

# Bacterial Profile and pattern of antimicrobial drug resistance in Diabetic foot ulcers at Tertiary care hospital

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## Abstract

**Background:** Worldwide diabetic foot is a major medical problem leading to disability and economic instability to family and country. **Objectives:** To assess the clinical and bacteriological profile of diabetic foot ulcer among rural Indian residents. Also to know the prevalent pattern of antimicrobial resistance of most commonly isolated organisms to various currently in use antimicrobial agents. **Methodology:** From 78 cases of diabetic foot ulcer, identification of isolates was done using standard microbiological techniques. Antibiotic sensitivity testing were carried out followed by detection of ESBL, MRSA, AmpC and MBL producing strains as per the CLSI guidelines. **Results:** In our study, a total of 78 specimens were cultured and 97.4% ulcer showed growth of organisms (76/78). Of the total 139 isolates, 134 (96.4%) were aerobes and only 5 (3.6%) isolates were anaerobes. An average of 1.8 organisms per lesion was isolated. Among the aerobes, 93 (69.4%) gram negative and 41 (30.6%) gram positive organisms were isolated. *Pseudomonas aeruginosa* 23.9% was predominant organism followed by *Klebsiella species* (20.9%). A total 41 (30.6%) aerobic gram positive bacteria were isolated. *Staphylococcus aureus* was the most common gram positive organism 28.4% in which *Methicillin resistant S. aureus (MRSA)* was 57.9%. Among gram negative organisms 46(49.5%) isolates showed ESBL production. Among ESBL producing strains *Escherichia coli* 68.3% isolates recorded highest ESBL activity followed by *Pseudomonas aeruginosa* 62.5 % and *Klebsiella species* 53.6 %. **Conclusion:** The present study has shown that diabetic foot infections are polymicrobial in nature and commonly caused by multi drug resistant gram negative organisms.

**Keyword:** Bacterial isolates, Diabetic Foot Ulcer, Extended Spectrum Beta- Lactamase, Methicillin resistant S. aureus.

## Introduction

Diabetes Mellitus is a chronic disorder affecting a large segment of population and in present scenario it act as major public health problem in India [1]. Among diabetes related complications, foot ulceration is the most common, affecting approximately 15% of diabetic patients during their lifetime [2]. Limb amputation has major impact on the individual, not only distorting body image, but also with regard to loss of productivity, increasing dependency, and cost of treatment [3].

Selecting appropriate antimicrobial therapy for diabetic foot infections requires knowledge of likely etiologic agents [4]. Failure to identify and treat a relatively susceptible organism may result in the development of multidrug resistant strain of gram negative bacteria [5]. Infections of foot ulcers by Extended Spectrum Beta-Lactamase (ESBL) producing multidrug resistant gram

negative bacteria in diabetic patients have been described frequently [6]. Several studies found methicillin-resistant *Staphylococcus aureus* (MRSA) in as many as 15-30% of diabetic wounds [7, 8]. Infections with multi drug resistant organisms (MDROs) may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality [5].

The aim of this study was to determine the hierarchy of the organisms most commonly isolated from patients with diabetic foot ulcers and antibiotic susceptibility pattern of these organisms at the onset and therefore empirical selection of right kind of anti-microbial therapy help in a better outcome in patients.

To assess the potential risk factors for infection of diabetic foot ulcers with multidrug resistant organisms and the outcome of these infections were also studied.

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## Material and Methods

The present study was undertaken during the period from September 2010 to June 2012 in our Microbiology Department. 78 diabetic patients with foot ulcer admitted at surgical wards of Krishna Hospital, Karad were included in the study.

### Inclusion criteria:

- Type I and II diabetes of all the age groups and both sexes.
- Diabetic patients with foot ulcers of Grade I or more.

### Exclusion criteria:

- Non-diabetic patients with foot ulcers.
- Diabetic patients with foot ulcers of Grade 0.

A semi structured questionnaire was developed to record the medical history, examination details and investigation reports. Diabetic foot ulcers were categorized into six grades based on Wagner classification [9].

### Microbiological Method:

Culture specimens were obtained at the time of admission with proper history of antibiotic treatment either curettage of the base of ulcer, needle aspiration of abscess material or deep wound swab using aseptic technique. The specimen was kept in sterile culture tube and immediately transported to microbiology laboratory for processing of aerobic and anaerobic bacteria [10, 11,12].

### Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing of aerobic isolates was performed by the Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI 2011 Volume 31 No. 1) [13].

### Multi drug resistant gram negative bacilli:

The isolates which were resistant to beta lactam antibiotics containing oximino- group such as oximino-cephalosporins such as Cefazidime, Ceftriaxone, Cephalexin and Cefodoxime as well as oximino-monobactam such as Aztreonam, were taken as Multi drug resistant gram Negative organisms (MDROs) [14].

Gram-negative bacilli were subjected to first screening tests for the detection of ESBL. Confirmation of ESBL production was tested by Phenotypic Methods i.e. Double disc synergy test [14] and combined disc diffusion test [13]. Also *Escherichia coli* and *Klebsiella* bacilli were screened for detection of AmpC beta

lactamase production. Confirmation of AmpC production was done by using AmpC disc test [15]. Screening and confirmation of *Pseudomonas aeruginosa* and *Acinetobacter* species for the detection of MBL was done by Imipenem – EDTA Combined Disc test [16]. Further confirmation of metallo-beta lactamase was done by the Imipenem-EDTA double disc synergy test. [17]. *Staphylococcus* species were tested for Methicillin resistance using 30µg Cefoxitin discs by Kirby Bauer Disc Diffusion method [13]. Quality control was performed using control strains *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 for ESBL detection. *Pseudomonas aeruginosa* ATCC 27853 and ATCC *Staphylococcus aureus* 43300 were used for the detection of MBL and MRSA respectively according to National Committee for Clinical Laboratory Standards guidelines (CLSI 2011 Volume 31 No. 1) [13].

### Statistical methods:

Statistical analysis was carried out after the raw data entered into MS Excel and analyzed into frequency percentage distribution. Chi-square test and unpaired t test were applied for showing association between study variables. Two tailed p value of < 0.05 was taken as significant.

## Results

Mean age of the patients was 59.5±14.6 years. Maximum,70.51% cases were above the age of 50 years and as age increases, the chance of getting of Diabetic foot ulcer also increases ( $\chi^2=43.42$ , p = 0.0001\*). The proportion of male cases were higher,76.9% as compared to females, 23.1% and difference was statistically significant ( $\chi^2=9.07$ , p = 0.002\*).Majority of the patients were Type II Diabetes Mellitus i.e. 97.4%. Mean duration of ulcer was 18.9±25.1 days and ranged from 3 days to 180 days. Most common associated complication was neuropathy seen in 44 (56.4%) cases. Majority of the ulcers belong to Grade III and IV i.e. 42(53.8 %) and shows right leg ulcer predominance in 53(67.9%) cases.

In our study, a total of 78 specimens were cultured and 97.4% ulcer showed growth of organisms (76/78). A total of 139 isolates were obtained with 2 specimens being sterile .An average of 1.8 organisms per lesion was isolated. 34 (44.7%) cases had growth of single organism while the rest were polymicrobial with 19.7% yielding 3 or more organisms.134 (96.4%) isolates were aerobes whereas 5 (3.6%) of the isolates were anaerobes. [Table 1]

**Table No. 1: Bacterial Isolates with Percentage**

Bacterial isolates	Percentage
Total number of isolates	139
<b>Aerobes</b>	134 (96.4%)
Gram positive organisms	41 (30.6%)
1) Methicillin sensitive <i>Staphylococcus aureus</i>	16 (42.1%)
2) Methicillin resistant <i>Staphylococcus aureus</i>	22 (57.9%)
3) <i>Streptococcus pyogenes</i>	3(2.2%)
Gram negative organisms	93 (69.4%)
1) <i>Pseudomonas aeruginosa</i>	32 (23.9%)
2) <i>Klebsiella</i> species	28 (20.9%)
3) <i>Escherichia coli</i>	19 (14.2%)
4) <i>Acinetobacter</i> species	7 (5.2%)
5) <i>Proteus</i> species	5 (3.7%)
6) <i>Citrobacter</i> species	2 (1.5%)
<b>Anaerobes</b>	5 (3.6%)

Table no.1 shows that in our study, a total of 78 specimens were cultured and 97.4% ulcer showed growth of organisms (76/78). A total of 139 isolates were obtained with 2 specimens being sterile. Also it shows the frequency of isolation of different organisms from diabetic foot ulcers. Gram negative organisms were most frequently isolated 93 (69.4%) followed by gram positive. A total 41 (30.6%) aerobic gram positive bacteria were isolated. *Staphylococcus aureus* was the most common gram positive organism (28.4%).

The ratio of gram positive to gram negative was 1.2:2. Only Gram positive organisms, were found in 35 (46 %) cases and 14 (18.4%) had only gram negative organisms. The remaining cultures had growth of both gram positive and negative organisms. *Staphylococcus aureus* was the most common gram positive organism (28.4%). High levels of resistance were noted to: Penicillin, Ampicillin, Erythromycin, Cotrimoxazole, Ciprofloxacin. Maximum sensitivity was seen to Netilmicin, Linezolid. Table No. 2

**Table No 2: Antimicrobial Susceptibility Pattern of *Staphylococcus Aureus***

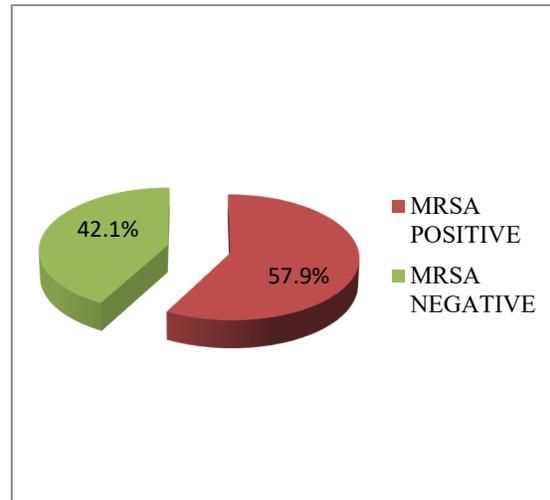
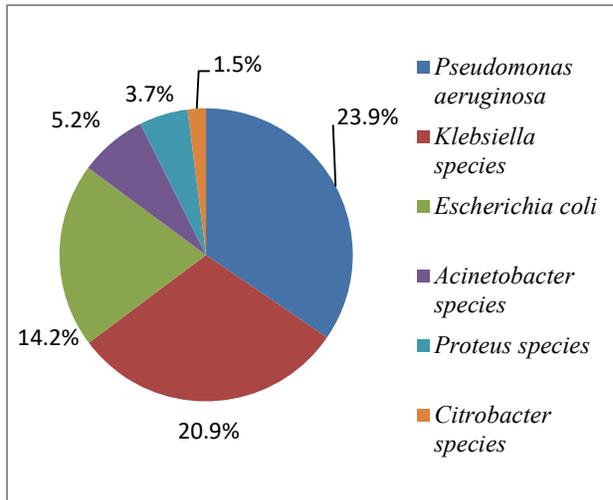
Drug	<i>Staphylococcus aureus</i> (38)
Penicillin	3(7.8%)
Cotrimoxazole	4 (10.5%)
Ampicillin	3(7.8%)
Ciprofloxacin	3(7.8%)
Ofloxacin	10(26.3%)
Norfloxacin	8(21.6%)
Levofloxacin	15(39.4%)
Azithromycin	5(13.2%)
Erythromycin	4(10.5%)
Amoxycillin+Clavulanic acid	6(15.9%)
Imipenem	28(73.7%)
Tobramycin	18(47.4%)
Cefoxitin	16(42.1%)
Linezolid	24(63.2%)
Teicoplanin	18(48.6%)
Netilmicin	28(75.7%)
Methicillin sensitive	16(42.1%)
Methicillin resistant	22(57.9%)

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Table no.2 shows in *Staphylococcus aureus* isolates high levels of resistance was noted to: Penicillin, Ampicillin, Erythromycin, Cotrimoxazole, Ciprofloxacin. Maximum sensitivity was seen to Netilmicin, Linezolid.

Fig No: 1 Distribution of Aerobic Gram Negative Bacteria

Fig No: 2 MRSA detection in Staphylococcus Aureus



Most of the gram negative bacteria were resistant to various classes of antibiotics. Imipenem was the most effective antimicrobial agent against all the isolated gram negative bacterial species. Table No. 3

MRSA were seen in 22(57.9%) isolates of *Staphylococcus aureus*. Figure No.2. Among gram negative organisms, 46(49.5%) isolates showed ESBL production. Figure No.3. Of the total 19 isolates of *Escherichia coli*, 13(68.3%) were ESBL positive, 4(21.5%) were AmpC positive and 4(21.5%) were both ESBL and AmpC positive. Similarly of the 28 isolates of *Klebsiella* species, 12(42.9%) were ESBL positive, 3(10.7%) were positive for AmpC and 3(10.7%) were both ESBL and AmpC positive.

Of the total 32 isolates of *Pseudomonas aeruginosa*, 13(40.6%) were only ESBL positive, 7(21.9%) were MBL positive and 7(21.9%) were both ESBL and MBL positive. Similarly of the 7 isolates of *Acinetobacter* species only 5(71.4%) were positive for MBL. ESBL were not detected in *Acinetobacter* species isolated.

Table No 3: Antimicrobial Susceptibility Pattern of Gram Negative Organisms

Rug	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella species</i>	<i>Acinetobacter species</i>	<i>Proteus species</i>	<i>Citrobacter Species</i>
Cefalorid	0	0	0	0	2(40%)	0
Cefodoxime	0	0	1(3.6%)	0	2(4%)	0
Cefoxitin	0	10(31.3%)	7(25%)	1(14.3%)	2(40%)	0
Ofloxacin	0	2(10.5%)	0	0	1(20%)	1(50%)
Ciprofloxacin	0	1(5.3%)	2(7.1%)	0	1(20%)	1(50%)
Norfloxacin	3(9.4%)	2(5.3%)	2(7.1%)	1(14.3%)	2(40%)	1(50%)
Levofloxacin	7(21.9%)	5(29.4%)	8(28.6%)	2(28.6%)	2(40%)	1(50%)
Ticarillin	3(9.4%)	0	1(3.6%)	0	3(60%)	1(50%)
Imipenem	16(50%)	16(94.1%)	24(85.7%)	6(85.7%)	5(100%)	2(100%)
Meropenem	17(51.1%)	15(88.2%)	12(42.8%)	3(42.9%)	4(80%)	2(100%)
Cefotaxime	11(34.3%)	0	1(3.6)	1(14.3)	4(80%)	0
Piperacillin+ Tazobactam	22(68.7%)	10(58.8%)	3(10.7%)	3(42.9%)	5(100%)	1(50%)

Amikacin	7(21.9%)	5(29.4%)	19(67.9%)	1(14.3%)	4(80%)	0
Ceftazidime	14(43.7%)	0	2(7.1%)	2(28.5%)	4(80%)	0
Amoxicillin+ Clavulanic acid	8(25%)	5(29.4%)	0	1(14.3%)	2(40%)	0
Gentamicin	6(18.7%)	5(29.4%)	9(32.1%)	1(14.3%)	3(60%)	1(50%)
Ceftriaxone	9(28.1%)	0	2(7.1%)	1(14.3%)	2(40%)	0
Aztreonam	9(28.1%)	0	1(3.6%)	2(28.5%)	1(20%)	0
Tobramycin	20(62.5%)	8(47%)	21(75%)	5(71.4%)	2(40%)	1(50%)

Table no.3 shows most of the gram negative bacteria were resistant to various classes of antibiotics. Imipenem was the most effective antimicrobial agent against all the isolated gram negative bacterial species.

The association of study factors with MDROs is presented in Table No 4. There were no significant differences in the demographic characteristics between the two groups. Duration of diabetes shows borderline significant association with MDRO infection. As the duration of diabetes increases, the increase in the incidence of infection with MDRO is high (p value 0.0527; t value 1.968). Increase in the grade of ulcer did not show any association with MDROs (p value 0.315).

**Table No 4: Association of Study Characteristics - MDRO and Non MDRO**

	MDRO	Non MDRO	Value
n	50	28	
Age (yrs)	58.66+14.69	60.96+14.45	P 0.506
Male	37 (74%)	22 (78.6%)	P 0.860
Female	13 (26%)	6 (21.4%)	
Duration of diabetes (yrs)	7.2+8.75	3.6+3.83	P 0.0527
Duration of ulcer (days)	20.5+19.59	15.9+33.00	P 0.448
Grade of ulcer			
I	4 (8%)	3 (10.7%)	P 0.315
II	11 (22%)	8 (28.6%)	
III	11 (22%)	7 (25%)	
IV	13 (26%)	9 (32.1%)	
V	11 (22%)	1 (3.6%)	
Uncontrolled blood sugar	40 (80%)	22 (78%)	P 0.880
Controlled blood sugar	10 (20%)	6 (21.4%)	
Smokers	16 (32%)	11 (39.3%)	
Non-smokers	34(68%)	17(60.7%)	P 0.0539
Associated Complications			
Neuropathy	32 (%)	12 (60%)	P 0.399
Duration of hospital stay (days)	25.4+17.36	17+14.89	P 0.0345
Medical Treatment	13 (26%)	16 (57.2%)	P 0.0129
Surgical Treatment	34 (74%)	12 (42.8%)	
Outcome			
Amputation	16(32)	3(10.7)	P 0.035
Non-amputation	34(68)	25(89.3)	

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Duration of hospital stay have been reported to increase with MDRO infections than Non-MDROs (p value 0.0345; t value 2.153) increasing the cost of management. Significantly more patients with MDRO infections required surgical treatment (p value 0.0129). It was seen that the outcome of amputation was more when associated by infection with MDROs as compared to non-MDRO and was statistically significant (p value 0.035).

Considering a fasting blood glucose level of <110 mg/dl and postprandial level of <160 mg/dl as glycemic control, the number of patients with glycemic control on day of admission was compared between those with and without MDRO infections.

No significant association of glycemic control with MDRO and non-MDRO infections had seen (p value 0.880).

**Discussion**

The mean age of the patients in our study was 59.5±14.6 years. Most of our patients did not have access to diagnostic facilities as they were from rural areas. This is the reason for higher mean age of patients in our study.

Majority of the patient 39 (50%) were in age group 50 to 70 years, which are comparable with study of Ekta Bansal et al [18] (51.3%). Males were found to be more common victims and the ratio of male to female was 3.3:1. Male preponderance have observed in similar studies [18,19,5].

In the present study mean duration of diabetes was 6.5 years which is in close comparison to the study of Mamatha Samaga et al [20].

Majority of the ulcers belonged to Grade III and IV. 12 patients had extensive gangrene Grade V. Two cases with cellulites till thigh had to undergo above knee amputation.

As the grading of ulcer increased, the number of bacteria isolated also increased. Our study results are in comparison to study by Samir Paul et al [19] who has shown maximum number of cases 27 (36%) with Grade III ulcers having maximum isolation of organisms.

In the present study peripheral neuropathy was seen in 44(56.4%) of cases. The feet were the target of peripheral neuropathy leading chiefly to sensory deficit and autonomic dysfunction.

In the present study 16 (20.5%) patients had blood sugar level within controlled limits with isolated of one

organism per case. And 62 (79.5%) patient had poor glycemic control at the time of admission with an average of 1.6 organisms per case was isolated. High blood sugar level and increased number of isolation of organisms per case was seen in the study by Mamatha Samaga et al [20] which correlates with present study.

A total of 139 organisms were isolated from positive cultures of which, 134 (96.4%) were aerobes and only 5(3.6%) isolates were anaerobes. This result correlates with the study of Mamatha Samaga et al [20] and EM Shankar et al [8] which shows the predominance of aerobic infections than anaerobic infection.

In present study polymicrobial infection was noted in (55.3%) cases these findings correlates with similar study by Gadepalli et al [21], Samir Paul et al [19], Banashankari et al [5] and are in contrast to findings of Dhanasekaran et al [22] who documented 84% of monomicrobial infection.

In present study an average of 1.8 organisms per case was isolated. Study by Ekta Bansal et al [18] showed 1.5 isolates per case while, Gadepalli et al [21] showed 2.3 organisms per sample.

The ratio of gram positive to gram negative was 1.2:2. Our study results are in comparison with most of the authors. [21,5,8,22].

In our study the predominant gram positive cocci isolated was *Staphylococcus aureus* 38 (28.4%) followed by *Pseudomonas aeruginosa*, this was in accordance to the studies conducted in Indian tertiary care hospital [18,23].

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The most common gram negative organism was *Pseudomonas aeruginosa* 32 (23.9%). The same results are seen in study by Umashankari et al [14] (20.9%), Ekta Bansal et al [18] (22%) and Samir Paul et al [19] (26.7%).

Most of the gram negative bacteria were resistant to various classes of antibiotics. In present study ESBL production were noted in 46 (49.5%) gram negative bacteria.

As compared to study by Samir Paul et al [19] we have observed high prevalence of ESBL detection in *Pseudomonas aeruginosa* (62.5%). In present study of the total 19 isolates of *Escherichia coli* 4(21.5%) were AmpC positive. Similarly of the 28 isolates of *Klebsiella* species 3(10.7%) were positive for AmpC.

Our study showed 14(43.8%) MBL production by *Pseudomonas aeruginosa* and 5(71.4%) by *Acinetobacter* species which is in close concordance to the study by S. Murugan et al [17]. Of the total 38(28.4%) isolates of *Staphylococcus aureus* 22 (57.9%) were MRSA positive. The result of the study of MRSA by Umashankari et al [14] in 53%, Ekta Bansal et al [18] 55.5% and Gadepalli et al [21] 56% are comparable with the present study (57.9%).

Maximum number of cases 4(57.2%) infected with MRSA positive *Staphylococcus aureus* required surgical treatment i.e. debridement and amputation as compared to MRSA negatives 1 (14.3%). Similarly 24(72.7%) cases infected with ESBL producing bacteria required surgical treatment as compared to cases infected with non-ESBL producing bacteria 6 (35.3%).

The findings are in correlation with study by Gadepalli et al [21] who showed significantly more patients with MDRO required surgical treatment.

The present study confirms that MDRO infection is extremely common in hospitalized patients with diabetic foot ulcer. This is in accordance with the report of Heurtier et al [24].

It was seen that the outcome of amputation was more when associated by infection with MDROs as compared to non-MDRO and was statistically significant. MDRO infection in foot ulcers was associated with the requirement for surgical treatment (P value 0.0129).

MDRO infections have been reported to increase hospital stay and cost of management [21], we found

duration of hospital stay increased significantly in MDROs (p 0.0345).

Almost two-thirds of our patients were infected with MDROs and the prevalence of both MRSA isolates and ESBL-producing gram-negative bacteria was high. The only factors significantly associated with MDRO infection were the presence of neuropathy, duration of diabetes.

The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogens. The increasing prevalence of MDROs is disconcerting because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome.

**Conclusion**

In conclusion, the prevalence of MDROs was alarmingly high and was associated with increased requirement for surgical treatment. These findings suggest that prospective multicenter studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcers taking into consideration the etiology of ulcers. In addition, the results alert, us that proper management of antibiotics must be implemented to decrease the incidence of MDRO infection in this population, lest we soon run out of effective antimicrobials for our patients.

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