



Research Article

Clinico Microbiological Spectrum of Diabetic Foot Ulcer with Multidrug Resistant Organisms at Tertiary Care Hospital, Jaipur

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Abstract

Background: Diabetes is one of the major health problems that have become a big burden in all over the world. Amongst the diabetic population, about 8– 20% experience foot ulcer in life time. DFIs are predominantly polymicrobial and multidrug-resistant (MDR) with the ability to form biofilm, which is an important virulence factor and results in treatment failure

Objective: To determine the bacteriological profile and susceptibility pattern of organisms and to assess the magnitude of infection by Multidrug resistant organisms (MDRO'S) like Extended spectrum beta lactamase (ESBL) and Methicillin- resistant Staphylococcus aureus (MRSA)

Materials and Methods: A total of 73 pus and exudates sample were collected from diabetic foot patients. Specimens were collected with a sterile swab stick from each patient. Significant isolates were identified by conventional methods according to the standard laboratory protocol. The antibiotic sensitivity test along with screening for MRSA and ESBL producing GNB were done.

Results: The maximum number of diabetic foot cases 30(46.15%) were seen between age of 51-60 years. A total of 90 organisms were isolated from 65 clinical samples out of which 65(68.39%) were gram negative bacilli and 25(31.56%) gram positive cocci. Pseudomonas species (25.65%) was predominant among gram negative bacilli and Staphylococcus aureus (16.84%) was the predominant among gram positive cocci. Gram negative organisms showed highest sensitivity to Polymixin B (100%), Gram positive organisms showed highest sensitivity to Vancomycin (100%). Out of 65 isolates 16(24.6%) were ESBL producers. Out of 16 isolates 7(43.7%) were MRSA. Multidrug resistance was seen in 88.8% of isolates.

Conclusion: Early identification of the risk factors and timely institution of appropriate treatment indispensable to avoid amputations. There is a need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy and reduce the risk of complications.

Keywords: Diabetic foot ulcers, Extended spectrum beta lactamase, Methicillin- resistant Staphylococcus aureus, Multidrug resistant organisms.

Introduction

Diabetes is one of the major health problems that have become a big burden in all over the world.

Diabetes mellitus (DM) is a serious and complex illness that affects almost every vital organ in the body. There are approximately 415 million people

worldwide suffering from diabetes. It has been estimated that this number will increase to 642 million by 2040¹.

Amongst the diabetic population, about 8– 20% experience foot ulcer in life time. It is one of the most common causes of hospital admissions in diabetics. Diabetic foot ulcers have 15 –45% higher risk of amputation of limb as compared to foot ulcers secondary to other aetiologies². Diabetic foot involvement including infections and foot ulcers are one of the frequently seen and disabling complications of diabetes leading to significant morbidity and mortality²⁻⁴.

Diabetic neuropathy and micro or macro ischemia are the two main risk factors that cause diabetic foot ulcer (DFU). Impaired microvascular circulation limits the access of phagocytic cells to infected area, and this result in poor concentration of antibiotics in infected tissue⁵.

DFIs are predominantly polymicrobial and multidrug-resistant (MDR) with the ability to form biofilm, which is an important virulence factor and results in treatment failure⁶.

Diabetic foot infections are often polymicrobial. *Escherichia coli*, *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Enterococcus* spp are the most frequent pathogens contributing to progressive and widespread tissue destruction. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been commonly isolated from 10-40% of the diabetic wounds^{3,4}.

Infection with MDR pathogens is also responsible for the increased duration of hospitalization, cost of management, morbidity and mortality of the diabetic patients^{5,7,8}. Therefore this study has been performed to determine the common etiological agents of diabetic foot infections and in vitro susceptibility to routinely used antibiotics and to assess the magnitude of infection by Multidrug resistant organisms (MDRO'S) like Extended spectrum beta lactamase (ESBL) and Methicillin-resistant *Staphylococcus aureus* and their correlation with type of ulcer and severity of diabetes.

Materials and Methods

Study Population: A total of 73 pus and exudates sample were collected from diabetic foot patients attending hospital both OPD and IPD were included in the study. Specimens were collected from under the margin of the ulcer and base of the ulcer with a sterile swab stick from each patient in a sterile container under aseptic conditions by standard protocols. The study has been carried out at department of Microbiology, S.M.S Medical College Jaipur.

Inclusion criteria:

- i) Diabetic patients complicated by diabetic foot ulcer
- ii) Patient who gives a valid consent.

Exclusion criteria:

- i) Diabetic patients without complicated by diabetic foot ulcer

Sample Processing

Samples were taken using two swab sticks, first swab stick subjected to gram staining to observe for the presence of pus cells and organisms. From second swab stick, sample was inoculated on Nutrient agar, Blood agar, Mac-Conkey agar and Thioglycollate broth. The inoculated media were incubated aerobically at 37° C for 18-24 hours and were observed for growth.

Methodology

Significant isolates were identified by conventional methods according to the standard laboratory protocol, including colony morphology, gram staining and biochemical reactions. All gram negative bacilli were identified to species level by their characteristic appearances on the media, Gram's stain, Oxidase test, Motility and biochemical reactions as per standard laboratory protocol. All gram positive organisms were identified to species level by their characteristic appearances on the media, Gram's stain, and Catalase test followed by Coagulase test. *Enterococcus* was identified by Bile Esculin disc test and it was also confirmed by Salt tolerance test (6.5% NaCl.)

Antimicrobial Susceptibility Testing

The antibiotic sensitivity test was performed by modified Kirby Bauer disc diffusion technique⁹ with commercially available Hi-Media antibiotic discs according to Central Laboratory Standard Institute (CLSI) guidelines on Mueller Hinton agar plates. The antibiotics which were used in our study were based on the standard protocol of the hospital and departmental policies as per CLSI guideline¹⁰.

Detection of extended spectrum beta-lactamases¹¹

Detection of extended spectrum beta-lactamases was done according to CLSI guidelines by two methods Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and Double Disc Synergy Test (DDST)

Phenotypic confirmatory disc diffusion test (PCDDT)

Lawn culture of isolate was made on Muller Hinton Agar (MHA) plate; discs of ceftazidime and ceftazidime + Clavulanic acid were placed on the surface of MHA and incubate overnight at 37°C. An increase of ≥ 5 mm in zone diameter of ceftazidime + Clavulanic acid in comparison to the zone diameter of ceftazidime alone confirmed the organisms to be ESBL producers

ESBL detection by Double Disc Synergy Test (DDST)-

Lawn culture of isolate was made on Muller Hinton Agar (MHA) plate. Disc of ceftazidime (30 μ g), cefotaxime (30 μ g) and co-amoxycylav (20 μ g Amoxycillin and 10 μ g Clavulanic Acid) were placed at a distance of 20mm from centre to centre in a straight line, with co-amoxycylav disc in the middle. The plate was incubated aerobically overnight and the results were read on the following day. Isolates which shows an enlargement of the zone of inhibition greater than 5mm on the co-amoxycylav side of the disc compared to the result seen on the side without co-amoxycylav was confirmed as ESBL producers. ESBL production was interpreted if the inhibition zone around the test antibiotic disc increased towards the co-amoxycylav disc.

Screening of MRSA was done by cefoxitin disc diffusion test: All strains were tested with 30 μ g cefoxitin discs (Hi-Media) on Mueller–Hinton agar plates¹². The bacterial suspension for each strain was adjusted to 0.5 McFarland. The zone of inhibition was calculated after 16–18 hr incubation at 35 °C. Zone size were defined according to CLSI criteria (23)(24): susceptible, ≥ 22 mm; resistant, ≤ 21 mm. ATCC 25923 MSSA was used as negative control.

Statistical Analysis: The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. The difference in proportion was analyzed by using chi square test. Significance levels for tests were determined as 95% ($P < 0.05$)

Results

A total of 73 clinically suspected cases of diabetic foot ulcer were enrolled in our study among that 65 (89.04%) were significant growth and 03 (4.1%) were sterile while contaminants grown in 05 (6.8%) samples. Out of 65 significant growth 42 (64.61%) were male while 23 (35.38%) were female. The maximum number of diabetic foot cases 30(46.15%) were seen between age of 51-60 years of age followed by elderly people ≥ 60 years of age (32.30%) and more number of cases seen in patients with duration of 4-8 weeks of foot ulcer. According to Wagener's classification the maximum number of cases were observed in Grade 2 of diabetic foot ulcer 24(36.9%) followed by 16 cases (24.6%) in Grade 3 of diabetic foot ulcer.

Out of 65 cases 42 (64.61%) were monomicrobial growth and 23(35.38%) were polymicrobial growth. A total of 90 organisms were isolated from 65 clinical samples out of which 65(68.39%) were gram negative bacilli and 25(31.56%) gram positive cocci. *Pseudomonas species* (25.65%) was predominant among gram negative bacilli and *Staphylococcus aureus* (16.84%) was the predominant among gram positive cocci.

Out of 90 isolates 80 were resistant to 3 or more classes of drugs which accounts for 88.8% of

MDRO. Among gram negative organisms 16 out of 65 (24.6%) were ESBL producers. Most of them were *Proteus species* and *Enterobacter species* while Methicillin resistant *Staphylococcus aureus* was reported in 43.7%

Gram negative organisms showed highest sensitivity to Polymixin B (100%), Tigecyclin (100%) followed by Meropenem(69.2%)They showed least sensitivity to amikacin (15%).

Pseudomonas species showed highest sensitivity to Colistin (100%), followed by Cefepime (42.66%), while the least sensitive was Ciprofloxacin (20.8%).

Gram positive organisms showed highest sensitivity to Vancomycin (100%) followed by

Doxycycline (93.7%) and Linezolid (89.2%) They showed least sensitivity to ciprofloxacin (12.5%)

Among gram negative organisms out of 65 isolates 16(24.6%) were ESBL producers. Most of them were *Proteus species* and *Enterobacter species*. Among the *Staphylococcus aureus*, out of 16 isolates 7(43.7%) were MRSA. The MRSA displayed high level of resistance to Erythromycin (88%), Penicillin (75%) and Quinolones (88%).

Multidrug resistance was seen in 88.8% of isolates predominantly occurring in *Acinetobacter species*, *Proteus species* and *Escherichia coli*.

Table 1 Total number of sample tested under study

Samples	Number (n=73)	Percentage (%)
Growth Positive	65	89.04%
Sterile	3	4.1%
Contamination	5	6.8%
Total	73	100%

Table 2 Duration of Diabetic Foot Ulcer under Study

Duration of Diabetic foot ulcer	No. of Cases (n=65)	Percentage (%)
2-4 weeks	21	32.30%
4-8weeks	30	46.15%
8-12 weeks	9	13.84%
>12 weeks	5	7.69%

Table 3 Distribution of Bacteria in Positive Culture (n= 95)

Gram negative Bacteria	Number (n=65)	Percentage (%)
<i>Pseudomonas spp</i>	24	25.26%
<i>Escherichia coli</i>	13	13.68%
<i>Enterobacter spp</i>	12	12.63%
<i>Proteus spp</i>	07	7.36%
<i>Acinetobacter spp</i>	04	4.21%
<i>Citrobacter spp</i>	02	2.10%
<i>Klebsiella spp</i>	02	2.10%
<i>Burkholderia spp</i>	01	1.05%
Gram positive Bacteria (n=30)		
<i>Staphylococcus aureus</i>	16	16.84%
<i>Enterococcus spp</i>	5	5.26%
<i>Micrococcus species</i>	5	5.26%
CONS	3	3.15%
<i>Streptococcus spp</i>	1	1.05%

Table 4 Antibiotic Sensitivity of Gram Negative Organisms

Antibiotics	Organisms(n=65)				
	Escherichia coli (n=13)	Enterobacter spp (n=12)	Pseudomonas spp (n=24)	Proteus spp (n=7)	Acinetobacter spp (n=4)
Amikacin	(2)15.3%	(5)41.6%	(6)25%	(1)14.3%	(1)25%
Ampicillin	0	0	-	0	0
Aztreonam	-	-	(6)25%	-	-
Cefepime	0	(1)8.3%	(10)41.66%	(1)14.3%	(2)50%
Cefotaxime	0	(1)8.3%	-	(1)14.3%	-
Ceftazidime	-	-	(7)29.16%	-	-
Cefoperazone+ Salbactam	(6)46.1%	(5)41.6%	(10)41.66%	(3)42.8%	(1)25%
Ciprofloxacin	0	(2)16.6%	(5)20.8%	0	(1)25%
Cotromox/TMP	(3)23.6%	(5)41.6%	-	0	(1)25%
Gentmycin	(4)30.7%	(6)50%	-	(1)14.3%	(1)25%
Imepenem	-	-	(8)33.33%	-	-
Piperacillin/ Tazobactam	(6)46.7%	(9)75%	(10)41.66%	(7)100%	(2)50%
Tobramycin	-	-	(10)41.7%	-	-
Tetracyclin –E	(4)30.7%	(6)50%	-	0	(1)25%
Meropenem	(9)69.2%	(9)75%	-	(6)85.7%	(3)75%
Tigecycline	(13)100%	(10)83.3%	-	(5)71.4%	(4)100%
Polymixin-B	(13)100%	(13)100%	(24)100%	0	(4)100%
Colistin	(13)100%	(13)100%	(24)100%	0	(4)100%

Table 5 Antibiotic Sensitivity of Gram Positive Organisms

Antibiotics	Staphylococcus aureus (n=16)	Enterococcus species(n=5)
Ampicillin	(4)25%	(1)20%
Cefepime	(10)62.5%	0
Ciprofloxacin	(2)12.5%	0
Clindamycin	(7)43.7%	(2)40%
Cotimox/TMP	(6)37.5%	(1)20%
Piperacillin-tazobactam	(10)62.5%	0
Erythromycin	(2)12.5%	(2)40%
Gentamycin	(11)68.7%	(2)40%
Tecoplanin	(14)87.5%	0
Cephoxitin	(9)56.2%	(1)20%
Doxycillin	(15)93.7%	(3)60%
Linezolid	(13)81.2%	(5)100%
Vancomycin	(16)100%	(5)100%

Table 6 Magnitude of infection by Multidrug resistant organisms (MDRO’S) like Extended spectrum beta lactamase and Methicillin- resistant Staphylococcus aureus

MDRO	Number of isolates	Percentage (%)
Present	80	88.89
Absent	10	11.11
Total	90	100.00

Discussion

Severe DFIs are usually polymicrobial in nature, where as mild and moderate DFIs are mostly monomicrobial¹³. Our study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers, especially by multidrug-resistant organisms. Appropriate

selection of antibiotics based on the anti-biograms of the isolates from the lesions is most critical for the proper management of these infections.

In this study, samples from 73 cases were taken out of which 3 samples were sterile and 5 samples were contaminated, Potent pathogens were grown in 65 patients sample out of which 42(64.61%)

showed monomicrobial and 23(35.38%) showed polymicrobial growth. We found high rate of mono-microbial growth (64.61%) as compared to polymicrobial growth (35.38%). Our findings are in concordance with Sudhir K et al⁵ and Jitendranath A et al³ who also reported high rate of mono-microbial growth as compared to polymicrobial growth. However Umadevi .S et al¹⁴ and Saseedharan S et al¹⁵ reported high rate of polymicrobial growth as compared to monomicrobial growth.

The monomicrobial nature of infection is associated with the duration of the ulcer and antimicrobial treatment. Earlier in the infection, the monomicrobial state prevails and as the infection progresses with time, a polymicrobial state arises.

In this study we found that the age group most commonly affected by the diabetic foot ulcer was 51 -60 years (46.15%), followed by 61 or more years (32.30%) which is consistent with the study conducted by Jitendranath et al³, P. Ramakant et al¹³, Khare et al¹⁶ and Patil .S et al¹⁷ who also reported the same findings. The number affected increases in elderly age group and less common in younger age group.

In this study most cases of diabetic foot ulcer were of 4-8 weeks duration i.e 30 cases (46.15%) followed by 2-3 weeks in 21 cases(32.30%). Most commonly right foot was involved. Our results are concordance with Khare J et al¹⁶ and Katherivel M et al¹⁸ who also reported most cases of diabetic foot ulcer were of 4-8 weeks duration.

In this study maximum no. of cases were observed in Grade 2 of diabetic foot ulcer(36.9%) followed by Grade 3 (24.6%) which is similar with the study conducted by Jitendranath A et al³ Khare et al¹⁶, Ramakant P et al¹³ who reported maximum cases of grade 2 of diabetic foot ulcer in 40%, 36%, 35.5% respectively. However Patil S et al¹⁷ reported 60% in Grade 2 of diabetic foot ulcer.

We also observed that maximum monomicrobial growth was isolated from grade 2 followed by grade 3 as the grade progresses polymicrobial wound infection was found to be more common. It

can be interpreted that to start with, there is a poor response to injury in a patient with diabetes. This may be because of several factors like vascular insufficiency, neuropathy and poor diabetic control. The poor defence leads to a rapid increase in the number of microbes, with progressive deterioration of diabetic foot wound.

In this study *Pseudomonas species*(25.65%) was predominant among gram negative bacilli and *Staphylococcus aureus* (16.84%) was the predominant among gram positive cocci .Our results are consistent with study conducted by Ramakant et al¹³, Mehta et al¹⁹, Turhan et al²⁰ in which *Pseudomonas* was the commonest organism isolated . However Chittur R et al⁷ reported *Proteus species* (24.26%) as a predominant among gram negative bacilli in their study. Majority of *Pseudomonas species* were isolated from grade 2 and grade 3 diabetic foot ulcers. *Staphylococcus aureus* (16.84%) was the second most common organism isolated in our study, majority of them were from grade 1 and grade 2 diabetic foot ulcers which are usually uncomplicated ulcers.

In this study, out of 90 isolates 80 were resistant to 3 or more classes of drugs which accounts for 88.8% of MDRO which is in accordance with the study of Sudhir K et al⁵ and Banu K et al⁴ who have reported 91% and 97.6% of MDRO's respectively. Multidrug resistance was predominantly seen in *Acinetobacter spp*, *Proteus spp* and *Enterobacter species*. Similar results were seen in study of Sudhir K et al⁵ and Yerat R et al⁷. Factors responsible for MDR may be frequent hospitalization, recent use of broad-spectrum antibiotics, inadequate surgical source reduction, chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means.

In this study 43.7% of Methicillin resistant *Staphylococcus aureus* was reported which is similar with the results of Sudhir K et al⁵, Yerat R et al⁷, Turhan et al²⁰ and Rawat V et al²¹ reported 41%, 40%, 44.2% and 40% respectively.

We also found 24.6% of ESBL producers in our study which is similar with the study conducted by Shanmugam et al²² and Turhan et al²⁰ 15% and 13.2% respectively.

In this study all *Pseudomonas spp* were sensitive to Colistin(100%), while few of them were sensitive to Cefepime (42.66%), Cefoperazon+Salbactam (42%) and Tobramycin(42%) which is consistent with the study of Sudhir K et al⁵ and Deepa T et al⁶.

In this study most of the *Enterobacter spp* and *Escherichia coli* were sensitive to Polymixin B(100%), Tigecyclin (100%) and Meropenem (69.2%) while most of them were resistant to Penicillin, Quinolones and Cephalosporins. Similar results were reported by Sudhir K et al⁵, Khare J et al¹⁶ and Turhan V et al²⁰.

In this study *Staphylococcus aureus* were sensitive to Vancomycin(100%), Linezolid (89.2%), Doxycilin(93.7%) and Tecoplanin (87.5%). Vancomycin and Linezolid were the most effective antibiotics against gram positive organisms. Similar results were reported by Sudhir K et al⁵, Khare J et al¹⁶ and Turhan V et al²⁰. Among the *Staphylococcus aureus*, 43.7% were MRSA. Our result was similar to the study of Sudhir K et al⁵, Yerat R et al⁷, Turhan et al²⁰ and Rawat V et al²¹ where MRSA were reported 41%, 40%, 44.2% and 40% respectively. The MRSA displayed high level of resistance to Erythromycin (88%), Penicillin (75%) and Quinolones (88%).

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers, especially by multidrug-resistant organisms. Appropriate selection of antibiotics based on the anti-biograms of the isolates from the lesions is most critical for the proper management of these infections.

Conclusion

Diabetic foot ulcers are one of the most common and dreaded complications of diabetes. Thus it is utmost important to screen all elderly patients for diabetes and educate them about foot care. Early

identification of the risk factors and timely institution of appropriate treatment indispensable to avoid amputations. Clinicians should switch to culture report-based use of narrower spectrum therapy. This would improve the overall antibiotic efficacy and reduce the emergence of multidrug resistant organisms in diabetes.

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