A study of aerobic bacterial culture and antibiotic susceptibility pattern of diabetic foot ulcer

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ABSTRACT

Background: Diabetes mellitus is a chronic disorder that affects large segments of the population in the whole world.

Aim: The aim of the study was to isolate and identify aerobic bacteria from diabetic foot ulcers (DFU) and to determine their antibiotic susceptibility pattern. The reason for the increased incidence of DFU involves the interaction of several pathogenic factors such as neuropathy, abnormal foot ulcer, and peripheral arterial diseases.

Materials and Methods: A prospective study was conducted for a period of 6 months in Sir Sayajirao General Hospital and Medical College, Vadodara, Gujarat. 175 DFU patients attending the surgery ward were included in the study. Two swabs were taken from each patient and processed by standard techniques to isolate and identify various bacterial species. Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method for all the aerobic bacterial isolates.

Results: It was observed from the result that common pathogens isolated from the DFU were *Klebsiella* species and acinetobacter followed by *Pseudomonas* and *Proteus* species, as well as *Escherichia coli*. *Pseudomonas* species, isolates were highly resistant to most tested antibiotics except piperacillin-tazobactam, levofloxacin, and meropenem. Cefoperazone-sulbactam and levofloxacin are more active against *E. coli* followed by gentamycin. Ofloxacin is more active against *Staphylococcus aureus* followed by gentamycin and cefoxitin. Cefoperazone-sulbactam is active against *Proteus mirabilis* followed by piperacillin-tazobactam.

Conclusion: This study concludes that DFU infection is mostly polymicrobial in nature and appropriate antibiotic therapy is required for treatment of DFU.

Key words: Aerobic bacteria, antibiotic, diabetes, diabetic foot ulcer, monomicrobial, polymicrobial

INTRODUCTION

Diabetes mellitus (DM) affects large segments of the population all over the world.[1] The overall prevalence of diabetes has crossed over 200 million population, which is predicted to cross 300 million in the next 15–20 years. More than 1 million amputations for diabetes-related complications occur in a single year.[2] The reason for the increased incidence of diabetic foot ulcer (DFU) was due to several pathogenic symptoms such as neuropathy and peripheral arterial diseases.[3] In DFU patients, aerobic bacteria such as *Staphylococcus* species (spp.), *Streptococcus* spp., *Enterococcus* spp., and *Pseudomonas* spp. were predominant pathogens after isolation.[4]

MATERIALS AND METHODS

Study Design

It is hospital-based prospective study.

Ethics Approval

The study was carried out after getting approval from Institutional Ethics Committee for Human Research of Sir Sayajirao General (S.S.G.) Hospital and Medical College, Vadodara.

Sample Size and Duration

A total of 175 patient's samples with DFU were taken for a period of 6 months, from September 2014 to February 2015.

Study Area

The study was conducted in Bacteriology section, Department of Microbiology, S.S.G Hospital and Medical College, Vadodara.

Inclusion criteria

- Patients with DFU of Wegner's Grade 1 and above were included after taking patient's consent.

Exclusion criteria

- Ulcer without diagnosis of diabetes was excluded.
- Patient who is not willing to participate was also excluded.

Study Method
Collection of sample

Before collection of patient's sample, the foot ulcer was rinsed with normal saline. Then, the superficial exudate was collected using sterile cotton swabs. From each patient, 2 swabs were

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collected, one was used for isolation of bacteria whereas another swab was used to prepare Gram-stain slide.

For culture

The first swab collected was then inoculated on different media such as Blood agar, MacConkey agar, and Nutrient agar. All plates were incubated at 37°C (aerobically) and evaluated after 24 h. The organism isolated was identified using standard techniques, based on colony morphology, Gram staining of smear from colony and biochemical properties.

For antimicrobial susceptibility testing

Antimicrobial susceptibility test of the bacterial isolates was done using different antibiotics based on Kirby-Bauer disc diffusion method [Table 1].[5,6]

Data obtained were read after 24 h which compared by zone size interpretative chart supplied with antibiotic discs.

Collection of data

The data were compiled in Microsoft Excel sheet which was analyzed using Statistical Package for the Social Sciences (SPSS) computer software version 17.0 for Windows to determine any significant corelationship exist between infection rate, age, and gender.

RESULTS

A total of 175 patients with DFU were admitted to the surgical wards at S.S.G Hospital and Medical College, Vadodara and were considered after taking consent form.

From Table 2, we found that among 175 cases, 51 (29.1%) cases were of age group 41–50 years, out of 51, 40 (29.4%) were male,

and 11 (28.2%) were females. Which is highest in age group, and 38 (22.1%) were of age group 51–60 years, out of 38, 28 (20.6%) were male, and 10 (25.6%) were females.

From Table 3, 145 (82.9%) were positive and remaining 30 (17.5%) were negative for bacterial growth. Out of 145 samples, 122 (86.9%) were monomicrobial, and 23 (13.1%) were polymicrobial. A total of 160 aerobic bacterial species isolated, from which 22 were Gram-positive bacteria and 138 were Gram-negative bacteria.

DISCUSSION

Diabetic foot disability in diabetes can produce not only physical disability but also socioeconomic problems.[7] DFU is a multifaceted problem, primarily due to underlying neuropathy, ischemia, and infection. Infection usually follows ulceration or injury to the neuropathic or ischemic foot. Infection superimposed constitutes medical emergency threatening both limbs and life. A superficial infection is usually caused by aerobic bacteria, and deep infection is caused by anaerobic bacteria.[8] In the present study, an attempt is made to know the microbial flora of DFU. The results obtained are compared with other studies and discussed as follows.

Age-wise Distribution

From Table 2, DFU was more common in age group of 41–50 years (19.1%), followed by 51–60 (22.1%) years, which was not similar to study of Ramani et al.,[9] Chincholikar and Pal,[8] as they reported 51–60 years (40%) and 61–70 years (21.9%). However, the age group has changed in our study from 51–60 to 41–50, which is correlated with the study of Prabhu and Prabhu.[10]

Sex-wise Distribution

From Table 4, it was observed that DFU was more common in males (77.7%) than female (22.3%) which were similar to other scientist's studies. The male predominance is due to peripheral neuropathy, peripheral vascular diseases, and outdoor occupation.[11,12]

Table 1: Antibiotics used for testing susceptibility of bacterial isolates

For Gram-negative organisms	For Gram-positive organisms
Cefoperazone-sulbactam	Penicillin
Piperacillin-tazobactam	Oxacillin
Gentamycin	Cefoxitin
Levofloxacin	Vancomycin
Cefepime	Linezolid
Cefotaxime	Gentamycin
Amikacin	Azithromycin
Meropenem	Clindamycin

Table 2: Age group of patients of DFU

Age group	n (%)		
	Male	Female	Total
21–30	21 (15.4)	5 (12.8)	26 (14.6)
31–40	17 (12.5)	6 (15.4)	23 (13.1)
41–50	40 (29.4)	11 (28.2)	51 (29.1)
51–60	28 (20.6)	10 (25.6)	38 (22.1)
61 and above	30 (22.1)	7 (18.0)	37 (21.1)
Total	136 (100)	39 (100)	175 (100)

DFU: Diabetic foot ulcer

Table 3: Total number of obtained bacterial isolates from DFU in percentage %

Name of the microorganism	Total number of isolates (%)
S. aureus	9 (5.6)
MRSA	6 (3.8)
CONS	3 (1.9)
MRCONS	3 (1.9)
Enterococcus species	1 (0.6)
Pseudomonas species	24 (15.0)
Proteus species	20 (12.5)
E. coli	22 (13.8)
Klebsiella species	30 (18.8)
Citrobacter species	3 (1.9)
Morganella species	2 (1.3)
Providencia species	7 (4.4)
Acinetobacter species	27 (16.9)
Enterobacter species	3 (1.9)
Total	160 (100)

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, MRSA: Methicillin-resistant Staphylococcus aureus, CONS: Coagulase-negative Staphylococci, MRCONS: Methicillin-resistant coagulase-negative Staphylococci, DFU: Diabetic foot ulcer

Comparison of Monomicrobial and Polymicrobial Flora with Different Scientist's Work

It is reported that 39–90% of all DFU are polymicrobial in nature.[15,16] In the present study, monomicrobial etiology was 72%, and polymicrobial etiology from Table 5, it was observed

Table 4: Sex-wise distribution of patients with DFU

Study series	n (%)	
	Total numbers of patients	
	Male	Female
Anandi et al.[1]	70 (65.4)	37 (34.9)
Chincholikar and Pal[8]	71 (67.6)	34 (32.4)
Present study	136 (77.7)	39 (22.3)

DFU: Diabetic foot ulcer

Table 5: Comparison of monomicrobial and polymicrobial flora obtained from patients with DFU

Study series	Monomicrobial (%)	Polymicrobial (%)
Pathare et al.[13]	0	100
Raymando and Mendoza[14]	45	54
Chincholikar and Pal[8]	30.5	69.5
Anandi et al.[1]	19.6	64.4
Present study	72	10.9

DFU: Diabetic foot ulcer

Table 6: Average number of species isolated from patients with DFU

Study series	Average number of species
Ramani et al.[9]	3.0
Raymando and Mendoza[14]	2.4
Louie et al.[15]	5.8
Pathare et al.[13]	3.1
Sharp et al.[16]	5.8
Sapico et al.[17]	4.7
Present study	0.83

DFU: Diabetic foot ulcer

that was 10.9%. Several factors such as vascular insufficiency, neuropathy, poor control of diabetes, and poor defense lead to rapid increase in the number of microbes.[13]

Number of Species Isolated by Various Workers

As shown in Table 6, we found that average number of organisms isolated per specimen was 0.83, which are partially correlates with other scientist's work.

Comparison of Aerobic Organism Isolated by Various Scientists

From Table 7 mentioned below, out of 175 patient's sample, 160 aerobic organisms were isolated, and compared with the above scientist's work, and we found that our study partially correlates with other scientist's work.

Antibiotic Sensitivity Pattern

From Table 8, mentioned below, It was observed that *Staphylococcus aureus* showed 44.4% sensitivity to clindamycin, 77.7% sensitive to azithromycin, and 88.8% sensitive to oxacillin. They are 100% sensitive to cefoxitin, vancomycin, linezolid, and gentamycin.

From Table 9, it was found that in Gram-negative bacteria, *Pseudomonas* were sensitive to levofloxacin in 62.5% while 100% sensitive to meropenem, whereas less sensitive to gentamycin 33.3% and amikacin 25%.

CONCLUSION

Nowadays in diabetic patients, DFU infection is considered one of the most threatening and disabling complications all over the world. DFU was polymicrobial in nature. It is essential to assess the magnitude of DFU to avoid further complications such as limb amputation, sepsis, or maybe mortality. Culture and sensitivity should be performed to identify the pathogen and for appropriate choice of antibiotic therapy.

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Table 7: Comparison of aerobic organism isolated with %

Study versus microorganism	Chincholikar and Pal[8]	Pathare et al.[13]	Ramani et al.[9]	Present study
Staphylococcus aureus (n=9)	31.3	19.2	60	5.6
Enterococcus spp. (n=1)	4.4	4.4	6.7	0.6
Pseudomonas spp. (n=24)	19.4	5.4	11.7	15.0
E. coli (n=22)	15.6	8.9	2.4	13.8
Klebsiella spp. (n=30)	8.13	13.9	12.3	18.8
Proteus spp. (n=20)	6.3	16.7	1.6	12.5
Citrobacter spp. (n=3)	2.6	1.5	9.2	1.9
Morganella spp. (n=2)	1.3	-	-	1.3
Providencia spp. (n=7)	1.9	0.1	1.2	4.4
Acinetobacter spp. (n=27)	1.3	-	0.6	16.9
Enterobacter spp. (n=3)	1.9	10.2	1.8	1.9

S. aureus: Staphylococcus aureus, E. coli: Escherichia coli

Table 8: Antibiotic susceptibility pattern obtained from Gram-positive bacterial isolates from DFU

Gram-positive bacteria and their total no.	Antibiotic sensitivity (%)							
	Penicillin	Oxacillin	Cefoxitin	Vancomycin	Linezolid	Gentamycin	Azithromycin	Clindamycin
S. aureus (*n*=9)	3 (33.3)	8 (88.8)	9 (100)	9 (100)	9 (100)	9 (100)	7 (77.7)	4 (44.4)
CONS (*n*=3)	0 (0)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.3)	0 (0)
MRSA (*n*=6)	0 (0)	0 (0)	0 (0)	6 (100)	6 (100)	0 (0)	0 (0)	0 (0)
MRCONS (*n*=3)	0 (0)	0 (0)	0 (0)	3 (100)	3 (100)	2 (66.7)	1 (33.3)	1 (33.3)
Enterococcus spp. (*n*=1)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)

S. aureus: Staphylococcus aureus, MRSA: Methicillin-resistant *Staphylococcus aureus*, CONS: Coagulase-negative *Staphylococci*, MRCONS: Methicillin-resistant coagulase-negative *Staphylococci*, DFU: Diabetic foot ulcer

Table 9: Antibiotic susceptibility pattern obtained from Gram-negative bacterial isolates from DFU

Gram-negative bacteria and their total no.	Antibiotic sensitivity (%)							
	Cefoperazone-sulbactam	Piperacillin-tazobactam	Gentamycin	Levofloxacin	Cefepime	Cefotaxime	Amikacin	Meropenem
Pseudomonas spp. (*n*=24)	12 (50)	24 (100)	8 (33.3)	15 (62.5)	11 (45.8)	11 (45.8)	6 (25)	24 (100)
Proteus spp. (*n*=20)	19 (95)	18 (90)	5 (25)	9 (45)	11 (55)	10 (50)	3 (15)	20 (100)
E. coli (*n*=22)	19 (86.4)	21 (95.5)	13 (59.1)	14 (63.6)	8 (36.4)	1 (4.5)	3 (13.6)	22 (100)
Klebsiella spp. (*n*=30)	28 (93.3)	29 (96.7)	8 (26.7)	23 (76.7)	20 (66.7)	9 (30)	3 (10)	29 (100)
Citrobacter spp. (*n*=3)	2 (66.7)	3 (100)	0 (0)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	3 (100)
Morganella spp. (*n*=2)	2 (100)	2 (100)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	2 (100)
Providencia spp. (*n*=7)	7 (100)	7 (100)	1 (14.3)	4 (57.1)	3 (42.9)	3 (42.9)	0 (0)	7 (100)
Acinetobacter spp. (*n*=27)	25 (92.6)	23 (85.2)	7 (25.9)	15 (55.6)	7 (25.9)	3 (11.1)	1 (3.7)	27 (100)
Enterobacter spp. (*n*=3)	3 (100)	3 (100)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)	3 (100)

E. coli: Escherichia coli, DFU: Diabetic foot ulcer

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