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Original Research Article

Bacterial and clinical profile of diabetic foot ulcer using optimal culture techniques

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ABSTRACT

Background: Diabetic foot ulcers (DFU) are the complications of diabetes mellitus. The diabetic foot ulcer infections are polymicrobial in nature. If they are not recognized and controlled it leads to many devastating consequences like limb amputation, sepsis, and even mortality. Hence, the present study was undertaken to determine the bacterial and clinical profile of diabetic foot ulcer using optimal culture techniques and the antimicrobial sensitivity pattern of the isolates.
Methods: A total number of 103 patients with a foot ulcer of Wagner's grade II or more and evidence of purulent exudates or edema were included in the study. Swab samples were obtained from the base of ulcers and were sent for bacteriological study. The specimen was processed in the microbiology laboratory for Gram stain, aerobic culture, and anaerobic culture. The organisms isolated were identified by standard procedures and antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method.
Results: A total no of 253 organisms were isolated from 103 patients. Out of these, 217 aerobes were isolated, and the most common organism isolated from gram positive bacteria was *Staphylococcus aureus*, 53 (24.42 %). *Pseudomonas aeruginosa*, 42 (19.35 %) was the predominant organism isolated from gram negative bacteria. Among the total 36 anaerobes, *Bacteroides fragilis* group, 17 (47.22 %) was the most common organism isolated. All the gram positive aerobic organisms were found to be sensitive for vancomycin. Among the gram negative organisms, *E. coli, Proteus mirabilis, Proteus vulgaris*, showed more sensitivity to cefotaxime (90.62%, 91.66%, 75%) respectively. Metronidazole was the drug of choice in case of anaerobes followed by imipenem.
Conclusions: It is necessary to identify the etiological factors and susceptible microorganisms responsible for causation of DFU. So that prompt management of diabetic foot ulcers is possible for successful outcome. Appreciation of the causative organisms in diabetic foot and their antibiotic sensitivity is essential for institution of appropriate antibiotic therapy.

Keywords: Aerobes, Anaerobes, Antimicrobial sensitivity testing, Diabetic foot ulcer

INTRODUCTION

Diabetes mellitus (DM) is one of the major public health problems whose prevalence is rapidly rising all over the globe at an alarming rate. Nowhere is the diabetic epidemic more pronounced than in India, as the WHO reports show that 69.2 million people had diabetes in the year 2015.[1] Patients with DM are prone to multifarious complications such as diabetic foot ulcer (DFU). DFU is a common complication of DM that has shown an growing trend over previous periods.[2-4] At some time in their life, 15% of people with diabetes mellitus develop foot ulcers that are highly susceptible to infection.[5] On estimation, the prevalence of this complication ranges

from 4%-27%.[6-8] DFU is considered as a major source of morbidity and a leading cause of hospitalization in patients with diabetes.[9] DFU can lead to infection, gangrene, amputation, and even death if necessary care is not provided.[10]

The microbiology of diabetic foot ulcers with acute infections who have not recently received antibiotics show aerobic gram positive *Cocci* as isolates.

In other patients however a polymicrobial infection involving gram negative and obligate anaerobic organisms is likely to occur.[11] The antibiotic therapy for treating diabetic foot ulcers needs to be guided appropriately in the light of causative organism and its sensitivity pattern to various drugs. And this, calls upon a need for a well-planned bacteriological study of diabetic foot ulcers.

The present study aims to assess the role of aerobic and anaerobic bacteria in the causation of diabetic foot ulcers in tertiary care hospital in Sangli and Miraj. The antimicrobial spectrum of these isolates would surely benefit the clinicians to achieve spectacular success in the treatment of diabetic foot ulcers. This will surely decrease the morbidity and economic burden of the disease by halting the major cause of non-traumatic lower limb amputations.

METHODS

This study was carried out in Government Medical College and Hospital, Miraj & PVPGH, Sangli from June 2007 to December 2007. A total number of 103 patients with diabetic foot ulcers attending the surgery, out-patient & in-patient department were enrolled for the study. Diabetic patients with a foot ulcer of Wagner's grade II or more and evidence of purulent exudates or edema were included in the study. Detailed history of the patient was collected.

Sample collection

Swab samples were obtained from the base of ulcer after cleaning with normal saline & then rubbing the swab over the lesion. Three swabs were collected from the same site. One swab was placed in a sterile test tube, second was placed in Stuart's medium & third was transferred to a sterile tube containing RCM. Discharge was aspirated with a sterile needle & syringe aseptically. In case of patients undergoing any surgical intervention, the surgeon was requested to send the specimen to the laboratory. Samples after collection were immediately transported to the laboratory for processing.

Processing of sample in the laboratory

In the laboratory, the sample was processed immediately by using standard gram staining procedure to study the morphology of the organisms.

Aerobic culture study

The sample was inoculated on blood agar, chocolate agar, Mac Conkey agar & then incubated at 37^0C for 24 hours. The colonies obtained were then processed as per standard conventional bacteriological methods (as per Mackie McCartney).[12]

Antibiotic susceptibility tests (AST) of the isolates was done by Kirby- Bauer disc diffusion method. Following antibiotic discs were used depending on the gram character of the isolate (as per the CLSI guidelines 2007).[13]

Gram positive

Vancomycin (Va) [30 µg], Penicillin (P) [10 units], Oxacillin (Ox) [1 µg], Cotrimoxazole (Co) [1.25 µg + 23.75 µg], Erythromycin (E) [15 µg], Ofloxacin (Of) [5 µg] & Ciprofloxacin (Cp) [5 µg].

Gram negative

Ampicillin (A) [10 µg], Amikacin (Ak) [30 µg], Ofloxacin (Of) [5 µg], Gentamicin (G) [10 µg], Ciprofloxacin (Cp) [5 µg], Cefotaxime (Ce) [30 µg] & Ceftriaxone (Ct) [30 µg].

Pseudomonas

Imipenem (Im) [10 µg], Piperacillin (Pi) [100 µg], Ceftazidime (Cf) [30 µg], Gentamicin (G) [10 µg], Ofloxacin (Of) [5 µg] & Ciprofloxacin (Cp) [5 µg].

Anaerobic culture study

The sample was inoculated on following medias, fresh blood agar (nonselective), Neomycin blood agar (selective).[14,15] A gentamicin [10 µg] & metronidazole [5 µg] were placed on the non-selective blood culture plate for presumptive identification of anaerobes. Both the plates were incubated for 48 hours at 37^0C in a Dynox anaerobic jar. *Pseudomonas aeruginosa* (strict aerobe) was used as biological indicator for testing the method for effective anaerobiosis. All the cultures were examined after 48 hours & if no growth occurred, they were further incubated up to 96 hours before discarding them.

Colonies sensitive to metronidazole & resistant to gentamicin were presumptly identified as obligate anaerobes & their characteristics were noted. An individual colony of each type was examined for its gram character & was sub-cultured on the chocolate agar for aerotolerance and incubated in 10% CO_2 for checking aerotolerance.

Blood agar for purity testing

Colony was subcultured and incubated anaerobically for isolation of organism. Following antibiotic discs were

placed on the 1st quadrant of the purity plate, viz., Kanamycin [1000 µg], Colistin [10 µg] & Vancomycin [5 µg]. These discs helped in the preliminary identification of anaerobes & serve to verify the gram stain. Pure isolates were further processed as per conventional techniques as per (Bailey & Scotts).

Antibiotic susceptibility tests (AST) of the isolates was done by Kirby- Bauer disc diffusion method. Following antibiotic discs were used (as per the CLSI guidelines 2007) - Penicillin (P) [10 units], Piperacillin (Pi) [100 µg], Metronidazole (M) [5 µg], Ceftriaxone (Ct) [30 µg], Clindamycin (Cl) [2 µg] & Imipenem (Im) [10 µg].

RESULTS

A total of 103 patient's foot ulcer samples were analysed. Table 1 presents the demographic data of the patients and their clinical characteristics. Out of 103, 81 were males and 22 were females, ratio was 3.68:1.

Table 1: Patients' demographic data and clinical characteristics.

Patient characteristics	N (%)
Age (in years)	
31 to 40	04 (3.88)
41 to 50	18 (17.47)
51 to 60	41(39.8)
61 to 70	31 (30.9)
71 & above	09 (8.73)
Sex	
Males	81 (78.64)
Females	22 (21.36)
Duration of disease (years)	
Unknown	7 (6.79)
1 to 5	25 (24.27)
5 to10	41 (39.8)
More than 10	30 (29.12)
Predisposing factor	
Trauma	78 (75.72)
Neuropathy	63 (61.17)
Vasculopathy	13 (12.62)
Smoking	53 (51.46)
Signs & symptoms	
Fever	37 (35.92)
Foul smell	42 (40.77)
Crepitations	14 (13.59)
Wagner's grade	
II	62 (60.19)
III	32 (31.07)
IV	08 (7.77)
V	01 (0.97)

The mean age group ± SD affected was 58.31±9.74 years within a range of 34 to 76 years. A maximum of 41 patients were suffering from diabetes mellitus for more than five years, mean duration was 9.02 years ± (SD) 5.09. Among 103 patients, maximum number of patients (75.72 %) had a history of trauma followed by neuropathy in 63 patients while vasculopathy was present only in 13 patients and 53 patients had a history of smoking. Majority of the patients (42) had ulcer with foul smell as the common symptom. Majority of the patients (62) presented with ulcer of Wagner grade II, followed by grade III in 32, grade IV in 8 and grade V in 1 patient.

A total number of 253 organisms were isolated from 103 patients. The average no of isolates was 2.45 per sample.Out of these, majority were aerobes 217 (85.77%) while only 36 (14.22 %) were anaerobes. Out of the total 217 aerobes isolated, the most common organism isolated from gram positive bacteria was *Staphylococcus aureus*, 53 (24.42 %). *Pseudomonas aeruginosa*, 42 (19.35 %) was the predominant organism isolated from gram negative bacteria as shown in Table 2. Among the total 36 anaerobes, *Bacteroides fragilis* group, 17 (47.22 %) was the most common organism isolated as shown in Table 3.

Table 2: Spectrum of aerobes isolated.

Organism	Number (%)
Staphylococcus aureus	53 (24.42)
Coagulase negative staphylococcus	21 (9.67)
Streptococcus spp	09 (4.14)
Pseudomonas aeruginosa	42 (19.35)
Escherichia coli	32 (14.74)
Proteus mirabilis	24 (11.05)
Proteus vulgaris	12 (5.52)
Klebsiella pneumoniae	11 (6.06)
Klebsiella oxytoca	06 (2.76)
Citrobacter koseri	05 (2.30)
Citrobacter freundii	02 (0.92)
Total	217 (100)

Table 3: Spectrum of anaerobes isolated.

Organism	No (%)
Gram negative rods	
Bacteroides fragilis group	17 (47.22)
Gram positive cocci	
Peptococcus spp	11 (30.55)
Peptostreptococcus spp	07 (19.44)
Gram positive rods	
Clostridium perfringens	01 (2.77)
Total	36 (100)

In the present study, all the gram positive organisms, *Staphylococcus aureus, Coagulase negative Staphylococci , Streptococci spp* were highly sensitive to vancomycin (92.45%, 95.23%, 100%) respectively.

Among the gram negative organisms, *E. coli, Proteus mirabilis, Proteus vulgaris*, showed more sensitivity to

cefotaxime (90.62%, 91.66%, 75%) respectively. *Klebsiella pneumoniae* showed sensitivity to ofloxacin (81.81%), *Klebsiella oxytoca* showed sensitivity of 83.33% to both ofloxacin & amikacin. *Citrobacter koseri* was only 60% sensitive to both ofloxacin & amikacin, *Pseudomonas aeruginosa* showed a high degree of resistance to most of the antibiotics but was sensitive to imipenem (90.47 %) as given in Table 5. Table 6 presents the antibiogram of anaerobes. It was observed that metronidazole was the drug of choice in case of anaerobes followed by imipenem.

Table 4: Antibiogram of gram positive aerobic organisms.

Organism	Va	P	Ox	Co	E	Of	Cp
Staphylococcus aureus	49 [92.45]	03 [5.66]	18 [33.96]	11 [20.75]	21 [39.62]	24 [45.28]	13 [24.52]
Coagulase negative staphylococcus	20 [95.23]	04 [19.04]	05 [23.81]	04 [19.04]	12 [57.14]	13 [61.90]	09 [42.85]
Streptococcus spp	09 [100]	04 [44.44]	-	04 [44.44]	03 [33.33]	02 [22.22]	02 [22.22]

Table 5: Antibiogram of gram negative aerobic organisms.

Organism	A	Ak	G	Ce	Ct	Pi	Of	Im	Cf	Cp
Pseudomonas aeruginosa	----	----	11 [26.19]	----	----	32 [76.19]	06 [14.28]	38 [90.47]	27 [64.28]	04 [9.52]
Escherichia coli	11 [34.37]	28 [87.50]	27 [84.37]	29 [90.62]	27 [84.37]	----	21 [65.62]	----	----	17 [53.12]
Proteus mirabilis	08 [33.33]	19 [79.16]	16 [66.66]	22 [91.66]	20 [83.33]	----	17 [70.83]	----	----	10 [41.66]
Proteus vulgaris	04 [33.33]	07 [58.33]	05 [41.66]	09 [75]	08 [66.66]	----	07 [58.33]	----	----	04 [33.33]
Klebsiella pneumoniae	03 [27.27]	08 [72.72]	07 [63.63]	04 [36.36]	03 [27.27]	----	09 [81.81]	----	----	05 [45.45]
Klebsiella oxytoca	01 [16.66]	05 [83.33]	04 [66.66]	02 [33.33]	01 [16.66]	----	05 [83.33]	----	----	02 [33.33]
Citrobacter koseri	01 [20]	03 [60]	02 [40]	02 [40]	01 [20]	----	03 [60]	----	----	02 [40]
Citrobacter freundii	00 [00]	02 [100]	00 [00]	01 [50]	00 [00]	----	01 [50]	----	----	00 [00]

(No= no of isolates & [No] = % of isolates, sensitive to respective antibiotics).

Table 6: Antibiogram of anaerobic organisms.

Organism	P	Pi	M	Ct	Cl	Im
Bacteroides fragilis	02 [11.76]	14 [82.35]	17 [100]	10 [58.82]	11 [64.70]	15 [88.23]
Peptococcus	05 [45.45]	10 [90.90]	11 [100]	08 [72.72]	06 [54.54]	10 [90.90]
Peptostreptococcus	03 [42.85]	07 [100]	07 [100]	04 [57.14]	04 [57.14]	06 [85.71]
Clostridium perfringens	00 [00]	01 [100]	01 [100]	00 [00]	00 [00]	01 [100]

(No= no of isolates & [No] = % of isolates, sensitive to respective antibiotics).

DISCUSSION

Diabetic foot ulcer is the most common complication of diabetes mellitus. It may develop as a result of neuropathy, ischemia or both and when infection complicates a foot ulcer, the combination can become limb and life threatening.[16]

In the present study we enrolled 103 patients having diabetic foot ulcers. The mean age of the patients

participated in the study was 58.31±9.74 years and majority of the patients (39.8%) were between 51 to 60 years. These findings are similar to the studies conducted by Ramani et al on 75 diabetic foot ulcers and found the mean age group affected was 58 years.[17] The prevalence of foot ulcers in the late 50's might be due to the occurrence of neuropathy, vasculopathy and altered immune responses in diabetic individuals and they are more evident in the later age groups as the disease progress.[18]

The present study showed male preponderance with 81 males & 22 females. Male preponderance in the present study could be explained on the basis that the males spend more time working outdoors, exposing their foot to more traumas.[19] This observation was comparable with the studies of Viswanathan et al.[20]

The present study showed that the mean duration of diabetes was 9.02±5.09 years that was comparable with the results of Viswanathan et al in which the mean duration of diabetes in patients was found to be 9.1±6.7 years.[20] Increased incidence of diabetic foot lesions with increasing duration of diabetes was also noted by previous studies.[21,22] The present study found predisposing factors like history of trauma in 78 patients, neuropathy in 63 and vasculopathy in 13 and smoking was concomitantly present in 53 patients. This was comparable to Reiber et al series in which 77% of patients had a history of trauma.[23] The high percentage of trauma seen in this study is due to lack of proper hygiene, barefoot walking, low socioeconomic status and lack of access to proper health care system.

The previous studies done by Ramani et al and Viswanathan et al found that neuropathy was much more common than vasculopathy in patients with diabetic foot lesions which was also confirmed by the present study.[24,25] Kundaje et al also found that increased incidence of foot ulceration was observed in smokers as compared to nonsmokers. This finding was also consolidated by the present study.[24] In present study, 37 patients were presented with fever, 42 had ulcer with foul smell and subcutaneous gas was evident in 14 cases, which correlates with the above studies.[24,25] Sapico et al in 1980 observed that out of 20 patients studied, 4 patients (20%) had ulcer with foul smell and 3 (15%) had history of fever on presentation.[26]

In the present study, it was seen that majority of the foot ulcers in the patients were of Wagner grade II, followed by grade III, IV & V respectively which was similar to the observations of Sharma et al and Anandi et al.[27,28] The present study isolated a total 253 organisms out of which aerobes were 217 (85.77%) & anaerobes 36 (14.22%) which goes in consonance with the studies of Ramani et al, Citron Ellie et al, Pathare et al.[17,29,30]

In this study, *Staphylococcus aureus*, was the most common isolate observed in diabetic foot ulcers, that was in accordance with the findings of previous studies of Ramani et al, Vijay et al, Abdulrazaka et al and Sharma et al.[31-33] *Coagulase negative Staphylococci* are also being increasingly recognized as pathogens in case of diabetic foot infections as reported by Diane et al.[29] *Pseudomonas* (17.5%) was reported as commonest isolate followed by proteus (14%) in a study conducted by Sharma et al which is similar to the present study.[33] The present study also showed a prevalence of *Citrobacter spp* in only 3.22% which was similar to Alavi et al.[34]

The present study isolated 36 anaerobes, the most predominant was *Bacteroides fragilis* group in 17, followed by *Peptococcus spp* in 11, *Peptostreptococcus spp* in 7 & *Clostridium spp* in 1. Other studies also showed similar results.[31,32,35,36]

Staphylococcus aureus (92.45%) and *coagulase negative Staphylococci* (95.23%) were most sensitive to vancomycin followed by ofloxacin. This result was similar to that obtained by Raja et al.[37] *Staphylococcus spp* were resistant to penicillin, which was also demonstrated by Rama Ramani et al.[17]

Streptococci spp were 100% sensitive to vancomycin followed by 44.44% sensitive to penicillin & cotrimoxazole both. These findings coincided with that of Raja who found 100% sensitivity to vancomycin & penicillin whereas 91% to cotrimoxazole.[37]

In the present study, *Proteus spp* were most sensitive to cefotaxime followed by ceftriaxone. Sharma et al also found that *Proteus spp* were most sensitive to cefotaxime whereas Raja et al showed 98% sensitivity to ceftriaxone.[33,37]

E. coli showed 90.62% sensitivity to cefotaxime & 87.5% to amikacin whereas Anandi et al, found *E.coli* to be 100% sensitive to both these antibiotics.[28] In present study, *Pseudomonas aeruginosa* showed a high degree of resistance to most of the antibiotics but was sensitive to imipenem (90.47%). Similar results were also shown by Sharma et al.[33] In our study it was observed that metronidazole was the drug of choice in case of anaerobes followed by imipenem which is in concordance with the results of Ramani et al.[17]

CONCLUSION

There is a high occurrence of foot ulcers within the population of people with diabetes. Foot ulcerations may lead to infections, lower extremity amputations and are major causes of disability to patients, often resulting in significant morbidity, extensive periods of hospitalization, and mortality. In order to diminish the detrimental consequences associated with diabetic foot ulcers, a high standard of care must be provided and appreciation of the causative organisms in diabetic foot and their antibiotic sensitivity is essential for institution of appropriate antibiotic therapy.

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