

Original Research Article

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## Bacteriological Profile of Diabetic Foot in a Tertiary Care Centre in Trivandrum, India

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### ABSTRACT

#### Keywords

*Klebsiella*,  
*Staphylococcus aureus*, Diabetic foot, Imipenem, Vancomycin.

#### Article Info

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Diabetic foot is one of the complications of diabetes leading to the prolonged hospitalization due to which complications like Neuropathy. In this study conducted from Jan 2016 to March 2016 a total Forty clinically diagnosed diabetic foot infected patients with ulcers were included. All the diabetic foot patients' ulcers were classified based on the Wagner's classification from 0 to 5 groups. All the organisms were identified according to standard methods. They were confirmed with Vitek 2. Gram negatives isolates were identified 59% of the time. *Klebsiella* spp was the most common gram negative organism while *Staphylococcus aureus* were the most common gram positive organism. Imipenem, Meropenem, Vancomycin and Linezolid were the antibiotic which was most susceptible.

### Introduction

Diabetic foot is one of the complications of diabetes leading to the prolonged hospitalization due to which complications like Neuropathy, peripheral vascular diseases, foot ulceration and infection with bacteria leading to sepsis further to the development of gangrene, which sometimes requires amputation of the gangrene foot. (Stephanie Wu, 2007). Due to development of foot ulceration the life span risk is high i.e. 25% in diabetic foot infected patients (Prompers, (2008).

Diabetes is a metabolic disorder of endocrine system. In future it is predicted that 240 million people were going to be suffered worldwide which may lead to more diabetic foot infections with complications (Girish M Bengalorkar, 2011). Fifteen (15%) percent of people with diabetes will develop a foot ulcer at some time during their life and for most of them foot amputations were necessary (Leila Yazdanpanah, 2015). The organisms that occur on diabetic foot infections will develop resistance to commonly used

antibiotics largely due to their indiscriminate usage (Vimalin Hena, 2010).

So in this background this present scientific study was taken up among the diabetic foot ulcer patients from in and around Venjaramoodu. To know the common aerobic bacteria causing infections in diabetic foot patients and their antibiotic sensitivity pattern to overcome their drug resistance for the control of this infections. So as to reduce morbidity & mortality rate among the diabetic foot infected patients

### **Materials and Methods**

So this study was conducted for knowing the common aerobic bacteria which caused infections among the diabetic foot patients in Sree Gokulam Medical College and Research Foundation, Trivandrum, India

In this study conducted from Jan 2016 to March 2016 a total Forty (40) clinically diagnosed diabetic foot infected patients with ulcers were included as if they received no antibiotics. All the diabetic foot patients' ulcers were classified based on the Wagner's classification from 0 to 5 groups. From all the diabetics' ulcers on the lower extremities, pus & exudates specimens were collected with two sterile swabs from each patient in a sterile container under aseptic conditions by following standard protocols and procedures. Then all the above collected samples were transported immediately to the Department of Microbiology, SGMC & RF for their processing for the isolation and identification of aerobic bacterial organisms. From the first smear a direct gram stain was done to observe for the presence of pus cells and organism and the second swab was cultured on Blood agar, Chocolate agar, MaConkey agar and Mannitol salt agar and incubated at 37 C for 48 hours and observed for growth. The isolates were further

identified by standard protocol. From the growth we do a gram stain and process based on whether the organism was gram positive or gram negative. Gram positive organism we do catalase test, coagulase test, bile esculin test and susceptibility to optochin and bacitracin. Gram negative organism hanging drop for motility, oxidase test, catalase test, indole, methyl red, Voges-Proskauer, citrate utilization, urease production oxidative fermentative test (Hugh-Leifson medium) for glucose, utilization of 10% lactose, gelatin liquefaction, lysine and ornithine decarboxylation, arginine dihydrolase test, growth at 42°C and 44°C, esculin hydrolysis and ONPG test (Washington W, 2006).

All the isolates identified were confirmed by Vitek 2 automated system.

The sensitivity test was performed by Kirby-bauer disc diffusion method using commercially available discs (Himedia). The results were interpreted as per the CLSI guideline (Performance Standards for Antimicrobial Disk Susceptibility Tests., Jan. 2016)

### **Results and Discussion**

From the table-I among the total 40 patients Wagner Grade – 2, 16 (40%) and Grade – 3, 11 (27.5%) ulcers were predominant. Followed by Grade – 1, 6 (15%), Grade – 4, 4 (10%), Grade – 5, 3 (7.5%).Grade -0 ulcers were nil.

Sample received from diabetic foot were two different types i.e. there were 22 pus and 18 exudates samples.

Maximum cases were seen in the age group was 41- 60 years(27) 67.5%.Least age group was 21 – 40 years(4) 10%.Males (29) 72.5% were maximum when compare with females

(11)27.5%. Among the total (40) specimens processed for culture thirty nine (39) 97.5% were culture positives & one (01) 2.5% were culture negative.

It was observed that among all the gram positive organisms the predominant one is *Staphylococcus aureus* (9) 56.25% followed by coagulase negative *Staphylococcus* (5) 31.25% and low incidence was *Enterococcus* spp (2) 12.5% were observed.

Gram-negative organisms were isolated among which the predominant organism is *Klebsiella pneumoniae* (10) 27.77% followed by least *Proteus vulgaris* (1) 2.77% and remaining gram negative isolates were *Pseudomonas aeruginosa* (8) 22.22%, *Escherichia coli* (6) 16.66%, *Pseudomonas* spp (5) 13.88%, *Proteus mirabilis* (4)11.11%, *Citrobacter* spp (2) 5.55 %.

Monomicrobial growth was seen in 27 (69.2%) of cases while polymicrobial growth was seen in 12 (30.7%) of cases. Among the polymicrobial growth the most common combination was *Klebsiella* + *Staphylococcus aureus* (4)

From the above table it is shown for *Staphylococcus aureus* 100% isolates were resistant to Penicillin. There were 5 cases of MRSA which showed 100% sensitivity to Vancomycin and Linezolid. Erythromycin, Gentamicin and Clindamycin were sensitive in 44% of the cases.

*Streptococcus* spp were sensitive strains and it was 100% sensitive to Penicillin and Erythromycin.

*Enterococcus* spp were 100% sensitive to Vancomycin and Linezolid. While 50% were sensitive to Amoxicillin, clavulinate and High level gentamicin. The antibiotic sensitivity pattern of isolated gram negative

bacteria showed that 90% of *Klebsiella* were sensitive to Imipenem and Meropenem, 85% were sensitive to Piperacillin tazobactam, 85% were sensitive to Cefoperazone sulbactam, 65% were sensitive to Ceftazidime and Cefipime, 55% were sensitive to Amikacin, 45% were sensitive to Ciprofloxacin, 20% were sensitive to Cotrimoxazole and 10% sensitive to Piperacillin. The second most common isolate *Pseudomonas aeruginosa* showed highest sensitivity to Imipenem (87.5%) and Meropenem (87.5%). Similarly *E. coli* and *Pseudomonas* spp showed highest sensitivity to Meropenem and lowest sensitivity to Piperacillin. Resistance pattern of all the isolates when analysed as a group against various classes of antibiotics showed that the isolates exhibited high resistance to Piperacillin and Cotrimoxazole while, the resistance against ceftazidime and cefipime was >40%. On the other hand, isolates showed a low level of resistance against piperacillin tazobactam, cefoperazone-sulbactam and ceftazidime. Extremely low level of resistance was observed against imipenem and meropenem.

Diabetic foot ulcer infections in the diabetic patients were one of the emerging infections who attended to the General surgery outpatient and inpatient department of SGMC, Venjaramoodu. This was a serious problem in them, which lead to high morbidity and may lead to amputation of foot. A wide range of aerobic bacteria complicated the ulcers by their infections among the diabetic foot ulcer patients, and the infected diabetic foot ulcers were difficult to control due to the drug resistant of the aerobic bacterial pathogens.

In our study the ulcers were mostly on the distal phalanges. Infections of the lower extremities in diabetic patients commonly occur on the plantar surface of the forefoot, in particular the toes and metatarsal heads.

All the study group diabetic foot ulcer patients have been classified according to Wagner's classification, (Table-II) into 0 – 5 Grades, and it was observed in this study majority of the cases were belongs grade – 2(40%) and less cases belongs to Grade – 5, 3(7.5%). Followed by moderate cases belongs to Grade – 3, 11(27.5%), Grade –1, 6(15%), Grade – 4, 4(10%) and cases belongs to Grade – 0 were nil and the mean value & “p” value proved statistically significant.

In a study of Ozer *et al* they observed that maximum cases belongs to Grade – 4 and least cases belongs to Grade – 1 followed by moderate cases belongs to Grade 3,2,5. (Ozer)

In a study by VK Sharma *et al* which was observed by them that majority of the cases were belongs to grade – 2. (Sharma, 2006)

So the observations observed in this study were correlated with the study of VK. Sharma *et al*. that majority of cases belongs to Grade-2 (Sharma, 2006).

The total 40 diabetic foot ulcer infected cases age and sex wise distribution in this study was observed that patients mean age in years is  $52.75 \pm 9.87$  and among the total cases males were predominant than females.

In a study of Ozer *et al* mean age in years is  $59.72 \pm 10.17$  and males were predominant.

In a study of Ravishenkar Gadepalli *et al* mean age in years is  $53.9 \pm 12.1$  and males were predominant. (Ravisekhar Gadepalli, 2006)

So this study was correlated with the study of Ravishenkar Gadepalli *et al* and Ozer *et al* reason might be the old age groups were more prone for diabetes (Ravisekhar

Gadepalli, 2006). It was observed that among the culture positive cases monomicrobial infections (with one organism) 27 (69.2%) were maximum than polymicrobial infections (more than one organism) 12 (30.7%).

In the study of Anandi *et al* monomicrobial infections were lower than polymicrobial infections. (Anandi C, 2004) In the study of Dhansekar *et al* monomicrobial infections were more than polymicrobial infections.

The observations observed in this study were correlated with the study of Dhansekar *et al*.

Among total isolates Gram negative organisms were more predominant than Gram positive organisms. These observations observed in this study were correlated to the studies of Sivaramanumadevi *et al* and Shanker *et al*. (Sivaraman, 2011; Shanker, 2005)

Though previous studies (Frykberg, 2003; (RG, 2003 Nov 28; Ge *et al.*, 2002) (Ge Y, 2002 Dec;19(12):) showed Gram-positive aerobes as predominant agents in diabetic foot infections, we frequently isolated

Gram-negative bacteria (59.7%) compared to Grampositive bacteria (41.3%). Similar to our findings, Shankar *et al.* (2005) (Shanker EM, 2005;) and Gadepalli *et al.* (2006) (Ravisekhar Gadepalli, 2006) showed predominant involvement of Gram-negative isolates

It was observed in this study that the predominant gram positive isolate was staphylococcus aureus among gram positive organisms followed by Streptococcus spp and the low incidence gram positive organism is *Enterococcus* spp. It was correlated to the study of Sivaramanumadevi

*et al.* (Sivaraman, 2011) Whereas study done by Ozer *et al* (Ozer B) the predominant gram positive isolate was *Enterococcus*.

Among the total isolated gram negative organisms observed in this study *Klebsiella pneumoniae* was predominant followed by least incidences *Proteus vulgaris*.

Diabetic patients with foot ulcers have several factors that may be associated with a high risk of multidrug resistant microorganism's carriage, such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission.

**Table.1** Grading of diabetic foot ulcers according to Wagner's classification.

Wagner's grade	No. Of cases	Percentage (%)
0	0	0
1	6	15%
2	16	40%
3	11	27.5%
4	4	10%
5	3	7.5%
Total	40	100%

**Table.2** Showing isolated gram positive organisms in this study.

S.no	Name of the organism	No. of isolates	Percentage (%)
1	<i>Staphylococcus aureus</i>	09	56.25%
2	<i>Streptococci spp</i>	05	31.25%
3	<i>Enterococcus spp</i>	02	12.5%
	Total	16	100%

**Table.3** Showing isolated gram negative organisms in this study.

S.No	Name of the organism	No. of isolates	Percentage (%)
1	<i>Klebsiella pneumonia</i>	10	27.77%
2	<i>Pseudomonas aeruginosa</i>	08	22.22%
3	<i>Escherichia coli</i>	06	16.66%
4	<i>Pseudomonas spp</i>	05	13.88%
5	<i>Proteus mirabilis</i>	04	11.11%
6	<i>Citrobacterspp</i>	02	5.55%
7	<i>Proteus vulgaris</i>	01	2.77%
	Total	36	100%

**Table.4** Showing organisms having polymicrobial growth

Slnos	Organisms	Total nos
1	<i>Klebsiella + Staphylococcus aureus</i>	4
2	<i>E. coli + Streptococcus spp</i>	2
3	<i>Pseudomonas aeruginosa + Citrobacterspp</i>	2
4	<i>Pseudomonas spp + Proteus mirabilis</i>	2
5	<i>Klebsiella + Enterococcus</i>	1
6	<i>E.coli+ Pseudomonas spp</i>	1

**Table.5** Gram positive organism's antibiotic sensitivity and resistant pattern

Organism	Total nos	P		Ceph		AC		G		Van		E		Clind		LZ	
		S%	R %	S%	R%	S%	R%	S%	R%	S%	R %	S%	R%	S%	R %		
<i>Staphylococcus aureus</i>	9	0	100	66.6	33.3	66.6	33.3	44.4	55.5	100	0	44.4	55.5	44.4	55.5	100	0
<i>Streptococci</i>	5	100	0	100	0	100	0	60	40	100	0	60	40	60	40	100	0
<i>Enterococcus spp</i>	2	0	100	0	100	50	50	50	50	100	0	100	00	100	0	100	0

**Table.6** Antibiotic sensitivity of Gram negative organism

Antibiotics	Organisms				
	<i>Klebsiella</i> (10) Sensitivity (%)	<i>Pseudomonas aeruginosa</i> (8) Sensitivity (%)	<i>E .coli</i> (6) Sensitivity (%)	<i>Pseudomonas spp</i> (5) Sensitivity (%)	<i>Proteus mirabilis</i> (4) Sensitivity (%)
Amikacin	55	50	66.6	40	50
Ciprofloxacin	45	25	50	40	50
Cotrimoxazole	20	25	50	20	25
Piperacillin	10	37.5	50	20	25
Ceftazidime	65	75	66.6	60	75
Cefipime	65	75	83.3	60	75
Cefaperazonesulbactam	85	87.5	83.3	80	100
Piperacillin tazobactam	90	87.5	83.3	80	100
Imipenem	90	87.5	100	100	100
Meropenem	90	87.5	100	100	100

All most all Gram negative organisms in this study were found to be resistant to Gentamicin and Ciprofloxacin, Gadepalli, *et al.*, 2006 study also reported increasing resistant to these drugs by most of the Gram negative organisms while imipenem and Meropenem were found to be the most

susceptible antibiotic. There were 5 cases of resistance among all gram negative organism to Imipenem and Meropenem. One case of Multidrug resistance organism was observed. This is low compared to other studies done across the world probably because of good antibiotic stewardship

being followed in the institution.

Gram positive isolates in this study were uniformly susceptible to Vancomycin and Linezolid while Pencillin Amoxycillin lavulunate, Gentamicin, Erythromycin were shown to have higher resistance pattern. There were three cases of MRSA. None of the gram positive isolates were resistant to vancomycin (VRSA). No cases of Vancomycin resistant enterococci was also seen Vancomycin, hence was considered as important antibiotics for diabetic foot infections especially in settings with high resistance to other antibiotics. Imipenem, meropenem

Vancomycin, Linezolid were reported to be the most effective agents against to the bacteria isolated in diabetic footinfections in several studies similar with our study (Ozer *et al.*, 2010; Raja, 2007; Gadepalli *et al.*, 2006; NS, 2007; Ravisekhar Gadepalli, 2006).

So treatment of diabetic foot infections in areas with drug resistant should include a combination of these antibiotics.

In conclusion, isolation of gram positive and gram negative bacteria from cases of Diabetic foot need to be taken with all seriousness because if left untreated the chance of infection becoming severe leading to complications like amputation and septicemia is high.

In Gram-negative bacteria were the most common pathogens in diabetic foot infections. Imipenem, meropenem, were the most effective agents against Gram-negative organisms. Vancomycin was the most effective against Gram-positive organisms. Decisive therapy should be based on both the cultures and susceptibility data and the clinical response to the empirical regimen.

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