

## Study of bacteriology and antibiogram of diabetic foot infections

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### Abstract

**Introduction:** Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Diabetics exhibit a great frequency and severity of infection. A diabetic with foot complication spends 32.3% of the total income compared to 9.3% by the diabetic without foot complication. **Materials & Methods:** . The sample taken was pus and exudates from the base of the ulcer after cleaning the necrotic tissue with a saline gauze. Direct Microscopy, Culture, Biochemical reactions & antibiotic sensitivity, **Results:** The male to female ratio was 2.03 in our study, The ulcers occurred over a wide range of age from 16 years to 78 years with a mean age of 47 years. 64 out of 100 cases were between the ages of 45-65 years. There was only one case below 25 years of age and 3 cases above 75 years, Monomicrobial infections are more in grades-I & II and polymicrobial infections are more in grades- III & IV. **Conclusion:** The present study was conducted to know the bacteriology and antibiogram of diabetic foot infections in and around Mangode, Palakkad district. *Pseudomonas aeruginosa* was the most common organism isolated and constitutes 32.43% of the total isolates. Out of the 15 *Pseudomonas* isolates that were subjected to Screening of Amp - C  $\beta$  lactamase by diantagonism test only 4 isolates showed blunting of Cefotaxime zone of inhibition adjacent and to Cefoxitin and were considered screen positive.

**Keywords:** Diabetic Foot; Amp - C;  $\beta$  Lactamase.

### Introduction

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. It was once regarded as a single disease entity but it is now seen as a heterogeneous group of disease characterized by a state of chronic hyperglycemia resulting from a diversity of

etiologies, environmental and genetic factors acting jointly [19].

Diabetics exhibit a great frequency and severity of infection. Out of all the infections, the foot infections are the most common for prolonged hospital stay amongst the diabetics. Diabetic foot is characterized by a classical triad of neuropathy, ischemia and infection. The foot infections are

a serious medical problem requiring prompt attention, appropriate diagnostic evaluation and proper therapeutic strategies [19].

Infection may be superficial or deep. Superficial infections are usually monomicrobial while deep infections are polymicrobial. A diabetic with foot complication spends 32.3% of the total income compared to 9.3% by the diabetic without foot complication [5].

The bacteriology and antibiotic sensitivity pattern of various pathogens in diabetic foot infections has been undergoing changes over the period of years. There is a considerable difference in the bacteriology of diabetic foot world wide in the different geographical areas [32].

Hence, the present study was conducted to establish the bacteriology and antibiotic susceptibility of diabetic foot infections, and an attempt was also made to know the resistance pattern of *Pseudomonas aeruginosa* which is noted for its multidrug resistance.

## Materials & Methods

The material for the present study was collected from patients admitted in the departments of Surgery, Septic ward in Kerala Medical college Hospital

### *Study group*

Diabetic patients presenting with ulcer on the foot belonging to Wagner's grades 1, 2, 3 & 4 were included.

### *Sample Collection*

The samples were collected on the first day of admission. The sample taken was pus and exudate from the base of the ulcer after cleaning the necrotic tissue with a saline gauze.

The material was collected by swabbing the base of the ulcer with two sterile swabs, one for direct smear and the other for inoculating into liquid and solid media.

*The specimens were processed by :*

1. Direct microscopy
  - a) Gram's Staining
  - b) Hanging drop preparation and
  - c) Capsular staining.

2. Culture
3. Biochemical reactions and sugar fermentation tests
4. Antibiotic susceptibility of the isolates was performed by Kirby - Bauer disc diffusion method

## Results

In the present study 100 samples of pus and exudates were obtained from patients with diabetic ulcers of the foot following standard procedures. They were processed for the identification of the infective agent in the Department of Microbiology, Kerala medical college & Hospital, Palakkad

### *Blood sugar level*

Blood sugar levels mg% -- number of Cases  
<150 - 18, 150-200---42, 200-300--31 >300-9

18 out of 100 cases developed foot ulcers in spite of near normal blood sugar levels

### *History of onset of ulcer*

Only 37 out of 100 patients gave the history of some form of injury and the rest informed that the ulcer has started spontaneously

### *Cases with wagner's grading of ulcers*

More than half of the cases(52) presented with ulcers of grade - II. Grade 1 = 18,

Grade 3 =27, Grade 4= 0

### *Distribution Of Risk Factors In Cases*

76 cases out of 100 were having peripheral Neuropathy and 70 cases symptoms of arteriopathy. 11 of the total patients got foot infection without any known risk factor.

### *Frequency Of Isolates In Various Wagner's Grades*

Monomicrobial infections are more in grades-I &II and polymicrobial infections are more in grades- III & IV.

### *Frequency Of Isolates In Urban / Rural areas.*

Only 11.54% of urban cases were polymicrobial where as 60.81% of rural cases were polymicrobial infections.

**Table 1:** Sample collection - cases

Sample were collected from septic ward more patient 62 cases

Department	No of cases
Septic ward	62
Surgery	29
Endocrinology	9
Total	100

**Table 2:** SEX wise distribution cases

The male to female ratio was 2.03 in our study

Males	67
Females	33
Total	100

**Table 3:** Age wise distribution cases

The ulcers occurred over a wide range of age from 16 years to 78 years with a mean age of 47 years.

64 out of 100 cases were between the ages of 45-65 years. There was only one case below 25 years of age and 3 cases above 75 years.

Age group	No of cases
<25Y	1
26-35	9
36-45	15
46-55	28
56-65	36
65-75	8
>75y	3
Total	100

**Table 4:** Duration of diabetes in the study group

Out of 100 patients 15 are unaware of the disease and diagnosed at the time of admission. 32 reported the duration of less than 5 years, 33 reported a duration of 5-10 years and 20 had the history of diabetes for more than 10 years.

Duration	No of cases
Not known before	15
<5y	32
5-10y	33
>10y	20
Total	100

**Table 5:** Glycaemic Control

Majority of the cases were under insulin treatment

Method	No of cases
Insulin	71
Oral hypoglycemics	29
Total	100

**Table 6:** Bacterial isolates from cases

*Pseudomonas aeruginosa* was the most common organism isolated and constitutes 32.43% of the total isolates.

*Staphylococcus aureus* was the second most common organism with 34 isolates and forms 22.97% of the total isolates.

*Escherichia coli* 32 (21.6%) *Klebsiella* spp 18 (12.16%) and *Proteus* spp 15 (10.13%) forms the rest of the isolates. *Acinetobacter* spp was isolated in one case

	No of cases	No of isolates
<b>organism isolated in pure:</b>	23	23
<i>Pseudomonas aeruginosa</i>		
<i>Staphylococcus aureus</i>	15	15
<i>Escherichia coli</i>	2	2
<i>Klebsiella</i>	9	9
<i>Proteus</i>	3	3
<b>Organism isolated in combination :</b>	14	28
<i>Pseudomonas</i> + <i>Escherichia Coli</i>		
<i>Escherichia coli</i> + <i>Staph aureus</i>	10	20
<i>Escherichia coli</i> + <i>Proteus</i>	6	12
<i>Pseudomonas</i> + <i>Staph aureus</i>	5	10
<i>Pseudomonas</i> + <i>Proteus</i>	3	6
<i>Escherichia-coli</i> + <i>Klebsiella</i>	3	6
<i>Klebsiella</i> + <i>staph aureus</i>	2	4
<i>Pseudomonas</i> + <i>Klebsiella</i>	2	4
<i>Proteus</i> + <i>Staph aureus</i>	1	2
<i>Proteus</i> + <i>Klebsiella</i>	1	2
<i>Acinetobacter</i> + <i>Klebsiella</i>	1	2
Total	100	148

#### *Antibiogram of staphylococcus aureus* (N = 34)

*Staphylococcus aureus* is showing highest sensitivity to Roxithromycin (26) followed by Cefaperazone + Sulbactam (23) combination and Amikacin (20). The organism is showing lowest sensitivity to cloxacillin (7) and penicillin G (5) among all the antibiotics tested.

#### *Antibiogram of gram negative bacilli*

Out of the seven antibiotics tested gram negative bacilli are showing highest sensitivity to amikacin followed by cefaperazone + sulbactam combination, Roxithromycin and Gentamycin. All the gram negative bacilli are showing least sensitivity to cloxacillin.

- 35 *pseudomonas* isolates were sensitive to cefaperazone + sulbactam followed by Roxithromycin (34) and Amikacin (32)
- 26 Isolates of *E.coli* were sensitive to Cefaperazone + Sulbactam followed by Amikacin (22) and Roxithromycin (22)
- 14 isolates of *Klebsiella* were sensitive to Amikacin followed by Roxithromycin (11)

and cefaperazone + Sulbactam.

- 12 Isolates of *Proteus* were sensitive to Amikacin followed by Roxithromycin (9) and Cefaperazone + Sulbactam (8).

Single *Acinetobacter* isolate obtained was sensitive to cefaperazone + sulbactam Amikacin and gentamycin.

Out of the 15 *Pseudomonas* isolates that were subjected to Screening of Amp - C  $\beta$  lactomase by disc antagonism test only 4 isolates showed blunting of Cefotaxime zone of inhibition adjacent and to Cefoxitin and were considered screen positive.

#### Discussion

The present study was conducted to establish the bacteriology and antibiotic susceptibility of diabetic foot infections. Samples were collected from 100 cases admitted in septic ward, surgery and departments.

Out of 100 cases studied 67 were male and 33 were females with a male to female ratio of 2.03.

This correlates with the ratio of 1.95 by Viswanath et al. Chennai in 2002, 1.89 by C. Anandi et al Chennai 2004. Vijaya et al in 2000 reported a male to female ratio 2.63 from Bengaluru and Ekra Bansal et al. in 2008 presented a ratio of 3.68.

The mean age of patients with diabetic foot infections from the studies are over the world varies from 43 years to 57 years. The present study includes patients from 16-78 years with a mean age of 47 years Syed Md Alavi from Karachi, Pakistan Reported same value in 2007.

Peripheral neuropathy is commonly associated with diabetic infections and is one of the risk factor for the development of foot ulcers. In the present study 76 out of 100 cases presented with the signs and symptoms of neuropathy. This was same as Given by Ekta Bangal et al Chandigarh in 2008, Ravisekhar et al Delhi in 2006. and higher than Shanker et al., Chennai (56%) in 2005.

Diabetic foot infections are usually Polymicrobial in nature and always yield more than one isolate for sample on the whole. In the present study a total of 148 isolates were obtained from 100 cases making the number of isolates per sample 1.48. This was very close to 1.42 by Viswananth et al in Chennai in 2002 and 1.52 by Ekta. Bansal et al in 2008 from Chandigarh, but Citran. M et al. USA in 2004 reported 2.7 and N.A Pathera in 1998 as 3.07 from Mumbai.

Gram negative bacilli forms 77% of the total isolates. This was in contrast to the predominant isolation of Gram positive cocci (80%) by E.J. Goldstein et al. California. Hartmen Mecutem A et al., in 2004. observed 64% showing increasing trend of Gram negative bacilli in Diabetic foot infections. Pathare NA et al in 1998 from Mumbai reported 50% of Gram positive cocci in the first two grades and reduced to less than one fourth in the last two grades. Vijay D et al., Bengaluru (2002) 65%, Sasikala R et al., Pondicherry (2005) 77.94%, Ekta Bansal et al Chandigarith (2008) 77%, the last two being almost same as the present study.(Figure 1) .

*Pseudomonas aeruginosa* was the most common organism isolated and constitutes 32.43% of the total isolates. This correlates with the studies from various Geographical areas as 22% by Robert G et al, USA in 2002, 28% by Shankar EM et al. in 2005, 21.67% by Ekta Bansal, Chandigarh in 2008. Our study supports the conclusion by Shankar EM et al. from Chennai that recovery of multidrug resistant *Pseudomonas aeruginosa* is of serious concern, the first person to report this from South India.

*Staphylococcus aureus* was the second most common organism with 34 isolates and forms 22.97% of the total. This differs widely from 76% by E.J. Goldstem et al. USA in 1996 and 77% by Dang C.N. et al UK in 2003. but correlates with 24.5% by citron M et al., USA in 2004, 19% by Robert G et al. USA in 2002, 18.3% by Viswanath et al Chennai in 2002, 20% by Yoga R et al. Malaysia in 2006, 21.17% by Sasikala et al, Pondichery in 2005 and 18.88% by Ekta Bansal et al Chandigarh in 2008.(Figure 4).

More than 50% of Gram negative bacilli are sensitive to Amikacin, cefaperazone +sulbactam, Roxithanycin and Gentamycin. Amikacin was effective against 70.79% of Gram negative bacilli that was less when compared to 88.46% by Dushyanth Singh Uttaranchal 2007 and 81.14% by Robert E, USA, 2002. It was more than 52% sensitivity reported by Ravisekhar et al 2006, and 33.4% by Sasikala 2005.

Cefaperazone + Sulbactam was showing 69.91% effectiveness in the preset study and was less than 90.66% by Ravisekhar AIMS, 2006 and 97.5% Robert G, USA .

Gentamycin was sensitive against 53.09% of Gram negative bacilli which coincides with 54.4% by Robert G USA in 2002 Ciprofloxacin was effective against 36.25% bacteria which was 52% by Ravisekhar et al in 2006. Cephalexin was effective against 35.35% of Gram negative bacilli for which Dushyant Singh, Uttaranchal in 2007 reported 54.09% sensitivity. Roxithromycin was showing 67.25% effectiveness in the present study.(Figure 6)

*Pseudomonas aeruginosa* is showing maximum sensitivity to cefaperazone + sulbactam (72.91%) which was less than 93.5% reported by Robert E, USA, 2002 and 88.8% by Ravisekhar et al., New Delhi, 2006. The sensitivity to Roxithromycin was 70.83%. The third most effective antibiotic against *Pseudomonas* was Amikacin with 66.07% sensitivity which was more than 55.5% by Ravisekhar et al., 2006 and 33% by Sasikala et al., Pondichery but less than the studies of Robert E, 2002 (78.95%) Yoga R, Malasya (100%) and C. Anandi et al. 2004 (90%). The percentage sensitivity of Gentamycin and Ciproflaxcin were 52.08% and 31.25% in comparison to Robert E et al. (33.33% and 62.5%) Yoga R et al. (50% and 100%) and C. Anandi et al (90% and 90%). Cephalexin was effective in 43.75% of isolates which was 54.09% in 2007 by Dushyant Singh. Cloxacillin was the least effective antibiotic (4.1%) against *Pseudomonas* that was 0% in Saudi in 2007 given by Syed - Md Alavi. (Figure 7).



In the present study *Pseudomonas aeruginosa* was the predominate isolate with resistance to more than 50% of drugs. The 15 *Pseudomonas* isolate screened for Amp-C  $\beta$  lactamase by disc antagonism test. Showed 4 screen positive isolates Comparison with other studies is difficult to do, since the patient population is different. The prevalence was 22% in SS Hospital, Varanasi, compared to earlier studies in India that were 17.3% in Kolkota and 20% in Aligarh. As we found 4 out of 15 isolates as screen positive there is significant need to conduct a thorough study on the prevalence of Amp-C  $\beta$  lactamase in *Pseudomonas aeruginosa* in Mangode, Palakkad distric

### Conclusion

- The present study was conducted to know the bacteriology and antibiogram of diabetic foot infections in and around Mangode, Palakkad district.
- Out of 100 cases studied 67 were male and 33 were females with a male to female ratio of 2.03.
- The present study includes patients from 16-78 years with a mean age of 47 years.
- 76 Out of 100 cases presented with the signs and symptoms of neuropathy.
- A total of 148 isolate were obtained from 100 cases and the number of isolates per sample is 1.48.
- *Pseudomonas aeruginosa* was the most common organism isolated and constitutes 32.43% of the total isolates.
- *Staphylococcus aureus* was the second most common organism with 34 isolates and forms 22.97% of the total isolates.
- *Escherichia coli* 32 (21.6%) *Klebsiella* spp 18 (12.16%) and *Proteus* spp 15 (10.13%) forms the rest of the isolates. *Acinetobacter* spp was isolated in one case.
- The antibiotic sensitivity testing shows Amikacin, cefaperazone + sulbactam, Roxithromycin as the most effective antibiotics against Gram negative bacilli.
- *Staphylococcus aureus* is showing highest sensitivity to Roxithromycin followed by cefaperazone + sulbactam combination.
- 15 Isolates of *Pseudomonas aeruginosa* that were obtained from February 2008 were

subjected to disc antagonism test for the detection of Amp – C  $\beta$  lactamase and 4 of them were screen positive.

- This finding may be taken as base line for further evaluation of prevalence Amp-C  $\beta$  lactamase production in this area.

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