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## Bacteriological study of diabetic foot infections in tertiary care hospital

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### Abstract

**Aim:** The present study was undertaken to assess the role of aerobic and anaerobic bacteria in the causation of diabetic foot ulcers.

**Material and Method:** Foot ulcer patients were categorized into six grades based on Wagner classification. A semi structured questionnaire was developed to record the medical history, examination details and investigation reports. A detail medical history was taken of all the patients regarding age and sex, duration of diabetes, type of diabetes, duration of foot ulcer, grade of ulcer, smoking, hypertension and associated complications. Meticulous clinical examination was done.

**Results:** 16 (20.5%) patients had blood sugar level within controlled limits and 62 (79.5%) patients had poor glycemic control at the time an average of 1.6 organisms per case were isolated from patients having control. An average of one organism per case was isolated from patients with controlled blood sugar level.,

**Conclusion:** As the grading of ulcer increased, the number of bacteria isolated also increased. Polymicrobial infection was noted in (55.3%) cases. An average of 1.8 organisms per case was isolated. Majority of the isolates were aerobes. Anaerobes constituted only 3.6% and *Peptostreptococcus anaerobius* was the major anaerobe isolated. Majority of the isolates were aerobic Gram negative bacteria. *Staphylococcus aureus* was the commonest among the aerobic gram positive bacteria. *Pseudomonas aeruginosa* was the second common of the isolates.

**Keywords:** diabetic foot ulcers, polymicrobial infection

### Introduction

Infected foot ulcer is common cause of morbidity in diabetic patients, ultimately leading to dreaded complications like gangrene and amputations. Infection is most often a consequence of foot ulceration, which typically follows trauma to neuropathic foot. The alarming fact is that India has more people with diabetes than any other country and incidence of foot problem and amputation remain very high, accounting for up to 20 % of diabetes related hospital admission. This can be easily attributed to several practices prevalent in India, such as barefoot walking, inadequate facilities for diabetes care, low socioeconomic status and illiteracy <sup>[1]</sup>.

About 10-30% of diabetic patients with a foot ulcer will eventually progress to an amputation, which may be minor (foot sparing) or major. Conversely, an infected foot ulcer precedes 60% of amputations, making infections perhaps the most important proximate cause of this tragic outcome <sup>[2]</sup>. Mild or non-limb threatening infections can be treated with oral antibiotics (Cephalosporins, Clindamycin, Amoxicillin/Clavulanate and Fluoroquinolones), surgical debridement of necrotic tissue, local wound care (avoidance of weight bearing over the ulcer) and close surveillance for progression of infection <sup>[3]</sup>.

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. Emergence of resistance among organisms against commonly used antibiotics has been clearly outlined in various studies as being due to their indiscriminate use. There is direct relationship between total amount of certain antibiotics used in particular hospital during a certain period and number of resistant strains that emerge <sup>[4]</sup>.

The present study was undertaken to assess the role of aerobic and anaerobic bacteria in the causation of diabetic foot ulcers. To determine antimicrobial susceptibility profile of organisms isolated from patients with diabetic foot ulcers. The risk factors for infection of ulcers with MDRO's and the outcome of these infections were also studied. The antimicrobial spectrum of these isolates would assist clinicians in the therapy of this dreaded complication of diabetes.

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## Material and Method

The present study was undertaken during the period from September 2010 to June 2012 in the Department of Microbiology, Krishna Institute of Medical Sciences Karad. 78 diabetic patients with foot ulcer admitted at surgical wards of Krishna Hospital, Karad were included in the study. Foot ulcer patients were categorized into six grades based on Wagner classification. A semi structured questionnaire was developed to record the medical history, examination details and investigation reports. A detail medical history was taken of all the patients regarding age and sex, duration of diabetes, type of diabetes, duration of foot ulcer, grade of ulcer, smoking, hypertension and associated complications. Meticulous clinical examination was done. Neuropathy was assessed by ability to sense touch with 10 gram monofilament and tuning fork. The temperature of the patient was also recorded. The adequacy of peripheral circulation was done by palpating the posterior tibial artery and the dorsalis pedis artery. The foot was examined for the presence of callus or any other abnormality. Touch, pain and joint position sensation was examined in the foot. Foul smell, local rise of temperature, discharge and discolouration of the surrounding area was noted. The base of the ulcer was palpated to assess the depth of the ulcer. When osteomyelitis (to assess bone involvement) was suspected, the foot X-ray was done and the ulcer was graded according to the Wagner's classification.

The patient was made to lie in supine position on bed and ulcer site was examined. The wound was thoroughly cleaned with sterile saline. Depending upon nature of ulcer, culture specimen was obtained as either curettage of the base of ulcer, needle aspiration of abscess material or deep wound swab using aseptic technique so as to avoid contamination with the normal skin flora. The specimen was kept in sterile culture tube or bottle and immediately transported to microbiology laboratory for processing.

The heat fixed smears were stained by Gram stain technique and examined under oil immersion objective of the light microscope. The smears were examined for the presence of pus cells, Gram positive and Gram negative organisms. The size, shape, arrangement of bacteria was noted.

The specimens were culture inoculated onto appropriate culture media viz., Blood agar, Chocolate agar plate for isolation of fastidious bacteria, MacConkey agar plate for isolation of gram negative organisms. The inoculated media were incubated at 37 °C overnight. Bacterial isolates were identified by their characteristic colony morphology, Gram stain, motility, catalase test, oxidase test and biochemical reactions as described in Practical microbiology of Mackie McCartney 14th volume.

Simultaneously specimen were inoculated on freshly prepared Blood agar and Chocolate agar plates and a plate of *Pseudomonas aeruginosa* was kept as biological control. All the plates were incubated anaerobically for 48 hours at 37°C in an anaerobic jar with Gaspak (HiMedia LE002A-5NO LOT-11-098 3.5 L). All isolated organisms were identified using colony morphology, Gram stain and maintained as stock culture in Robertson's cooked meat media (RCM). Subculture from RCM was done on freshly prepared blood agar plate. Gentamicin disc (10 µg) was placed at the junction of primary well and first quadrant streaking while a Metronidazole disc (5 µg) was placed at the junction of first and second quadrant streaking to identify the anaerobes presumptively as all the anaerobes are known to be resistant to Gentamicin.

The Blood agar plates were immediately incubated

anaerobically for 48 hours at 37°C in an anaerobic jar with Gaspak (Anaerogas Pack 3.5 L LE 002A-5NO LOT 11-098). Colonies sensitive to Metronidazole disc (5 µg) were identified using Gram stain and morphology as obligate anaerobes. As the anaerobic culture setup is not established in our department, all isolates maintained in RCM were carried to anaerobic laboratory JNMC Belgaum. Identification of species was made according to Anaerobic laboratory manual of Wadsworth 3<sup>rd</sup> Edition [5].

Antimicrobial susceptibility testing of aerobic isolates was performed on Mueller Hinton agar plate using the Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI 2011 Volume 31 No. 1) [6].

ATCC *Staphylococcus aureus* 43300 was used as control strain for the test. Inoculum of test and control organisms was prepared and matched with turbidity 0.5 McFarland standard. Test and control organisms were inoculated onto Mueller-Hinton agar plates. *Staphylococcus* species were tested for Methicillin resistance using 30µg Cefoxitin discs by Kirby Bauer Disc Diffusion method. Plates were incubated aerobically at 35°C for 24 hours. MRSA: The diameter of zone of inhibition was measured and interpreted according to National Committee for Clinical Laboratory Standards guidelines (CLSI 2011 Volume 31 No. 1).

## Results

In the present study 16 (20.5%) patients had blood sugar level within controlled limits and 62 (79.5%) patients had poor glycemic control at the time of admission.

An average of 1.6 organisms per case were isolated from patients having poor glycemic control. An average of one organism per case was isolated from patients with controlled blood sugar level.

In the present study 16 (20.5%) patients had blood sugar level within controlled limits and 62 (79.5%) patients had poor glycemic control at the time An average of 1.6 organisms per case were isolated from patients having control. An average of one organism per case was isolated from patients with controlled blood sugar level.

**Table 1:** Correlation between grade of ulcer and bacterial isolates.

Grade	No. of cases	Monomicrobial infection	Polymicrobial infection			Total organisms	Isolates per case
			Two	Three	> Three		
I	7	5	2	-	-	9	1.2
II	17	6	7	4	-	32	1.8
III	20	11	4	3	2	36	1.8
IV	22	11	5	4	-	33	1.6
V	12	1	10	1	-	24	2

## Discussion

Diabetic foot is considered one of the most threatening and disabling complications for a diabetic patient as the lesions of the extremities can become so severe that the patient may risk the amputation of the toe, foot or leg. Successful treatment depends on correct evaluation of the patient, isolation of pathogens with their antibiotic susceptibility patterns.

In the present study, an attempt has been made to know the various etiological agents responsible for diabetic foot ulcers and their antibiotic susceptibility patterns. In the present study, we observed that the males were in majority 60(76.9%) and

females were 18(23.1%). The ratio of males to females was 3.3:1 showing diabetic foot ulcers to be more common in males. The male population predominated the studies and may be due to the fact that males are more involved in smoking and outdoor activities in the Indian scenario and are thus more prone to injuries which can predispose to ulcers [7].

In the present study mean duration of diabetes was 6.5 years which is in close comparison to the study of Mamatha Samaga. 26(33.3%) of the patients had diabetes mellitus for 2-5 years and 15(19.2%) for 6-10 years. Diabetic peripheral neuropathy results in foot deformity due to a motor deficit, anaesthesia from sensory impairment and increased susceptibility to sepsis following autonomic dysfunction. Incidence of neuropathy is different in different studies. In the above series the incidence of neuropathy is variable as this depends on the clinical evaluation. In the present study 27(34.6%) were smokers while 51(65.4%) were non smokers. Smoking has always been considered a factor in the etiology of foot lesions not only because of increased incidence of atheroma, but also because of added shift to the left of oxyhaemoglobin dissociation curve due to glycosylation [8].

In the present study 14(18%) had associated hypertension while 64(82%) were non-hypertensives. An average of 1.6 organisms per case was isolated from patient having poor glycemic control and one organism per case was isolated from patients with controlled blood sugar level. 62 patients had high blood sugar level (F >110, PP >160, R >200 mg/dl) and were labelled as poorly controlled, while 16 patients had blood sugar level within controlled limits (F 110, PP <160 and R <200 mm/dl).

The good control of blood sugar in diabetic patient is a desirable goal in the prevention of certain infections and to ensure maintenance of normal host defense mechanisms determining resistance and response to infection. There is a significant diminution in intracellular bactericidal activity of leukocytes with *Staphylococcus aureus* and *Escherichia coli* in patients with poorly controlled

diabetes. Good control of blood sugar may markedly improve healing of wounds in diabetic patients with good peripheral blood flow. Polymorphonuclear leucocyte functions such as chemotaxis, phagocytosis and intracellular killing improve, with lowering of blood sugar.

Blood glucose home monitoring is currently recommended to aid in the day to day management of diabetes. In present study, of the 78 infected ulcers, 76 ulcers yielded growth of organisms and two ulcers were bacteriologically sterile. A total of 139 organisms were isolated from positive cultures of which, 134 (96.4%) were aerobes and only 5(3.6%) isolates were anaerobes. Anaerobes were not isolated as the sole pathogenic agent.

In our study the predominant gram positive cocci isolated was *Staphylococcus aureus* and accounted for 38 (28.4%) of the infections followed by *Pseudomonas aeruginosa*, this was in accordance to the study conducted in Indian tertiary care hospital.

The results of our study suggests a definite increase in the prevalence of MDRO in Diabetic foot ulcer than most of the earlier standard results. This alarmingly increasing incidence of Multi Drug Resistant Organism (MDRO) is a potential risk factor in management of diabetic infection. Since improper management of MDRO might lead to devastating complications, which includes systemic toxicity, gangrene formation and amputation of lower extremity. Therefore patients with wounds infected with MDRO require an early

diagnosis and careful follow up, ensure that appropriate and effective medical and surgical regimen is readily available to the patients. In spite of various precautions taken to control and prevent the spread of MDRO, they seem to be an ever-growing problem and are associated with adverse prognosis.

These MDRO are frequently resistant to many classes of antibiotic so it is necessary for the clinicians to be completely aware of the prevalence rate of multi drug resistant organism (MDRO) and their management strategies. Hence surveillance about the prevalence of the MDRO should be done at regular intervals to assess the susceptibility patterns of the local strains, in order to adopt proper antibiotic policy to keep a check on the increasing drug resistance. In addition to proper cleansing, debridement, local wound care, a diabetic foot infection requires a carefully selected broad-spectrum antibiotic therapy.

### Conclusion

Diabetic foot ulcers were more common in 6<sup>th</sup> and 7<sup>th</sup> decade of life. Mean duration of diabetes mellitus in the study was 6.5 years. Majority of the patients with foot ulcers had diabetes mellitus for 2-5 years. Most of the foot ulcers were found in type II Diabetes Mellitus. Males were found to be more common victims. Most common associated complication was neuropathy.

Amputation procedure as a part of therapy was more commonly needed in smokers. In poorly controlled diabetics mean number of bacteria isolated from the lesions were maximum as compared to well controlled. Majority of the ulcers belonged to Grade III and IV. As the grading of ulcer increased, the number of bacteria isolated also increased. Polymicrobial infection was noted in (55.3%) cases. An average of 1.8 organisms per case was isolated. Majority of the isolates were aerobes. Anaerobes constituted only 3.6% and *Peptostreptococcus anaerobius* was the major anaerobe isolated. Majority of the isolates were aerobic Gram negative bacteria. *Staphylococcus aureus* was the commonest among the aerobic gram positive bacteria. *Pseudomonas aeruginosa* was the second common of the isolates.

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