

Original Research Article

Clinico-Microbiological Profile of Diabetic Foot Ulcer: The common Anatomical site

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ABSTRACT

Background: Diabetes is one of the oldest diseases known to mankind. Ulceration of the foot in diabetes is a common complication. Diabetic foot ulcers are at high risk of infection secondary to high glucose levels and poor tissue perfusion. The aim is to identify the microorganisms and the antibiotic susceptibility pattern involved in different grades of diabetic foot ulcer.

Materials and Methods: 50 patients with diabetic foot ulcers (DFU) were included in this study. Pus was processed for Gram positive, Gram negative and Fungal isolates by culture. Antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion test.

Results: Out of 135 isolates, Gram negative bacteria comprised the major group of 73 (54.1%) followed by Gram positive bacteria 54 (40%) and Fungus 08 (5.9%) were observed on culture. *Staphylococcus aureus* (61.1%) in the Gram positive group is the commonest pathogen followed by *Enterococcus spp* (27.8%) and *Streptococcus spp* (11.1%). 44.5% of *Staphylococci* were methicillin resistant. Multi-drug resistance was seen in 28.26% of isolates. We observed ESBL producer in 18 (58.06%) isolates out of 31 Gram negative strain. Elderly male with Type II diabetes (NIDDM) cases were frequently observed to develop foot ulcer.

Conclusion: Diabetic foot infections are polymicrobial in nature. *Pseudomonas aeruginosa* in Gram negative organisms followed by *Staphylococcus aureus* in Gram positive group and *Candida spp* in fungal were the predominant pathogens. Empirical antibiotic selection should be followed by culture guided adjunctive therapy. Erythromycin, Lincomycin, Imipenem or Cefoperazone + Sulbactam would be appropriate for empirical treatment.

Abbreviations: NIDDM – non insulin dependent diabetes mellitus.

Key words: NIDDM, Gram-negative bacilli, polymicrobial.

INTRODUCTION

Diabetes is one of the oldest diseases known to mankind ⁽¹⁾. It afflicts large number of people of all social conditions throughout the world ⁽²⁾. Its

complications pose a major threat to future public health resources throughout the world. The WHO has projected that the maximum increase in diabetes will occur in India ⁽³⁾. India has nearly 33

million diabetic subjects today, which is mainly from the urban population. The scenario is also rapidly changing in rural areas⁽⁴⁾. Diabetes India study confirms the WHO estimate of 35 million adults with diabetes in India today⁽⁴⁾. Ulceration of the foot in diabetes is a common complication⁽⁵⁾. They also have a combined infection involving bone and soft tissue called the fetid foot. The unique anatomy of the foot is the main reason that infection is potentially serious in this location⁽⁶⁾. Dorsal or plantar regions are the most common site of ulcer in diabetic patients followed by heel and plantar metatarsals⁽⁶⁾. Infection is an underlying cause for the development of ulcers and unless there is aggressive intervention, amputation becomes the end result⁽¹⁾. Fifty percent of all traumatic amputations of the lower extremities are associated with diabetes^{1, 2}. Microbiology of diabetic foot ulcers is known to be polymicrobial in nature. It is commonly stated that patients with diabetes mellitus are more susceptible to infection than normal individuals. It further leads to complications and death more frequently if not treated effectively. There are limitations in assessing the risk of infection and resulting complications for which optimal management requires a multidisciplinary approach. The present study was undertaken to assess the role of aerobic bacteria in the incidence of diabetic foot ulcers, the antimicrobial susceptibility of such isolates, would assist the clinician in instituting therapy to avoid the dreaded complications.

MATERIALS AND METHODS

Sample Collection

50 patients with diabetic foot ulcers (DFU) were included in this study. The study was conducted over a period of 18 months. Samples were collected after explaining the aim of the research and informed consent was obtained from the patient. Clinical history was taken regarding to the age, sex, and type of diabetes, duration of diabetes, any predisposing factor and antibiotics given. A note was taken on the investigations and

type of operation performed. Swabs were brought to the microbiology department immediately and processed within 30 minutes of collection.

Sample Processing:

Samples were collected deep from the base of the ulcer using 2 sterile swabs. One swab was used for gram staining and the other was used for culture⁽⁷⁾. Direct gram stained smears were examined under the microscope to evaluate relative number of microorganisms and their morphological characteristics. Any fungal elements observed were confirmed by KOH preparation. The samples for culture were inoculated onto 5% Sheep blood agar(SBA), Chocolate agar and Mac Conkey's agar medium and incubated at 37° C for 24 hrs in 7-10% CO₂ concentration and the plates were examined for growth. Sabouraud's dextrose agar slopes were used for culture of fungus. The organisms were identified by direct gram staining, Colony morphology and Biochemical reactions.

Antimicrobial susceptibility testing

The antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion test.⁽⁵³⁾ The panel antibiotic discs used were Ampicillin(10µg) , Erythromycin (15µg), Clindamycin(2µg), Netilmycin (30µg), Lincomycin (15µg), Vancomycin (30µg), Gentamicin(120µg), Amikacin(30µg), Ceftazidime (30µg), Imipenem(10µg), Piperacillin/Tazobactam(100µg+10µg), Cefoperazone/Sulbactam(75µg +30µg), Cefuroxime (30µg), Cefotaxime(30µg), Ofloxacin (5µg), Ciprofloxacin(5µg). Methicillin –Resistant *Staphylococcus Aureus* (MRSA) detection:

This was detected by using a 1µg Oxacillin disk. Bacterial lawn culture inoculums of the *Staphylococcus spp* was kept aseptically overnight incubation at 35-37° C. The zone of inhibition ≤ 10mm was considered to be resistant to oxacillin and penicillinase resistant penicillins. Gram negative bacteria, which were found to be resistant to 3rd generation cephalosporins were subjected to the detection of Extended spectrum β- lactamase producers (ESBL).

Study Design: Cohort Study (Prospective Observational study) with asking research questionnaire developed for this purpose.

Study Location: This study was undertaken in the Department of Microbiology, Government Wenlock Hospital Mangalore from 2006-2008.

Inclusion Criteria: Foot ulcer patients who diagnosed or suspected to have diabetes mellitus and confirmed by elevated fasting as well as postprandial blood sugar.

Exclusion criteria: Healthy people who were suspected with foot ulcer having normal fasting and postprandial blood sugar.

Ethical issues:

This study confirms to the ethical principles of medical research developed by the World Medical Association Declaration of Helsinki. Ethical clearance was given by the Research Committee, Government Wenlock Hospital Mangalore.

Data Analysis: All data obtained with questionnaire and microbiological culture analysis were analysed using the statistical software SSPS 16.0. The chi square test was used to compare between two groups. Statistical significance was accepted when P value is ≤ 0.05 .

RESULTS

Out of 135 isolates, gram negative bacteria comprised the major group of 73 (54.1%) followed by gram positive bacteria 54 (40%) and fungus 08 (5.9%), Table 1.

Table 1: Type of microorganisms isolated from the diabetic foot ulcer.

Type of Microorganisms	Number of Isolates	Percentage
Gram positive bacteria	54	40
Gram negative bacteria	73	54.1
Fungi	08	5.9

Staphylococcus aureus was the commonest pathogen amongst the gram positive aerobes comprising 33 (61.1%) number of isolates. The other gram positive organisms isolated were *Enterococcus spp* (15 nos, 27.8%) and *Streptococcus pyogenes* (6 nos, 11.1%), (Table 2).

Table 2. Gram Positive Bacteria Isolated from Diabetic Foot Ulcer.

Species	Number of Isolates (n=54)	Percentage
<i>S. aureus</i>	33*	61.1
<i>Enterococcus spp</i>	15	27.8
<i>S. pyogenes</i>	06	11.1

* Statistically significant ($p < 0.001$) when compared to the rate of isolation of other gram positive organisms

In our study, gram negative bacteria comprised of 73 (54.1%) of total aerobic isolates. They were predominant pathogens among the aerobic bacteria. *Pseudomonas aeruginosa* was in 19 (26.1%) isolates followed by *Klebsiella species* and *Citrobacter species*. (Table 3)

Table 3. Gram negative bacteria isolated from diabetic foot ulcers.

Species	Number of isolates n=73	Percentage
<i>Pseudomonas aeruginosa</i>	19	26.1
<i>Klebsiella pneumoniae</i>	17	23.3
<i>Citrobacter spp</i>	10	13.7
<i>Proteus mirabilis</i>	08	11.0
<i>Escherichia coli</i>	06	8.2
<i>Proteus vulgaris</i>	04	5.5
<i>Providencia spp</i>	03	4.1
<i>Morganella spp</i>	03	4.1
<i>Acinetobacter spp</i>	02	2.7
<i>Enterobacter spp</i>	01	1.3

All the isolates of *Staphylococcus aureus* were found to be sensitive to Netilmicin and Vancomycin. Majority of the isolates were highly sensitive to Lincomycin. Out of the 33 *Staphylococcus aureus* isolates, 15(45.5%) were detected to be Methicillin-resistant *Staphylococcus aureus* (Table 4). All *Enterococcus spp* were susceptible to Ampicillin and Vancomycin. Around 86.6% of the enterococcal isolates exhibited high level aminoglycoside resistance (HLAR).

Table 4. Antimicrobial susceptibility pattern of gram positive cocci.

Antimicrobial agents	S. aureus (n=33) (%)		Enterococcus spp (n=15)	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	11 (33.34)	22 (66.66)	15 (100)	-
Oxacillin	15 (45.45)	18 (54.55)	-	-
Erythromycin	22 (66.67)	11 (33.33)	10 (66.66)	5 (33.34)
Netilmicin	33 (100)	-	-	-
Lincomycin	30 (90.9)	9 (9.1)	-	-
Vancomycin	33 (100)	-	15 (100)	-
Clindamycin	23 (69.69)	10 (30.31)	-	-
Gentamicin (HLG)	-	-	13 (86.67)	2 (13.33)

The present study showed that all the gram negative aerobic isolates were susceptible to Imipinem and Cefoperazone-Sulbactam. *Pseudomonas aeruginosa*, the predominant gram

negative pathogen was susceptible to Imipinem (100%) and majority of the strains were susceptible to cefoperazone/sulbactam, piperacillin/ tazobactam and gentamicin, Table 5.

Table 5. Antibiotic susceptibility of gram negative bacteria

Antimicrobial agent	Pseudomonas aerogenosa n=19%	Klebsiella Spp n=17%	Citrobacter n=10%	P. mirabilis n=8%	E. Colli n=6%
Ampicillin	-	17(100)	3 (30)	1 (12.5)	0 (0)
Cefuroxime	-	14(82.4)	2 (20)	3 (37.5)	4 (66.6)
Cefotaxime	-	15(88.3)	3 (30)	5 (50)	3 (50)
Ofloxacin	-	16(94.2)	8 (80)	5 (62.5)	2 (33.3)
Gentamicin	16(84.2)	14(82.4)	2 (20)	7 (87.5)	2 (33.3)
Piperacillin	-	4(23.5)	2 (20)	8 (100)	1 (16.6)
Ceftazidime	-	7(41.2)	3 (30)	6 (75)	0 (0)
Amikacin	14(73.68)	14(82.4)	9 (90)	7 (87.5)	6 (100)
Ciprofloxacin	-	8(47.05)	8 (80)	8 (100)	2 (33.3)
Cefoperazone sulbactam	17(89.47)	17(100)	10 (100)	8 (100)	6 (100)
Imipenem	19 (100)	17(100)	10 (100)	8 (100)	6 (100)
Piperacillin Tazobactam	18(94.73)	-	-	-	-

Gram negative isolates that were resistant to third generation cephalosporins, were also screened for ESBL production. In our study 18 (58.1%) of the total 31 isolates were confirmed to be ESBL

producers. Among ESBL producing organisms, *Escherichia Coli* contributes to be the major one comprising 66.67% of the total. (Table 6)

Table 6 : Distribution of Extended spectrum β - lactamase producers(ESBL) strains among the different isolates.

Organism	No of strains isolated	ESBL	Percentage
Escherichia coli	6	4	66.67
Klebsiella spp	10	6	60
Citrobacter spp	7	4	57.2
P. mirabilles	4	2	50
P. vulgaris	4	2	50
Total	31	18	58.06

Maximum number of cases (23, 55%) among males belonged to the age group 40-59, followed by the age above 60 yrs (15, 36%) Our study showed a significant male preponderance (p< 0.001) in foot ulcers comprising of 42 (84%) numbers as compared to females comprising of

only 8 (16%) of the total. In females ulcers were mainly detected above 60 yrs of age. Gram negative organisms constituted the major pathogens in both male and female patients. (Table 7)

Table 7: Prevalence of bacterial pathogens in relation to age and sex

Sex	Age in Yrs	Type of isolates (%)		No .of organisms	Percentage
		Gm +ve (n=54)	Gm-ve (n=73)		
Male (n=42)*	<20	-	-	-	-
	20-39	4	7	11	8.7
	40-59	25	33	58	45.7#
	≥60	17	22	39	30.6
Female (n=8)*	<20	-	-	-	-
	20-39	-	-	-	-
	40-59	1	1	2	1.6
	≥60	07	10	17	13.4

* Statistically significant (p <0.01) when both the sexes were compared. # Statistically significant (p <0.05) when age groups were compared.

In our study it was found that majority of the patients belonged to Type II (NIDDM) category (45, 90%) in contrast to the Type 1 (IDDM) category where the number was only (5, 10%).

Predominant pathogens in Type I were Gram positive i.e. 8 (62%) whereas in Type II the predominant pathogens were Gram negative organisms I.e. 68(59.6%) (Table 8).

Table 8: Prevalence of bacterial pathogens according to the types of Diabetes.

Type	Age in Yrs	Type of isolates		No .of organisms	Percentage
		Gram +ve	Gram-ve		
Type 1 (IDDM) N=5	20-39	-	-	-	-
	40-59	6	4	10	7.8
	≥60	2	1	3	2.3
Type 2(NIDDM) N=45*	20-39	4	7	11	8.6
	40-59	20	30	50	39.3*
	≥60	22	31	53	42#

* Statistically significant (p <0.001) when two types diabetes were compared.

Out of 50 patients with diabetic foot ulcer, 38 patients had blood sugar level >200mg/dl (designated as uncontrolled group) and 12 cases showed the blood sugar level ≤ 200mg/dl as

controlled group. In uncontrolled group 2.7 organisms were isolated per sample and 2.16 organisms in controlled group.

Table 9: Mean Isolates in relation to the blood sugar level

Blood sugar level	Number of patients	Number of isolates	Isolates per case
≤200mg%	12	26	2.16
>200mg%	38	104	2.7

Duration of the diabetes was elicited in patients with foot ulcers. In majority of them (38, 76%) the duration was within 5 yrs. It was observed that

gram negative organisms were the predominant pathogens (59, 47%) with an average of 2.5 isolates per sample within this group. (Table 10)

Table 10: Prevalence of bacterial pathogens in relation to the duration of diabetes

Duration	Number of patients	Isolates		Isolates/case
		Gram positive	Gram negative	
Detected on admission	4	8	3	2.75
Up to 5 years	38	36	59	2.5
6-10 years	4	5	6	2.75
Could not be elicited	4	5	5	2.5

Out of 50 patients, 29 were smokers followed by 26 had neuropathic changes and 23 had vascular changes. It was seen that the no. of isolates per

patient was the maximum (2.6/ case; p< 0.05) in patients having vascular changes. (Table 11)

Table 11: Prevalence of bacterial pathogens in relation to the associated predisposing condition

Predisposing Conditions	Number of patients	Isolates (% age)		Isolates/case
		Gram positive	Gram negative	
Smoking	29	26 (44.06)	33 (55.94)	2.03
Vasculopathy	23	29 (48.34)	31 (51.66)	2.6
Neuropathy	26	23 (38.98)	36 (61.01)	2.26

As per Wagner's classification, Grade 1 ulcer was seen in 3 patients, Grade II in 16 patients, Grade III in 22 patients, Grade IV in 5 patients and only 4 patients with Grade V lesions. The mean number of isolates in grade I ulcers was 2.3, in Grade II 2.62, in Grade III 2.6, in Grade IV 2.0 and in Grade V it was 2.75. There was an increase in the mean number of isolates as the grade increased where maximum isolates was in Grade V ulcers except there was a slight drop in number of isolates in Grade IV (Table 12). It was also observed that isolation of Gram negative organisms in grade V was significantly higher ($p < 0.05$) than in Grade I (Table 12)



Fig I. Most common Anatomical location (medial aspect of foot) In diabetic foot ulcer and Wagner's grading.

Table 12: Incidence of bacterial pathogens in relation to grade of ulcers(Wagner's grading).

	Grade I	Grade II	Grade III	Grade IV	Grade V
No. of patients	3	16	22	5	4
Organisms isolated	7	42	57	10	11
Average per sample	2.3	2.62	2.6	2.0	2.75*
Gram positive organisms	4 (57.14)	21 (50)	21 (36.84)	4 (40)	4 (36.36)
Gram negative organisms	3 (42.85)	21 (50)	35 (63.15)	6 (60)	7 (63.63) [#]
*Statistically significant ($p < 0.05$) when grade V is compared to grade I. # Statistically significant ($p < 0.01$) when grade V is compared to grade I.					

DISCUSSION

Diabetes mellitus is very much prevalent in Indian population and so also the diabetic foot ulcers contributing 20% of hospital admissions⁽¹⁴⁾. The incidence of type 2 diabetes is rising in India. Epidemiological studies show the prevalence of type 2 diabetes is more in southern region in comparison to northern and eastern parts of the country⁽¹⁵⁾. In our study the gram negative organisms (51.4%), gram positive cocci (40%) and fungal isolates (5.9%) are major pathogens detected for infection of diabetic foot ulcer^(11, 14, 16). *Staphylococcus aureus* (61.1%) in the gram positive group is the commonest pathogen followed by *Enterococcus spp* (27.8%) and *Streptococcus spp* (11.1%) as isolated in our study

^(12,17). *Klebsiella pneumoniae* (23.3%) closely followed by *Pseudomonas aeruginosa* (26.1%)^(11, 14) are isolated among the gram negative group. Gram positive isolates are uniformly susceptible to vancomycin and few of the enterococcal (13.1%) group are resistant to aminoglycoside antibiotic in culture^(11,16). Methicillin resistant *Staphylococcus Aureus* (MRSA) is observed in 45.4% of the staphylococcal group⁽¹⁴⁾. Most of the MRSA infections are thought to be nosocomial in origin. Hence high risk diabetic foot ulcer patient are at greater risk of developing MRSA during repeated hospitalisation or lengthier hospital stay. Gram negative bacterial isolates in the present study were susceptible to Imipenem and Cefoperazone/Sulbactam^(11,14). Certain strains

of gram negative bacteria including *Pseudomonas aeruginosa* are resistant to first line antibiotics (Amikacin, Gentamicin and Ceftazidime) may be due to multidrug resistant nosocomial strains developed during indiscriminate use of antibiotics without culture. We observed ESBL producer in 18 (58.06%) isolates out of 31 gram negative strain, which is quite high rate of prevalence in comparison to other studies⁽¹⁶⁾. We also observed *Escherichia coli* constituting 66.67% of the ESBL producers could be due to widespread use of antibiotics in Tertiary care hospital⁽¹⁶⁾. The mean age is 57.5 years range is 29-75 years⁽¹⁴⁾ and the male: female ratio is being 5.3/1. Elderly male preponderance in the present study⁽¹⁸⁾ is observed as they used to walk long distances in bare foot for agricultural purpose. In a developing country like India due to lack of awareness of signs and symptoms diabetes are remained misdiagnosed/undiagnosed resulting in the complication of diabetic foot ulcer. In the present study Type II diabetes (NIDDM) cases are 45 (90%) in comparison with insulin dependent diabetes mellitus (IDDM) 5 (10%) cases⁽⁴⁾. Most of the cases are detected in the age group 40-59 years, which indicates as age and duration of diabetes increases culminates poor glycaemic control leading to foot ulcer and other complications^(9, 19). The combined effect of poor host defense and wound healing responses set the stage of peripheral neuropathy leading to foot problems. Smoking has always being considered as an etiology of foot ulceration because of the increased risk of atheroma and left shifting of oxyhaemoglobin dissociation curve due to glycosylation⁽²⁰⁾. Neuropathy is an unique complication of the diabetes mellitus that predisposes to foot ulceration due to trivial trauma⁽¹³⁾. Vasculopathy is producing venous insufficiency of medium and small size vessels leading to decreased blood flow and low oxygen saturation of the peripheral tissue enhancing the growth of microaerophilic and anaerobic organisms. Altered blood flow can affect the kinetic of local inflammatory response involving both cellular and

humoral factors. Tissue hypoxia directly alters the oxygen dependent bactericidal effects of leucocytes⁽⁸⁾. The Wagner's classification reveals that there is an increase in mean number of isolates as the grade increases except for decrease in grade IV ulcer may be due to some technical error during collection and processing of the isolates. Maximum isolates per case is found in grade V ulcer⁽¹⁰⁾. It can thus be interpreted that there is poor healing response to injury in a patient with diabetes. This may be due to vascular insufficiency, neuropathy and poor glycemic control. Most common Anatomical location (medial aspect of foot) in diabetic foot ulcer and Wagner's grading (Fig I and Table 12). The upright posture of human body is responsible for impaired venous return and stagnation⁶.

CONCLUSION

Diabetes mellitus has become a problem of epidemic proportions. Type 2 diabetes is by far the commonest form of the disease globally with developing countries like India at the forefront of this epidemic. India with its dubious distinction of being called "the diabetic capital of the world" is presently estimated to have over 30 million individuals affected by this deadly disease. Despite several advances in the field of diabetology, it is unfortunate that there exists a low awareness of the disease among the public. Infection of the diabetic foot is a common problem in clinical practice and is associated with significant morbidity. Early detection and appropriate treatment are the cornerstones for delaying the onset and progression of the diabetic complications. Optimal management requires a multidisciplinary approach including an intensive education program which can improve the foot care knowledge and behavior of high risk patients. Aggressive surgical debridement and wound management, carefully chosen microbial therapy and modification of host factors (i.e. hyperglycemia, arterial insufficiency) are equally important for a successful outcome. Diabetic foot infections are polymicrobial in nature. As the

Wagner's grade increase, the prevalence of isolates also increases. While *Pseudomonas aeruginosa* was the most frequent isolate among the gram negative organisms, *Staphylococcus aureus* was the most common among gram positive organisms. *Candida species* are the predominant isolates among fungal pathogens. Empirical antibiotic selection should be followed by culture guided adjunctive therapy. Erythromycin, Lincomycin, Imipenem or Cefoperazone + Sulbactam would be appropriate for empirical treatment.

Conflict of interest: None

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