gram negative bacilli (n=92)**

Antibiotic	<i>E.coli</i> (n=19)	<i>Kebsiella</i> spp (n=24)	<i>Pseudomonas</i> spp (n=16)	<i>Proteus</i> spp (n=25)	<i>Acinetobacter</i> spp(n=6)	<i>Citrobacter</i> spp (n=4)
Ampicillin	4(18%)	3(15.7%)	NT	5(20%)	NT	1(25%)
Amoxicillin/ clavulanic acid	5(22.7%)	3(15.7%)	NT	5(20%)	NT	1(25%)
Ampicillin/ sulbactam	6(27.2%)	4(21%)	NT	8(32%)	NT	2(50%)
Amikacin	13(59%)	7(36.8%)	12(75%)	19 (76%)	3(50%)	2(50%)
Gentamicin	10(45.4%)	7(36.%)	6(37.5%)	8(32%)	2(33.3%)	1(25%)
Ofloxacin	8(36.3%)	7(36.8%)	NT	11(44%)	NT	1(25%)
Ciprofloxacin	5(22.7%)	3(15.7%)	4(25%)	10(40%)	2(33.3%)	2(50%)
Co-trimoxazole	6(27.2%)	2(10.5%)	NT	11(44%)	2(33.3%)	2(50%)
Ceftazidime	6(27.2%)	2(10.5%)	6(37.5%)	12(48%)	1(16.6%)	1(25%)
Chloramphenicol	13(59%)	14(73.6%)	5(31.2%)	19(76%)	NT	2(50%)
Imipenem	20(91%)	18(94.7%)	14(87.5%)	24(96%)	5(83.3%)	4(100%)
Meropenem	22(100%)	19(100%)	16(100%)	25(100%)	6(100%)	4(100%)
Piperacillin/ Tazobactam	17(77.2%)	12(63%)	12(75%)	18(72%)	4(66.6%)	3(75%)
Polymyxin-B	22(100%)	19(100%)	16(100%)	25(100%)	6(100%)	4(100%)
Cefoperazone	6(27.2%)	5(26.3%)	7(43.7%)	9(36%)	3(50%)	2(50%)
Cefotaxime	12(54.5%)	4(21%)	2(12.5%)	4(16%)	3(50%)	1(25%)
Netilmicin	8(36.3%)	10(52.6%)	6(47.5%)	10(40%)	2(33.3%)	1(25%)

**Table 5: Antibiotic sensitivity profile of gram positive cocci (n=39)**

Antibiotic	<i>S.aureus</i> (n=25)	CONS(n=4)	<i>Enterococcus</i> spp (n=6)
Methicillin S	18(72%)	2(50%)	NT
Methicillin R	7(28%)	2(50%)	NT
Amoxicillin/clavulanic acid	20(80%)	3(75%)	2(33%)
Ampicillin/sulbactam	16(64%)	2(50%)	4(66%)
Gentamicin	14(56%)	2(50%)	NT
Gentmicin (HLAR)	NT	NT	5(83%)
Amikacin	21(84%)	2(50%)	5(83%)
Ciprofloxacin	13(52%)	3(75%)	3(50%)
Erythromycin	10(40%)	2(50%)	4(66%)
Cephalexin	14(56%)	2(50%)	3(50%)
Clindamycin	9(36%)	1(25%)	NT
Vancomycin	25(100%)	4(100%)	5(83%)
Linezolid	25(100%)	4(100%)	6(100%)
Cotrimoxzole	15(60%)	1(25%)	2(33%)
Cefuroxime	12(48%)	2(50%)	3(50%)

**DISCUSSION**

A wide range of bacteria can cause infection in these patients. While the foot infections with diabetes are initially treated empirically. The therapy which is directed at the known causative organism may improve the outcome. This prospective study was performed to

evaluate the diabetic foot infections, the causative pathogens and the antimicrobial sensitivity profiles of Isolates. In the present study, there was significant association between diabetic foot ulceration and clinical parameters like male gender, duration of diabetes more than 10 years, non insulin dependent diabetes mellitus

(NIDDM) and duration of foot infection more than one month. Other contributing factors such as neuropathy and the vascular diseases were present at the time of consultation.

Foot infections in diabetes were rarely due to single organism. Aerobic bacteria (*Staphylococcus* spp, *Streptococcus* spp and Enterobacteriaceae), anaerobic flora (*Bacteroides* spp, *Clostridium* spp, and *Peptostreptococci* spp) and fungi are the organisms that are isolated most often [4]. In this study a total of 136 organisms were isolated from 106 samples averaging 1.38 isolated per culture positive patients. This was nearly similar to study conducted by Banashankari *et al.* [3] who reported an average of 1.2 and this is slightly lower than the findings from Kavita A *et al.* [11] where culture yielded an average of 2.56.

Polymicrobial infections are now commonly seen in infected diabetic foot throughout the world [12]. Gram negative bacterial isolates (66.7%) predominated as compared to gram positive organisms (28.6%) in our study, which is in concordance with the study of Rawat *et al.* [9]. *Proteus* spp (18.3%) and *S.aureus* (18.3%) were the predominant pathogens. They were followed by *E. coli* (16.1%), *Klebsiella* spp (13.9%), *Pseudomonas* spp (11.7%), *Acinetobacter* spp. and *Enterococcus* spp (4.4%) each. The results were similar to study conducted by Banashankari *et al.* [3]. However, in a study conducted by Banno S *et al.* [7] *Pseudomonas* spp (21.9%) was the predominant isolate, followed by *Klebsiella* spp (19.4%) and *S. aureus* (14.5%).

Treatment of diabetic foot infection requires antibiotic therapy based on culture and surgical intervention. *Staphylococcus aureus* was found to be most sensitive to vancomycin, linezolid and Amikacin and least sensitive to clindamycin and cefuroxime and the results were similar to study conducted by Rawat *et al.* [9]. 28% of isolates were MRSA. Coagulase negative staphylococci (CONS) were most sensitive to vancomycin and linezolid but least sensitive to cotrimoxazole and clindamycin. *Enterococcus* spp were most sensitive to linezolid, vancomycin, amikacin and gentamicin but least sensitive to Co-trimoxazole and amoxicillin/clavulanic acid. All aerobic gram negative bacilli showed similar sensitivity pattern to imipenem, meropenem, piperacillin/tazobactam and polymyxin-B. The results were similar to study conducted at AIIMS, New Delhi [13] and Bangalore [3]. Most of the gram negative isolates showed resistant to multiple drugs. Our study confirms that MDROs are prevalent in hospitalized diabetic foot patients.

## CONCLUSION

To conclude, proper antibiotic therapy is an essential part of diabetic foot management and the prevalence of MDROs was alarmingly high and was associated with increased requirement for surgical treatment. This is

probably related to indiscriminate use of antibiotics. So, these findings suggest that prospective multicentre studies should be carried out to formulate guidelines for empirical antibiotic regimen in diabetic foot ulcers.

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