

## ORIGINAL ARTICLE

## Prevalence of Gram Negative Bacteria in Diabetic Foot -A Clinico-Microbiological Study

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**Abstract:** *Aim and Objective:* To determine the bacterial spectrum in diabetic foot lesions and analyze the antibiotic susceptibility pattern of the isolated bacteria. *Methods and Methodology:* Tissue samples/discharge/pus/ were cultured from 202 patients admitted for the treatment of diabetic foot infections. Specimens were tested by gram stain, culture and antibiotic sensitivity. *Results:* A total of 202 specimens were cultured, yielding 246 bacteria at the end of 18-24hrs. Gram negative aerobes were the most frequently isolated bacteria constituting 162 isolates (66%), followed by gram- positive aerobes 78 isolates (32%). Enterobacteriaceae group and *P. aeruginosa* strains were largely susceptible to imipenem (100%), piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin. More than 70% of staphylococcus aureus was sensitive to methicillin. Cefoperazone + sulbactam showed about 67% sensitivity, while ciprofloxacin and amikacin were only 23% and 44% sensitive. MRSA was isolated in 20 cases (47% of S.aureus) and Methicillin resistant coagulase negative staphylococcus in 2 cases (15% of coagulase negative staphylococcus). Methicillin resistant organisms were sensitive to vancomycin (95%). *Conclusion:* Diabetic foot infections are predominantly due to gram positive bacteria like *Staphylococcus aureus* or polymicrobial. There is a growing trend of isolating gram negative bacteria in these naïve lesions of the diabetic foot. The need for adequate gram negative antibacterial coverage at the commencement of diabetic foot therapy is essential to prevent and treat limb/life threatening infections.

**Keywords:** Diabetic foot, gram negative bacteria, culture sensitivity pattern.

### Introduction

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 upto 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, atleast ones in such patient's lifetime. Majority of the diabetic foot lesions are initially treated empirically based on the clinical knowledge of the treating surgeon and on the prevalence of the microbial pattern in the locality and the hospital. It would be prudent if the treatment is directed based on the hierarchy of the organisms most commonly isolated and the most common antibiotic sensitivity pattern of these organisms, at the onset and thus help in a better outcome. Worldwide, several studies have been conducted with respect to the bacteriology and antibiotic sensitivity pattern.

A number of studies have found that *Staphylococcus aureus* and other gram positive aerobes are the main causative pathogen usually isolated in more than 60% of cases [1]. However, many ongoing studies including the two recent prospective studies reported a predominance of gram-negative aerobes [2-3]. It is of significant importance in health care system because, in the routine practice of management of diabetic foot infections, surgeons / physicians mostly prescribe the antibacterial coverage of gram positive bacteria and the anaerobes. Failure to identify and treat a relatively susceptible organism may result in the development of multidrug resistant strain of gram negative bacteria. The role of anaerobes is particularly unclear, because in many studies specimens were not collected appropriately for anaerobic culture or due to lack of anaerobic culture setup in many institutions. Among those that did use appropriate methods, some report that anaerobes play a minimal role [4] while others have detected 95% prevalence of anaerobes<sup>2</sup> in a study with *Bacteroides fragilis* being the predominant anaerobe isolated [5-6]. Of major concern is the increasing incidence of multidrug-resistant organisms (MDRO), particularly methicillin-resistant *S. aureus* (MRSA) [7-8]. The prevalence of MRSA is as high as 20-30% [9]. The study was conducted to determine the bacterial culture and antibiotic sensitivity pattern in various diabetic foot lesions.

### Material and Methods

202 cases of type 2 diabetes, admitted for treatment of diabetic foot infections at M.S.RAMAIAH hospitals, Bangalore during the period of Dec2008 to Dec 2009 were included in the study. Patients with foot infections 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 the following informations were collected - demographic characteristics, duration of diabetes, duration of the diabetic foot lesion, associated comorbidities like hypertension, COPD, Arterial diseases, venous disorders, peripheral neuropathy, retinopathy, nephropathy. Vital parameters of the patients were recorded. Local examination of size of an ulcer/wound, laterality, WAGNER stage [10], depth of the ulcer/wound with probing whenever necessary and assessment for signs of severity of infection of the ulcer (foul smelling discharge, necrosis, crepitations, cellulitis and systemic signs) were tabulated (Table-1). Plain Radiographs of the foot were taken in Wagner grade >2 ulcers for detection of involvement of the adjacent bone and osteomyelitis whenever possible. Preliminary tests including haemogram, fasting and postprandial blood sugars, HbA1c, BUN, S.creatinine were done. Each wound was thoroughly irrigated with saline before acquiring the infected tissue from the lesion. Pus or discharge were also swabbed and sent. Specimens were sent immediately to laboratory in a sterile container for staining and culture and sensitivity. Empirical antibiotics were started. Wound debridement/disarticulation of toes or amputations were performed based on the extent and severity of the lesions. The outcome of the disease in terms of resolution of infection/worsening of infection and the events of amputation were analyzed.

**Microbiological analysis:** To avoid the chance of isolation of only colonizing (rather than pathogenic) bacterial flora, all ulcers were thoroughly washed with saline. Specimens were collected by Scrapings from ulcer base, wound curettage, or aspiration rather than swab techniques (necrotic tissue or the bony fragments were also picked). Specimens were tested by gram stain, (KOH, ZN stain if suggestive) and Aerobic cultures were plated onto MacConkey agar and Blood agar incubated at 37°C in ambient air. Isolates were identified at the end of 18-24hrs by standard methods [11]. Antimicrobial susceptibility testing of aerobic isolates was performed by the 'Kirby Bauer disc diffusion' method as recommended by the Clinical and Laboratory Standards Institute (CLSI). *Staphylococcus* species were tested for methicillin resistance by using oxacillin disc recommended by the National Committee for Clinical Laboratory Standards [12-13]. All patients received intravenous antibiotics to cover gram positive, gram negative aerobes and anaerobes till the culture sensitivity study reports were available. The results were analyzed.

### Results

Sl. No.	Characteristics	No	Percentage
1.	Age(yrs) – 40 – 50	102	50%
	50-60	56	27%
	60-70	34	17%
	>70	10	6%
2.	Sex – male	156	77%
	female	46	23%
3.	Smoker	134	66%
4.	Alcoholic	43	22%
5.	Diabetes mellitus		
	<10 years	132	65%
	10-20 years	47	23%
	>20 years	23	12%
6.	Duration of ulcer(months) <3months	177	88%
	>3months	25	12%
7.	Size of ulcer(cm <sup>2</sup> ) <5	134	66%
	>5	68	34%
8.	Poorly controlled (HbA1c >8)	67	33%
9.	Hypertension	129	64%
10.	Obesity	54	27%
11.	Peripheral vascular disease	43	21%
12.	IHD	45	22%
13.	COPD	34	17%
14.	Asso osteomyelitis (clinically,radiographically,intraoperatively)	133	66%
15.	Nephropathy(s.creat>1.8)	35	17%
16.	Neuropathy	154	76%

202 diabetic patients were evaluated (Table-1). 158(78%) of the cases were in the age group of 40-60 years with a mean age of 54 years.

Majority of the study patients were Males - 156 (77%). Mean duration of diabetes was 8.2years +/- 2.3 with majority not having the disease for more than a decade-132 (65%). Nearly 1/3<sup>rd</sup> of the cases had poor glycemetic control (HbA1c >8) - 67(33%) at presentation. All the nephropathy patients were seen to have poor glycemetic control. Essential hypertension was the most common associated co morbid condition seen in patients (64%). A raised S.Creatinine >1.8mg/dl was seen in 35 patients (17%), and about 4 of them were on hemodialysis and 1 on CAPD. S.Creat of 5mg/dl-8mg/dl was seen in 3 of stage5 CKD, awaiting transplant.

Sl. No	Charecteristics	No	%age
1	Diabetic foot – right	99	49%
	Left	86	43%
	Bilateral	17	8%
2	Sole	148	73%
	Toes	165	82%
	Dorsum	89	44%
	leg	65	32%
3	Wagner Classification		
	2	92	46%
	3	61	30%
	4	45	22%
	5	24	12%
4	Associated cellulitis	87	43%

A casual predominance of right leg ulcers (n=99, 49%) with majority of lesions located over toes (n=165, 82%) and sole (n=148, 73%) was witnessed. Majority of the ulcers belonged to WAGNER 2 category (n=92, 46%) and 13 patients had extensive gangrene (WAGNER 5) (Table-2). They all had to undergo amputation. Ascending cellulitis of the foot and leg was seen in 43% of cases and was managed along with the ulcer proper. 1 case of which had cellulitis till the thigh had to undergo AKA, however patient succumbed to postop pulmonary edema

Bacteria category	Percentage
N isolates	246
Aerobic and facultative isolates	<u>240(98%)</u>
Gram negative	162(66%)
Proteus species	45(18%)
E.Coli	40(16%)
Pseudomonas aeruginosa	32(13%)
Acinetobacter	17(7%)
Klebsiella species	16(7%)
Citrobacter species	4(2%)
Enterobacter species	8(3%)
Gram positive	78(32%)
S.aureus	43(19%)
Enterococcus species	22(9%)
Coag neg staph	13(5%)
contaminants	<u>6(2%)</u>

and pneumonia. In our study, a total of 202 specimens were cultured and isolated 246 organisms as shown in (Table-3), with 10 specimens being sterile. 64% had growth of single organism, while the rest were polymicrobial and about 5% yielding 3 or more organisms. 240(98%) had grown aerobic facultative organisms and 6(2%) of the growth could not be categorized as aerobes. This may be due to anaerobic organisms. In our study, the gram negative aerobes were isolated in 162 cases (66%) with predominant organisms being proteus (n=42, 18%), E.coli (n=40, 16%), pseudomonas (n=32, 13%). Among gram positive organisms Staph aureus was isolated in 43 cases (19%) followed in decreasing

order by enterococcus and coagulase negative staph. Thus a ratio of 1.5:1.0 for gram negative to gram positive was seen in aerobes.

With tissue specimen collection being the most frequent method (56%) and less use of swabs for culture has resulted in minimal contaminants and hence reflected in minimal isolation of Staph epidermidis and other commensals. Gram positive organisms only were found in 18.4 % (n=45), and 34% (n=96) had only gram negative organisms. The remaining cultures had grown both gram positive and negative organisms.

	<b>Proteus sp(n=45)</b>	<b>E.coli (n=40)</b>	<b>Pseudom (n=32)</b>	<b>Acinetobac (n=17)</b>	<b>Klebsiella (n=16)</b>	<b>Citrobacter (n=4)</b>	<b>Enterobacter (n=8)</b>
Amikacin	15, 33%	22, 55%	24, 75%	8, 47%	10, 63%	3, 75%	4, 50%
Amox-clav	14, 32%	11, 27%	6, 19%	5, 30%	9, 56%	2, 50%	4, 50%
Cefoperzone	16, 34%	12, 28%	16, 50%	7, 41%	4, 25%	1, 25%	3, 38%
Cefoxatime	6, 13%	7, 18%	4, 13%	2, 12%	3, 19%		
Ceftazidime	16, 34%	9, 23%	15, 49%	12, 71%	8, 50%	1, 25%	4, 50%
Ciproloxacin	40, 90%	22, 55%	20, 62%	11, 64%	10, 63%	2, 50%	5, 62%
Cefuroxime	6, 13%	2, 5%	1, 3%	4, 24%	4, 25%	--	--
Gatifloxacin	13, 31%	18, 45%	10, 31%	5, 30%	3, 19%	--	--
Netilmicin	15, 33%	13, 29%	12, 37%	7, 41%	7, 43%	1, 25%	3, 38%
Gentamicin	10, 22%	4, 12%	16, 50%	7, 41%	3, 19%	--	--
Imepenem	45, 100%	40, 100%	32, 100%	17, 100%	16, 100%	3, 75%	4, 50%
Piperacillin	--	--	21, 63%	12, 71%	13, 81%	3, 75%	5, 62%
Piperacillin-tazobactam	--	--	24, 75%	12, 71%	11, 69%	3, 75%	5, 62%

<b>Antibiotic</b>	<b>S. aureus (n=43)</b>	<b>Coag Neg Staph (n=13)</b>
Methicillin sensitive	23, 54%	6, 46%
Methicillin resistant	20, 47%	2, 15%
Cefoperazone sulbactam	20, 47%	5, 38%
Amikacin	19, 44%	3, 23%
Ciprofloxacin	10, 23%	4, 30%
Cloxacillin	5, 11%	2, 15%
Gentamicin	2, 5%	1, 8%
Clindamycin	14, 33%	3, 23%
Linezolid	12, 28%	4, 30%
Vancomycin	3, 7%	1, 8%
Erythromycin	6, 12%	1, 8%
Ceftazidime	2, 5%	0
Cefazolin	1, 2%	0
Cephalexin	1, 2%	0

The results of susceptibility pattern are summarized in Table – 4 and table 5.

Sensitivity pattern demonstrated that aerobic gram negative isolates like *Enterobacteriaceae* group and *P. aeruginosa* strains were largely susceptible to imipenem(100%), piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin. Piperacillin-tazobactam and the quinolones were active against more than 60% of the gram-negative organisms, while amoxicillin-clavulanate, cefoxatime, and cefuroxime were the least active of the antimicrobial tested.

*S.aureus* exhibited a high frequency of resistance to the antibiotics tested including methicillin (47%), erythromycin (34%). High levels of resistance to erythromycin, and ciprofloxacin (23% each) were found in *Enterococcus* species. However, no high-level aminoglycoside resistance was observed in the enterococcal isolates. All the isolates were uniformly susceptible to vancomycin, clindamycin, amikacin and linezolid. Multidrug resistant pseudomonas to ciprofloxacin, amikacin was isolated in 2 cases. Gram positive multidrug resistant organisms (MDRO) were isolated in 22 cases, comprising of 20 cases of MRSA and 2 cases of Coag Neg Staph. Methicillin resistant organisms were sensitive to vancomycin in more than 95% of the cases. Surgical debridement was done in 164(81%). More than 95% of the patients were treated and cured of the infection with minimal debridement and the antibiotics. Below knee amputation had to be conducted in 11cases (5%) and above knee amputation in 2 cases (.9%). 6 patients who were amputated were seen harboring MDRO strains. All MDRO cases were treated with vancomycin with good healing rates.

### Discussion

Most studies advocate treatment of the clinically infected diabetic foot wounds with the small-spectrum antimicrobial therapy to avoid development of the resistant strains/organisms [14]. Any delay in identifying the pathogens of this limb-threatening infection can result in potential life threatening situation. It has been studied that procuring tissue as the source of specimen for culture and sensitivity has yielded pathogenic organisms by eliminating the contaminants and hence is more sensitive and specific method than swab cultures [15-16]. Although some advocate the isolation with swab is reliable, it has to be done at utmost care as there is every possibility of isolating only contaminants [17]. We have followed the tissue technique for acquiring the specimens and hence very less sterile growth (n=10) and few contaminant growth. In our study, a total of 246 organisms were isolated from 202 samples with an average of 1.2 organisms per case. A study by vishwanathan et al., [18] yielded an average of 1.21organisms per case. Polymicrobial isolations are now commonly seen in badly infected diabetic foot throughout the world [19].

Gram negative organisms predominated in our study population. 162(66%) isolated bacteria were gram negative followed by gram- positive aerobes constituting 78 isolates (32%), which is in almost equal concordance with a study by Shankar et al.,[20] where Gram-negative bacteria (57.6%) were isolated more often than gram-positive ones (42.3%). *Proteus*, gram negative (19%) and *S.aureus*, gram positive (18%) were the predominantly isolated pathogens. In similar distribution *E.coli* (16%), *P.aeruginosa* (13%) was isolated.

A study by Ramani et al [21] also made similar observation and found *proteus* (20.73%), *klebsiella* (12.35%), *pseudomonas* (11.73%) as the most common pathogens. Prabhakar et al [22] also showed a predominant gram negative (*proteus*, *E.coli*) growth. Our sensitivity pattern of *proteus*, *E.coli*, *pseudomonas* showed more or less similar sensitivity to Imipenem, piperacillin, piperacillin+ tazobactam, ciprofloxacin, ceftazidime, and amikacin resembling the result of a study conducted at AIIMS, New Delhi [23]. More than 50% of gram positive organisms were sensitive to methicillin. Cefoperazone + sulbactam showed about 47% sensitivity, while ciprofloxacin and amikacin were only 23% and 44% sensitive. Similar results were seen in a study by Ekta bansal et al., [24]. We had isolated 20 cases of MRSA constituting 47% of *s. aureus* and 2 cases i.e., 15% of Methicillin resistant Coagulase negative *staphylococcus*. This is in accordance with the report of Heurtier et al [25]. Almost one third (22 of 78%) of our patients in whom gram positive bacteria like *s.aureus* and coagulase negative *staphylococcus* were isolated, were actually infected with these MDROs. Our study confirms that MDRO infection is common in hospitalized patients with diabetic foot ulcers. The prevalence of MRSA isolates was higher in our population as compared with previous studies. The sensitive antibiotic is vancomycin and hence the drug of choice in MRSA infections [25]. Amikacin, ciprofloxacin, clindamycin and linezolid were the other antibiotics commonly sensitive to gram positive organisms.

By confirming the microbiological profile of diabetic foot infections, we believe this study would aid the clinicians in the selection of the appropriate antibiotic for the organisms cultured based on the available results of this study. Aggressive hospital antibiotic protocols need to be formed to address such an issue to prevent catastrophes, resistant development, help the multi disciplinary care (physiotherapy etc) to facilitate better outcome. Such a protocol has to be administered at outpatient level, so that an appropriate therapeutic intervention is carried out, the moment a patient reaches the hospital.

### Conclusion

Diabetic foot infection is a polymicrobial infection of varying severity based on the extent, duration of the infection and the status of glycemic control. Gram negative organisms, like *proteus* was the most frequently isolated organism. Staph was the most common gram positive organism. A combination of Cefoperazone+sulbactam, anaerobic coverage with one of piperacillin+ tazobactam / ciprofloxacin / amikacin would be essential for the empirical treatment.

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