

Original Research Article

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Study on Bacterial Spectrum of Organism Causing Diabetic Foot Ulcers with its Antibiogram in Dr. B.R. Ambedkar Medical College, Bangalore, India

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ABSTRACT

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Diabetic foot is one of the serious complications associated with diabetes and affects quality of life in respective patients in all ages and races. Diabetes mellitus is a disorder that shares the phenotype of hyperglycaemia. The prevalence of diabetes depends on many etiological factors such as age, sex, heredity, diet, socio-economic conditions and physical activity, environmental factors, life style choices etc. Diabetes is multi factorial disease in which various factors act in complex manner. A prospective study was carried out in department of microbiology at DR.B R Ambedkar Medical College & Hospital, Bangalore. Total of 120 samples with the clinical diagnosis of Diabetic Foot ulcer were collected and processed. Of the total 120 diabetic foot patients samples studied 80 were males and 40 were females, the male: female ratio being 2:1. The maximum number of patients having diabetic foot infections belonged to the age group of 56-65 years. Among the total pathogens isolated, 52.38% gram negative isolates and gram positive isolates i.e *Staphylococcus aureus* accounted for 47.62% and was the most common organism isolated, followed by *Pseudomonas aeruginosa* & *Klebsiella spp*, *Escherichia coli*.

Introduction

Diabetic foot is one of the serious complications associated with diabetes and affects quality of life in respective patients in all ages and races. Diabetes mellitus is a disorder that shares the phenotype of hyperglycaemia. The prevalence of diabetes depends on many aetiological factors such as age, sex, heredity, diet, socio-economic conditions and physical activity, environmental factors, life style choices etc. Diabetes is multi factorial disease in which various factors act in complex manner (Kasper Dennis *et al.*, 2005).

Neuropathy, peripheral arterial disease, and pressure overload make the sufferers prone to

ulcer. Among persons with diabetes mellitus, the risk of developing a foot ulcer is estimated to be 15%. Based on recent studies, the annual population based incidence ranges from 1.0% to 4.1% and the prevalence range from 4% to 10%, suggesting the life time incidence as high as 25% (Singh *et al.*, 2005).

Patients with diabetes lose the protective sensations for temperature and pain, impairing awareness of trauma such as abrasions, blistering, or penetrating foreign body (Lipsky *et al.*, 2004). Motor neuropathy can result in foot deformities that contribute to local pressure from footwear, making skin ulceration even more likely. Once the skin is

broken (typically on the plantar surface), the underlying tissues are exposed to colonization by pathogenic organisms. The resulting wound infection may begin superficially, but with delay in treatment and impaired body defense mechanisms caused by neutrophil dysfunction and vascular insufficiency, it can spread to the contiguous subcutaneous tissues and to even deeper structures (Abdulrazak *et al.*, 2005). So the present study is designed to isolate and identify bacterial causes in diabetic patients and assess their susceptibility to antibiotics.

Materials and Methods

A prospective study was carried out in department of microbiology at DR.B R Ambedkar Medical College & Hospital, Bangalore, India.

Swabs obtained from diabetic foot ulcers cases during one year period from Dec 2015-Jan2017 were collected & processed. Patients details including age, sex, registration no, unit, occupation, history of trauma, habits of bare foot walking, alcohol, smoking, socio-economic status, past history of the disease and duration of the disease, treatment taken and any complications, existing co-morbidities.

E.g. Hypertension, Tuberculosis, Malnutrition, Anemia, Peripheral vascular disease were obtained

Collection of specimen: Discharge from margins and edges of ulcer were collected with help of two sterile swabs, one of which was used for gram stain and other for culture before antiseptic dressing was applied. Then swabs were immediately transported to the laboratory for culture.

Laboratory Procedures: All samples were subjected to conventional culture methods.

All the samples were subjected to Gram's staining for microscopic examination and culture as per standard guidelines. The exudates were cultured on Blood agar and MacConkey agar. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, culture and biochemical properties using standard laboratory criteria. Antimicrobial sensitivity of the bacterial isolates was done on Mueller Hinton agar using Kirby Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI).

Results and Discussion

Total of 120 samples with the clinical diagnosis of Diabetic Foot ulcer were collected and processed. Out of 120 specimens, 105 (87.5%) specimens showed bacterial growth, while 15 (12.5%) specimens did not show any growth.

Of the total 120 diabetic foot patients samples studied 80 were males and 40 were females, the male: female ratio being 2:1. Age group ranged from 28 years to 70 years with an average of 55 years. The maximum number of patients having diabetic foot infections belonged to the age group of 56-65 years.

Table 2 Illustrates the bacterial isolates which shows 52.38% gram negative isolates and gram positive isolates i.e *Staphylococcus aureus* accounted for 47.62% and was the most common organism isolated, followed by *Pseudomonas aeruginosa* & *Klebsiella spp*, *Escherichia coli*.

The Result of the test for susceptibility to the commonly used antibiotics are shown in Table no. 3, 4 and 5. Antibiotic sensitivity pattern of *staphylococcus aureus* showed that Cefoxitin resistance i.e., Methicillin resistant *S.aureus (MRSA)* was 54%, combination of

Amoxicillin/Clavulanic acid showed 70% sensitivity while Gentamicin and Cefuroxime showed 65% and 50% sensitivity respectively. Erythromycin and Azithromycin showed 75% resistant pattern while Cefadroxil showed 55% resistance. Almost all isolates of *Pseudomonas aeruginosa* were sensitive to Piperacillin + Tazobactam, Cefipime and Imepenem, 80%, 76%, 100% respectively.

Foot ulceration is the most severe complication affecting diabetic patients which is not confined to certain superficial underlying subcutaneous tissue. Diabetic foot ulceration (DFU) arises from uncontrolled diabetes and incomplete health self-care (Mendes *et al.*, 2012; American Diabetes Association, 2011).

This study investigates clinical and microbiological findings of DFU in patients. The majority of the patients with DFU were male and older than 50 years, consistent with other reported studies such as Ilanes *et al.*, (2001) which shows maximum age group above 57 years. This may be due to factors such as the differences in life styles and professional activities and jobs, causing the feet to tolerate more pressure.

In the present study, 105 organisms were isolated from 120 patients and *S.aureus* accounts for the major organism isolated. The observations are similar with Chincholikar (2002) while differ significantly from Ekta *et al.*, (2008) in which the major organism are GNB which again indicated the role of

geographical variations in microbial etiology. *Staphylococcus aureus* was predominantly isolated organism (47.62%) followed by *Pseudomonas aeruginosa* (23.81%), *Klebsiella* (14.29%) and *E coli* (4.76%).

Other organisms like *Proteus mirabilis*, *P.vulgaris*, *Citrobacter spp* and *Acinetobacter spp* were also isolated. Almost similar results were obtained by Vvimalin Hena *et al.*, (2010) & Murtada Jeber *et al.*, (2013). In our study, Methicillin resistance was seen in 54% of the *S. aureus* which is also in concordance with findings by Gadepalli *et al.*, Ekta *et al.*, (2008) and Chincholikar (2002) in which MRSA was seen in 56%, 55.50% and 55.56% respectively. *Staphylococcus spp.*, being the predominant organism isolated showed linezolid and teicoplanin to be the most effective antibiotics with 100% sensitivity and low resistance was seen to amoxy-clav (40%,n=50)& gentamicin (44%,n=50) but a high resistance was exhibited to other antibiotics. Other studies have shown different susceptibility antibiotic patterns and approximately in most vancomycin and linezolid have shown good activity against the strains. The antibiotic sensitivity pattern of *Pseudomonas* was approximately similar to study by Ekta *et al.*, (2008). Most of the isolates were sensitive to almost all drugs used with Piperacilin/Tazobactam (80%:n=25),being most sensitive (80%,n=25),followed by Ceftazidime (72%,n=25), Cefepime (76%, n=25), Amikacin (64%, n=25) & low level of resistance seen to Flouroquinolones.

Table.1 Pattern of isolation of microbial growth from Diabetic Foot Ulcer

Total Patients examined	120
Patients with microbial growth n (%)	105(87.5%)
No growth n (%)	15(12.5%)
Gram Positive Isolates	47.62%
Gram Negative Isolates	52.38%

Table.2 Bacterial Pathogens isolated from 105 Culture Positive Diabetic Foot Ulcers

Organism	Isolates	(n=105) (%)
<i>Staphylococcus aureus</i>	50	47.62%
<i>Pseudomonas aeruginosa</i>	25	23.81%
<i>Klebsiella spp</i>	15	14.29%
<i>Escherischia coli</i>	5	4.76%
<i>Proteus mirabilis</i>	3	2.86%
<i>Proteus vulgaris</i>	3	2.86%
<i>Citrobacter spp.</i>	2	1.90%
<i>Acinetobactor spp</i>	2	1.90%

Table.3 Antibiogram of Staphylococcal isolates from diabetic foot ulcers (n= 50)

Anti microbial agent	(n=50)			
	S	%	R	%
Amoxycillin/Clav.acid	30	60.00%	20	40.00%
Azithromycin	15	30.00%	35	70.00%
Erythromycin	12	24.00%	38	76.00%
Cefuroxime	25	50.00%	25	50.00%
Cefoxitin sensitive	27	54.00%	23	46.00%
Cefadroxil	28	56.00%	22	44.00%
Gentamicin	28	56.00%	22	44.00%
Linezolid	45	90.00%	5	10.00%
Teicoplanin	50	100.00%	-	-

Table.4 Antimicrobial sensitivity of isolates of *Pseudomonas aeruginosa* (n = 25)

Anti microbial agent	n=25			
	S	%	R	%
Piperacilin/Tazobactam	20	80.00%	5	20.00%
Ceftazidime	18	72.00%	7	28.00%
Cefepime	19	76.00%	6	24.00%
Amikacin	16	64.00%	9	36.00%
Gentamicin	17	68.00%	8	32.00%
Ciprofloxacin	10	40.00%	15	60.00%
Levofloxacin	12	48.00%	13	52.00%
Doripenem	24	96.00%	1	4.00%
Imepenem	25	100.00%	0	0.00%

Table.5 Antimicrobial susceptibility pattern of gram negative bacteria except *Pseudomonas* (n = 105)

n=105						
Anti microbial agent	<i>Klebsiella</i> spp	<i>Escherischia coli</i>	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>	<i>Citrobacter</i> spp.	<i>Acinetobacter</i> spp
Imipenem	86.67%	80.00%	100.00%	100.00%	100.00%	100.00%
Amikacin	66.67%	60.00%	66.67%	33.33%	50.00%	50.00%
Gentamicin	73.33%	60.00%	66.67%	33.33%	100.00%	50.00%
Ciprofloxacin	46.67%	40.00%	33.33%	66.67%	50.00%	0.00%
Levofloxacin	40.00%	40.00%	33.33%	66.67%	100.00%	0.00%
Ofloxacin	40.00%	60.00%	33.33%	66.67%	100.00%	50.00%
Cefotaxime	33.33%	40.00%	66.67%	33.33%	50.00%	50.00%
Ceftriaxone	46.67%	60.00%	66.67%	33.33%	50.00%	100.00%
Cefipime	66.67%	80.00%	66.67%	66.67%	100.00%	100.00%
Co–trimoxazole	40.00%	40.00%	100.00%	66.67%	100.00%	50.00%
Ampicillin/ Sulbactam	80.00%	100.00%	66.67%	100.00%	100.00%	100.00%

All isolates were found 100% sensitivity to Imipenem. In present study, *Klebsiella* and *E. coli* were most sensitive to combination like Ampicillin + Sulbactam, Gentamicin, Cefipime, Imepenem and these organisms were resistant to other Cephalosporins, Cotrimoxazole and Flouroquinolone. Similar results were found by Ekta *et al.*, (2008). The antibiotic resistance shown in the present study may be due to such factors including hospitalization, recent use of broad-spectrum antibiotics, history of surgery and chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means. To alleviate this situation and also reduce the rate of amputation, clinicians should prescribe antibiotics rationally, timely, and sufficiently and there should be periodic supervisions on the drug consumption by the respective organizations (Farshad *et al.*, 2011).

It can be concluded that culture specimens for correct management of the DFI and knowledge of the susceptibility of

antimicrobial drugs are essential for the choice of appropriate antibiotics with maximum efficacy. Diabetic foot infections are polymicrobial in nature

Organisms in mixed infections showed multidrug resistance as compared to single isolated strain.

These multidrug resistant organisms are frequently resistant to many classes of antibiotics so it is necessary for the clinician to be completely aware of the prevalence rate of multidrug resistant organisms and their management strategies. So this study will help the clinicians to choose appropriate antibiotic or combination of antibiotics for the treatment of Diabetic Foot Ulcer.

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