

Original Research Article

Clinico-Microbiological Profile Of Diabetic Foot Ulcer

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ABSTRACT:

Background: Diabetes mellitus is one of the most important underlying condition contributing to high morbidity and mortality globally leading to a number of cumbersome clinical conditions, of which a Diabetic Foot Ulcer (DFU) is the most dreaded complication. A leading cause of hospitalization, in diabetic patients 36 the consequences are adverse leading to loss of a part of the lower limb or the whole limb. The risk of amputating a lower limb is 15 -46 times higher in a diabetic patient than in a non diabetic patient.

Aims and objectives: 1. To determine the genera of microorganisms found in diabetic foot ulcer. 2. To determine the antibiotic susceptibility pattern of organisms found in diabetic foot ulcer.

Materials and Methods: A total of 138 patients out of 193 were culture positive for DFU were included in the study. The details of the patient was recorded and a brief clinical history regarding the isolates was obtained from the laboratory information system. Details like age, gender, fasting and post prandial values and Hb A1c values were recorded. The isolates were collected and stored appropriately. The samples were stored in nutrient agar butts containing 1.5% nutrient agar and 6 ml of sterile glycerol and stored at -20 °C.

Observations and Results: A total of 193 patients with DFU were included in the study, out of which 138 (72%) were culture positive, Hence a total of 138 patients were enrolled in this study. Among the 138 patients, the ratio of right is to left limb DFU was found to be 67% and 33% respectively. Among the 138 patients, 92 (67%) were male and 46 (33%) were female. The age of patients ranged from 30 -80 years. A majority of patients (31%) belonged to the age group pf 41 -50 years. Of these 27 were males and 10 were females.

Conclusions: 193 diabetic foot ulcer samples were taken for this study out of which 138 were culture positive. B rining the rate of diabetic foot infection in our study to 72%.The total number of isolates in our study were 223. 51% of the cultures were polymicrobial (70/138) and 49% were monomicrobial (68/ 138). Our study shows diabetic foot ulcer infections are more common in males (67%) in comparison to fem ales (33%)

Keywords: Diabetic Foot Ulcer(DFU), Diabetes mellitus, Staphylococcus aureus, Gram positive Microorganisms, Gram Negative Microorganisms, Monomicrobial, Polymicrobial communities.

INTRODUCTION

Diabetes mellitus is one of the most important underlying condition contributing to high morbidity and mortality globally leading to a number of cumbersome clinical conditions, of which a diabetic foot ulcer (DFU) is the most dreaded complication. A leading cause of hospitalization, in diabetic patients (**Priyadarshini Shanmugam et al., 2013**)³⁶the consequences are adverse leading to loss of a part of the lower limb or the whole limb (**Kleopatra alexiadou et al ., 2012**)³. The risk of amputating a lower limb is 15 -46 times higher in a diabetic patient than in a non diabetic patient. (**David G Armstrong et al ., 1998**)⁸The ulcer can occur in both type 1 and type 2 diabetes.

A DFU is a result of cardinal factors like peripheral vascular disease, peripheral neuropathy, trauma, foot deformities and arterial insufficiency and also impaired resistance to infection. Foot ulcers lead to amputations in 85% of cases which is associated with risk factors like age, and duration and severity of diabetes.

A DFU usually gets infected in 40% - 80 % of individuals. 40 -70 % of all traumatic limb amputations of lower limb occurs in diabetic patients (**Kleopatra Alexiadon et al ., 2012**)³. Wounds and ulcers become severe and lead to limb amputations mostly due to the havoc of infection caused by various monomicrobial and polymicrobial communities. Few studies show that a colony forming unit of 10^5 is needed to elicit an infection. Poor glycaemic control is the primary cause of infection as the immune cells are ineffective in fighting the infection even in a small opening or sore leading to ulcer.

Staphylococcus aureus is a well known established pathogen (**Pradeep M.S. S et al., 2017**)³⁵along with *Enterococcus species* , among the gram positive microorganisms. Gram negative organisms showed a major predominance of *Enterobacteriaceae* family especially *Escherichia coli*, *Klebsiella pneumoniae* , *Morganella morganii* and *Proteus mirabilis* being the largest group of aerobic organisms along with *Enterobacter species*, *Pseudomonas species* , especially *Proteus species* and *Klebsiella species* were most commonly encountered. (**Jneid et al ., 2017**)²²Average of 1. 8 bacteria were reported in a diabetic foot ulcer out of which 51.2% were gram negative bacilli isolates . (**Fatemah sadeghpour et al., 2019**)³⁷ .

The prevention and cure of a diabetic foot ulcer is essential as it leads to many irreversible conditions like amputation of a lower extremity whereas the lifetime risk of a diabetic patient to develop a DFU is 25%(**Ambrose Atosona et al ., 2019**)¹⁰ and poses major threat to the life and lifestyle of patients . The risk of amputation in DFU is a matter of concern for the healthcare sector and the individual. A multidisciplinary approach is needed to treat a diabetic foot ulcer (**Sararogi et al .,2008**)³⁹ in Diabetic foot ulcer, assessment and management. Early detection, management and treatment will reduce the chances of amputation, which is the need of the hour DFU. A quick and effective treatment of a diabetic foot ulcer is the need of the hour. An empirical and judicious use of antibiotics for therapy and treatment of diabetic foot ulcer is necessary so as to avoid over use of antibiotics as the number of multidrug resistant organisms affecting foot ulcer is on rise. Hence this study was taken up to identify the genera of bacteria associated with diabetic foot ulcer and to see their antibiotic susceptibility pattern.

AIM AND OBJECTIVES

1. To determine the genera of microorganisms found in diabetic foot ulcer.
2. To determine the antibiotic susceptibility pattern of organisms found in diabetic foot ulcer.

MATERIALS AND METHODS

PLACE OF STUDY: Central Laboratory of Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai.

TYPE OF STUDY: The study was carried out in a prospective manner.

PERIOD OF STUDY: Six months (August 2019 to January 2020)

SAMPLE SIZE: As it was a time bound study, all DFU isolates were included.

STUDY PARTICIPATION CRITERIA:

Inclusion criteria:

All isolates from the pus samples of diabetic foot ulcer were included in the study.

Exclusion criteria:

Isolates of non diabetic patients with foot ulcer and infections are excluded.

INSTITUTIONAL ETHICS COMMITTEE: CSP/ 19/ MAY/ 77/ 155

SAMPLE

A total of 138 patients out of 193 were culture positive for DFU were included in the study. The details of the patient was recorded and a brief clinical history regarding the isolates was obtained from the laboratory information system. Details like age, gender, fasting and post prandial values and Hb A1c values were recorded. The isolates were collected and stored appropriately. The samples were stored in nutrient agar butts containing 1.5% nutrient agar and 6 ml of sterile glycerol and stored at -20 °C.

RESULTS

A total of 193 patients with DFU were included in the study, out of which 138 (72%) were culture positive. (Figure 1)

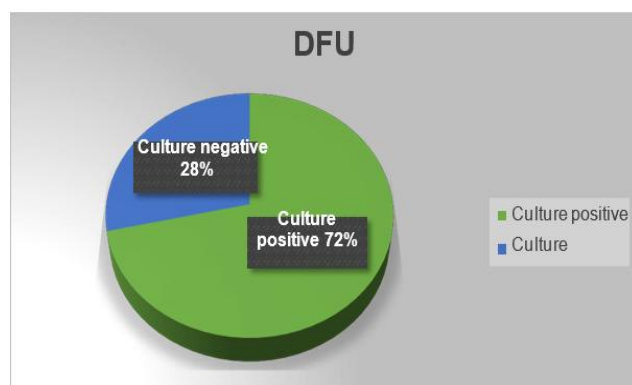


Figure- 1: Percentage of infected DFU

Hence a total of 138 patients were enrolled in this study. Among the 138 patients, the ratio of right to left limb DFU was found to be 67% and 33% respectively. (Figure-2)

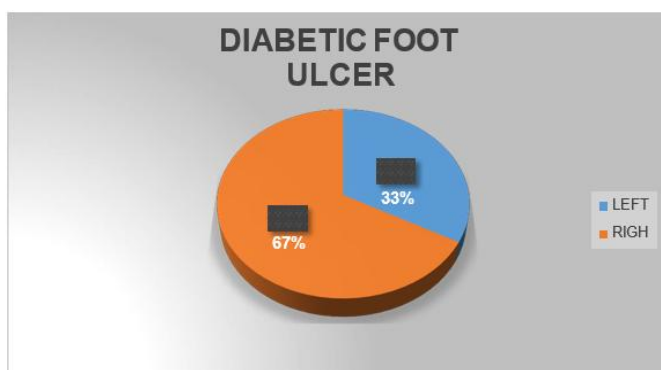


Figure- 2: Ratio of left and right diabetic foot ulcer

Among the 138 patients, 92 (67%) were male and 46 (33%) were female. The age of patients ranged from 30 -80 years. A majority of patients (31%) belonged to the age group of 41 -50 years. Of these 27 were males and 10 were females. (Table 1) (Figure 3)

Table- 1 Correlation of age and DFU

AGE	No of patients	Percentage
31- 40	9	7%
41- 50	43	31%
51- 60	41	30%
61- 70	28	20%
71- 80	17	12%

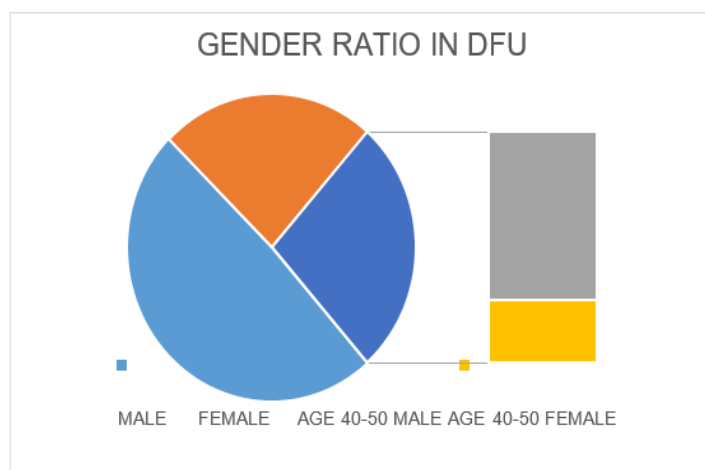


Figure- 3: Gender ratio in DFU with highest age group

Out of 138 patients, 223 isolates were obtained, out of which Gram positive cocci accounted for 42.6% and gram negative bacilli accounted for 57.4%. (Figure 4)

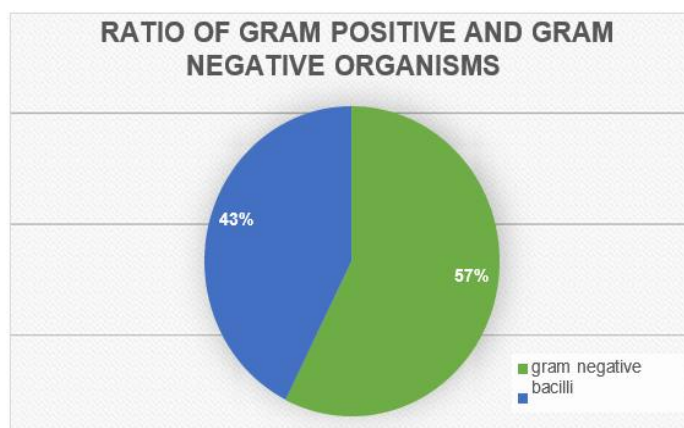


Figure- 4: Ratio of gram positive and gram negative organisms

Various species of microorganisms were isolated from the diabetic foot ulcer, which indicates the risk of infection in a diabetic foot ulcer.

From the gram positive organisms the most predominant organism was *Enterococcus faecalis* and from the gram negative bacilli the most commonest organism was *Escherichia coli*. (Table- 2) (Figure- 5)

Table- 2: Percentage of microorganisms (n= 223)

Organism	No of isolates	Percentage
Acinetobacter species	9	4%
Citobacter species	5	2%
Escherichia coli	37	17%
Enterobacter species	9	4%
Enterococcus faecalis	47	22%
Enterococcus faecium	3	2%
Klebsiella pneumoniae	25	11%
Morganella species	7	3%
Proteus species	13	6%
Providencia species	3	1%
Pseudomonas species	18	8%
Staphylococcus species	39	17%
Streptococcus species	7	3%

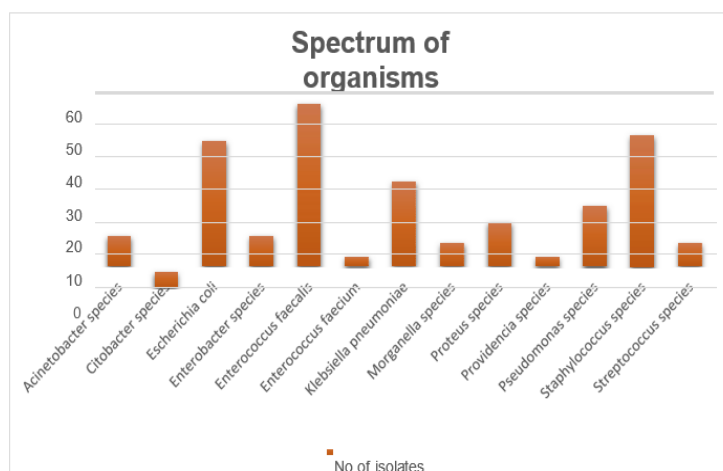


Figure- 5: Spectrum of organisms

In the DFU of the patients, 51% had more than one pathogen, hence of polymicrobial origin and 49% had monomicrobial infection.

Among the polymicrobial community, *Enterococcus faecalis* (50%) was found to be the most predominant organism and *Escherichia coli* (30 %) as the highest gram negative bacilli.

Among the monomicrobial community, *Staphylococcus aureus* (29. 5%) was found to be the highest among the gram positive organisms and *Escherichia coli* (13. 2%) to be the highest among gram negative bacilli .(Figure 6)

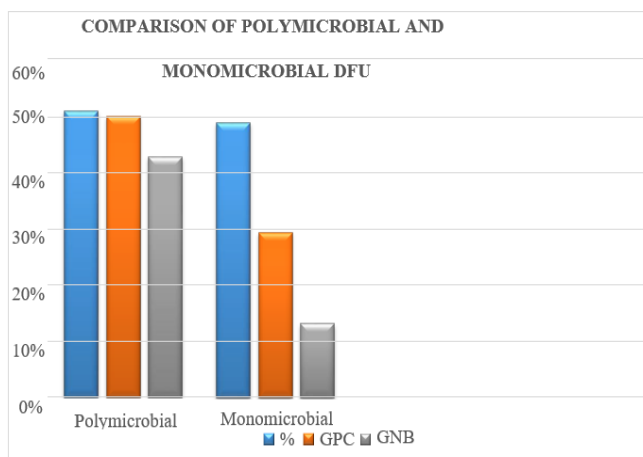


Figure- 6: Comparison and polymicrobial and monomicrobial microorganisms

Escherichia coli and *Klebsiella pneumoniae* were having the highest Hb A1c range among all the microorganisms.

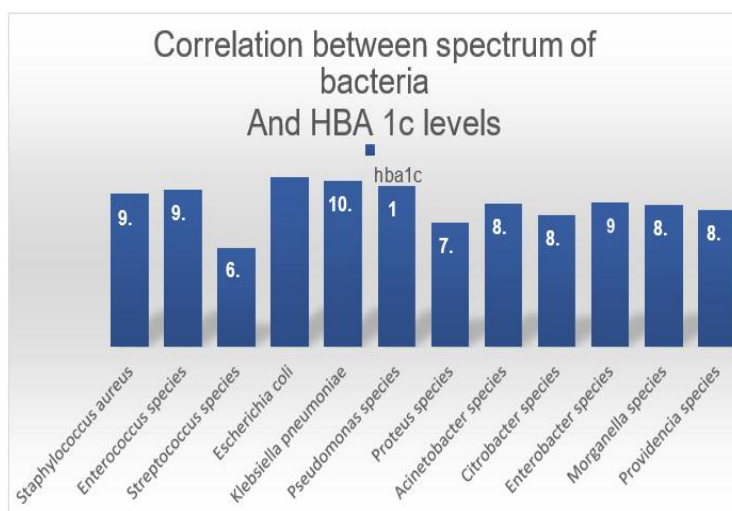


Figure- 7: Correlation between spectrum of bacteria and Hba1c levels

Group of patients with uncontrolled and controlled diabetes were observed along with correlation of Hb A1c levels. The range of Hb A1c was from 3 -18. The patients had the highest range of 11 -12. Hence a majority of patients belonged to uncontrolled diabetes group.

The highest number of people had a Hb A1c level of more than 8. (Table-3)

Table- 3: Range of HbA1c

HbA1c level	No of patients (n=136)
6 -7	20
7 -8	24
More than 8	92

(Data for 2 patients not available)

Keeping the Hb A1c range as 6 -7, 10 patients (14. 7%) and range 7 -8, 11 patients (16. 3%) had **monomicrobial** infection. 47 patients, (69%) had hba1c levels more than 8, having uncontrolled diabetes. A total of 68 (49 %) patients had monomicrobial infection.

Keeping the hba1c range as 6 -7, 13 patients (18. 5%), and range 7 -8, 13 patients (18. 5%) had **polymicrobial** infection. 42 patients (60%) had Hb A1c levels more than 8. A total of 70 (51%) has polymicrobial infections. (Table- 4).

Table- 4: Correlation between HbA1c levels and type of microbial community

Hb A1c level	Monomicrobial (n= 68) (49%)	Polymicrobial (n= 70) (51%)
6 -7	10 (14. 7%)	13 (20%)
7 -8	11 (16. 3%)	13 (20%)
More than 8	47 (69%)	42 (60%)

(Data for two patients not available)

Table- 5: Antibiotic susceptibility pattern for gram positive cocci

ANTIBIOTIC SUSCEPTIBILITY PATTERN FOR GRAM POSITIVE COCCI (n=98)						
Antibiotics	<i>Staphylococcus species</i> (n=39)		<i>Streptococcus species</i> (n=7)		<i>Enterococcus species</i> (n=51)	
	R	S	R	S	R	S
Ampicillin	26(66%)	13(33%)	-	7(100%)	18(35.2%)	33(64.7%)
Cefoxitin	12(31%)	27(69%)	2(28.5%)	5(71.4%)	51(100%)	-
Cefotaxime	13(33%)	26(66%)	-	7(100%)	-	-
Erythromycin	19(48.7%)	20(51.2%)	2(28.5%)	5(71.4%)	32(62.7%)	19(37.3%)
Gentamicin	12(30.7%)	27(69.2%)	7(100%)	-	19(37.3%)	32(62.7%)
Ciprofloxacin	22(56.4%)	17(43.5%)	-	7(100%)	23(45.1%)	28(54.9%)
Clindamycin	12(30.7%)	29(74.3%)	-	-	-	-
Vancomycin	-	39(100%)	-	7(100%)	-	51(100%)
Linezolid	-	39(100%)	-	7(100%)	-	51(100%)

Among the gram positive cocci, *Staphylococcus species* had a prevalence rate of 17% in which drug resistance was seen in 23% (MRSA). The organism showed 66% resistance and 34% sensitivity to β lactams such as ampicillin in overall picture. Among these, 63% showed resistance to Cefoxitin, hence Methicillin Resistant *Staphylococcus Aureus* (MRSA). A majority of MRSA was resistant to Cefotaxime and Ciprofloxacin (100% each) and also sensitive to Erythromycin (66%) and Clindamycin (55%). All the MRSA isolates were susceptible to Vancomycin and Linezolid (100% each), followed by Gentamicin (90%), but 55% resistance to ciprofloxacin. 30% of Coagulase negative *Staphylococcus aureus* were also resistant to Cefoxitin. All isolates of *Staphylococcus species* were 100% sensitive to Vancomycin and Linezolid. (Table 6).

Table- 6: Comparison of AST between *Staphylococcus species*

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCUS SPECIES (n= 39)						
Organisms	MRSA (n= 9)		MSSA (n= 20)		CONS (n= 10)	
	Resistance	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Ampicillin	9(100%)	-	10(50%)	10(50%)	7(70%)	3(30%)
Cefoxitin	9(100%)	-	-	20(100%)	3(30%)	7(70%)
Cefotaxime	9(100%)	-	-	20(100%)	4(40%)	6(60%)

Erythromycin	6 (66 . 6 %)	6(33. 4 %)	8(22. 3 %)	12(77 . 7 %)	5 (50 %)	5 (50 %)
Gentamicin	7 (77 %)	2 (23 %)	2 (10 %)	18 (90 %)	3 (30 %)	7 (70 %)
Ciprofloxacin	9 (100 %)	-	11 (55 %)	8 (45 %)	2 (20 %)	8 (80 %)
Clindamycin	5 (55 . 6 %)	4(44. 4 %)	3 (15 %)	17 (85 %)	4 (40 %)	6 (60 %)
Vancomycin	-	9 (100 %)	-	20(100 %)	-	10(100%)
Linezolid	-	9 (100 %)	-	20(100 %)	-	10(100 %)

Enterococcus species showed Erythromycin was 64% resistant in Enterococcus faecalis but 100 % resistant in Enterococcus faecium. High level Gentamicin was 97% sensitive in Enterococcus faecalis and Enterococcus faecium was more resistant to Cefotaxime, Erythromycin and Ciprofloxacin than Enterococcus faecalis. Vancomycin and Linezolid were 100 % sensitive in all isolates. (Table 7).

Table- 7: AST for Enterococcus species

ANTIBIOTIC SUSCEPTIBILITY FOR ENTEROCOCCUS SPECIES (n=51)				
Antibiotics	Enterococcus faecalis (n= 48)		Enterococcus faecium (n= 3)	
	R	S	R	S
Ampicillin	19(42. 6%)	33(60. 4%)	1(33. 4%)	2(66. 6%)
Cefotaxime	-	-	-	-
Erythromycin	30 (62. 5%)	18 (37. 5%)	-	-
High level gentamicin	3 (7%)	45 (97%)	-	3 (100%)
Ciprofloxacin	21 (43. 7%)	27(56. 2%)	2 (66. 6%)	1 (33. 4%)
Vancomycin	-	48 (100%)	-	3 (100%)
Linezolid	-	48 (100%)	-	3 (100%)

Escherichia coli was found more predominantly resistant to Cephalosporins followed by Quinolones like Ciprofloxacin. The organism showed high sensitivity to Carbapenams like Imipenem and Meropenem (97%) and a few isolates who were sensitive to Levofloxacin and Tobramycin were resistant to most of the drugs. (Table 8)

Table- 8: AST of Escherichia coli (n=37)

Antibiotics	R	S
Cefotaxime	29(78. 3%)	8(21. 6%)
Amikacin	-	37(100%)
Cefaperazone sulbactam	1 (2. 7%)	36(97. 2%)
Piperacillin	1(2. 7%)	36(97. 2%)

Tazobactam		
Ceftazidime	29(78. 3%)	8(21. 6%)
Ciprofloxacin	25(64. 1%)	12(35. 9%)
Cefepime	4(57. 1%)	3(42. 8%)
Imipenem	1(2. 5%)	36(97. 4%)
Meropenem	1(2. 5%)	36(97. 4%)
Polymyxin B	-	37(100%)
Levofloxacin	4(50%)	4(50%)
Tobramycin	-	8(100%)
Ertapenem	1(25%)	3(75%)
Cefotaxime	29(78. 3%)	8(21. 6%)

Klebsiella pneumoniae (more than 50%) was resistant to a few Cephalosporins but 100% sensitive to Cefepime. The organism was also resistant to Quinolones like Ciprofloxacin and Levofloxacin. *Proteus* species was 100% sensitive to Carbapenems and β lactamase inhibitors. *Proteus* species was 75% sensitive to Polymyxin B. *Providencia* species were sensitive to β lactam inhibitors upto a 100%. *Morganella* species was highly sensitive to Aminoglycosides and β lactamase inhibitors. (Table 9)

Table- 9: AST of gram negative bacilli

Antibiotics	<i>Klebsiella pneumoniae</i> (n= 25)		<i>Proteus species</i> (n= 13)		<i>Providencia species</i> (n= 3)		<i>Morganella species</i> (n= 7)	
	R	S	R	S	R	S	R	S
Cefotaxime	17(68 %)	8(32 %)	-	13(100 %)	3(100 %)	-	4(57. 1 %)	3(42. 8 %)
Amikacin	-	-	2(15. 3 %)	11(84.6 %)	2(66. 6 %)	1(33. 4 %)	1(14 . 2 %)	6(85. 7 %)
Cefaperazone sulbactam	10(40 %)	15(60 %)	-	13(100 %)	-	3(100 %)	1(14 . 2 %)	6(85.7 %)
Piperacillin Tazobactam	11(44 %)	14(56 %)	-	13(100 %)	-	3(100 %)	-	7(100 %)
Ceftazidime	17(68 %)	8(32 %)	-	13(100 %)	3(100 %)	-	4(57 . 1 %)	3(42. 8 %)
Ciprofloxacin	16(64 %)	9(36 %)	6(46. 1 %)	7(53. 8 %)	-	-	5(71. 4 %)	2(28. 5 %)
Cefepime	-	8(100 %)	1(50 %)	1(50 %)	-	-	1(100 %)	-
Imipenem	5(20 %)	20(80 %)	-	13(100 %)	-	-	2(28. 5 %)	5(71. 4 %)
Meropenem	6(24 %)	19(76 %)	-	7(100 %)	-	-	1(50 %)	1(50 %)
Polymyxin B	-	25(100 %)	-	-	-	-	-	-
Levofloxacin	11(84.6 %)	2(15.2 %)	2(50 %)	2(50 %)	-	-	-	2(100 %)

xacin	(%)							(%)
Tobramycin	9(36 %)	4(64 %)	-	3(100 %)	-	-	-	-
Ertapenem	1 (25 %)	3 (75 %)	-	-	-	-	-	-

Acinetobacter species was completely sensitive to Polymyxin B and also to Cefepime, Imipenem and Meropenem. It was 66% resistant to Aminoglycosides. Pseudomonas was sensitive to Carbapenams and Aminoglycosides, but 100% resistant to cefepime. Enterobacter species was 100% sensitive to β lactam inhibitors and Aminoglycosides. Citrobacter species was 100% sensitive to Carbapenams, Polymyxin B and Aminoglycosides and 60% resistant to Cephalosporins. (Table 10)

Table 10: Antibiotic susceptibility pattern of gram- negative bacilli (n=128)

Antibiotics	<i>Acinetobacter species (n=9)</i>		<i>Pseudomonas species (n=18)</i>		<i>Enterobacter species (n=9)</i>		<i>Citrobacter species (n=5)</i>	
	R	S	R	S	R	S	R	S
Ampicillin	-	-	-	-	9 (100 %)	-	5(100 %)	-
Cephalexin	-	-	-	-	9 (100 %)	-	5(100 %)	-
Cefotaxime	9(100 %)	-	-	-	5 (55 . 6 %)	4(44 . 4 %)	3 (60 %)	2 (40 %)
Amikacin	6(66 . 6 %)	3(33 . 4 %)	5 (27 . 7 %)	13 (72 . 3 %)	-	9 (100 %)	1 (20 %)	4 (80 %)
Cefazolin sodium	6(66 . 6 %)	3(33 . 4 %)	7 (38 . 8 %)	11 (61 . 2 %)	-	9 (100 %)	1 (20 %)	4 (80 %)
Piperacillin	4(44 . 4 %)	3(33 . 4 %)	7 (38 . 8 %)	11 (61 . 2 %)	-	9 (100 %)	1 (20 %)	4 (80 %)
Tazobactam	4(44 . 4 %)	3(33 . 4 %)	7 (38 . 8 %)	11 (61 . 2 %)	-	9 (100 %)	1 (20 %)	4 (80 %)
Ceftazidime	6(66 . 6 %)	3(33 . 4 %)	9 (50 %)	9 (50 %)	5 (55 . 5 %)	4(44 . 5 %)	3 (60 %)	2 (40 %)
Ciprofloxacin	7(77 . 7 %)	2(22 . 3 %)	10 (55 . 5 %)	8 (44 . 5 %)	2 (22 . 2 %)	7 (77 . 8 %)	2 (40 %)	3 (60 %)
Cefepime	2(22 . 2 %)	7(77 . 8 %)	3 (100 %)	-	-	-	1(33 . 3 %)	2(66 . 7 %)
Imipenem	4(44 . 4 %)	5(55 . 6 %)	4 (36 . 3 %)	7 (63 . 7 %)	-	9 (100 %)	-	5 (100 %)
Meropenem	3(33 . 3 %)	6(66 . 7 %)	4 (36 . 3 %)	7 (63 . 6 %)	-	9 (100 %)	-	5 (100 %)
Polymyxin B	-	9(100 %)	1 (44 . 5 %)	10 (55 . 5 %)	-	9 (100 %)	-	5 (100 %)
Levofloxacin	5(55 . 5 %)	4(44 . 5 %)	6 (100 %)	-	-	9 (100 %)	-	2 (100 %)
Tobramycin	7(77 . 7 %)	2(22 . 3 %)	5 (83 . 3 %)	1 (16 . 7 %)	-	9 (100 %)	1 (50 %)	1 (50 %)
Ertapenem	-	-	-	-	-	-	-	-

The percentage of MDR: Non-MDR is 12.1%.

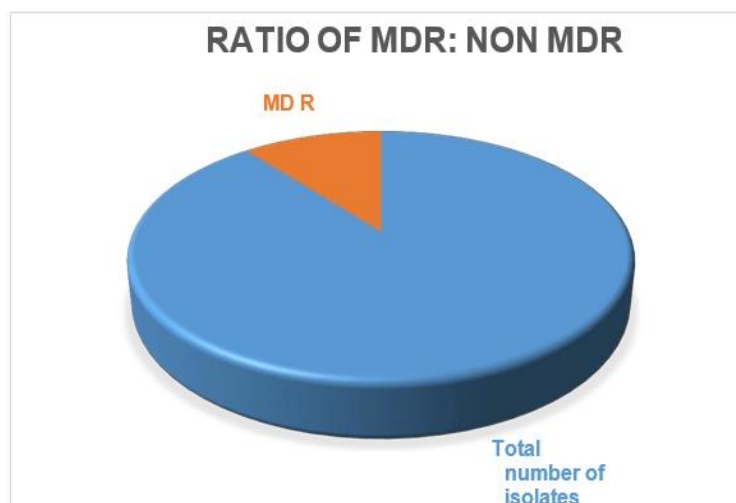


Figure- 8 Ration of MDR: Non MDR isolates

Acinetobacter species had the highest number of multidrug resistant isolates among all the organisms of 44.5%. It was followed by Klebsiella species having 44% drug resistance followed by Escherichia coli.

Table- 11: Number of multidrug resistant organisms in DFU

MULTI DRUG RESISTANT ORGANISMS IN DFU		
Acinetobacter species (n=9)	4	44.5%
Citrobacter species (n=5)	1	20%
Morganella species (n=7)	1	14.2%
Escherichia coli (n=37)	3	8.1%
Klebsiella species (n=25)	11	44%
Pseudomonas species (n=18)	7	38.8%

MDR Acinetobacter and was associated more with Hb A1c levels lesser than 8 in both monomicrobial and polymicrobial infections. MDR Escherichia coli was associated with monomicrobial infection with less than 8 Hb A1c. MDR Klebsiella was having high prevalence in more than 8 Hb A1c in both monomicrobial and polymicrobial infections. MDR Klebsiella was the most predominant organism in both monomicrobial and polymicrobial infections in both <8 and more than 8 Hb A1c levels.

Table 12: Correlati on of HbA1c level with organism

Organism	<8	poly	mono	>8	poly	mono
Acinetobacter species	3	2	2	1	-	-
Escherichia coli	2	-	2	1	1	-
Klebsiella species	5	3	1	6	6	1

With this correlati on we can observe that higher the Hb A1c level, more the chances of an MDR organism in DFU. This also signifies that mi croorgani sm l ike Aci netobact er species have high Multi drug resistance can affect peopl e with high Hb A1c levels and the patient may not respond to treatment. Organism s l ike Kl ebsi el la pneumoni ae and Escheri chi a coli are commonly found in both monomi crobi al and polym i crobi al i nfection of DFU and may aggravat e the ulcer.

DISCUSSION

Diabeti c foot is charact eri zed by numerous pat hol ogical compl i cations espec i all y ul cerations and infecti ons. Most of the diabeti c foot ulcers are managed by aggressive surgical debri dem ent with proper ant i bi oti c cover. If not, dependi ng on the organism this may lead to ost eomyel iti s, gangrene and even the need for am put ation of the limb. So, the i dent ifi cation of the isolate and the choice of em pi ri cal anti bi oti cs is very important and this i nform ati on should be accurat e and dependabl e.

193 diabetic foot ulcer samples were taken for this study out of which 138 were culture positive. Making the percentage of culture positi ve di abeti c foot infect ion to be 72%. Out of this 138 culture positive sample number of isolat es were 223. Thus 51% of cultures were polym i crobi al (70/ 138) and 49% were m onomi crobi al (68/ 138). Though there was higher rate of polym i crobi al growth but it was marginal. Unlike our study **Sekhar SM *et al .*, 2014⁴⁰** report ed equal rate of polym i crobi al and monomi crobi al growth. Other studies such as **Pradeep M. S. S *et al .*, 2017³⁴⁵** showed higher rate of monomi crobi al growth in diabet ic foot infect ions.

Our study shows di abeti c foot ulcer infect ions are more common in males (67%) in comparison to females (33%). This findings are support ed by **Javedh Shareef *et al .*, 2018⁴²** who found diabetic foot ulcer to be higher 63.38% in males when compared to fem ales . This may be due to factors such as the di fference in life styles and professi onal acti viti es and jobs, causing the feet to tolerat e more pressure (**Mojtaba Anvarin ejad *et al .*, 2015⁷**)

This study shows the highest rate of infect ion of diabetic foot in the age group 40 -50 years(31%) closely foll owed by 51 -60 years (30 %). Unlike our study, most other studies had publi shed a higher incidence of infecti ons in patients who are in the range of 51 -60 years (**Mojtaba Anvarin ejad *et al .*, 2015⁷** , **Ekta Bansal *et al .*, 2008¹²** , **Arun Anand *et al .*, 2016⁵**) In the age group 40 -50 years more infecti on were seen in males than in females.

Uncont roll ed or poorly cont rol led diabetes will reduce the effectiveness of immune cells as well as impair m icrovascul ar ci rcul ati on, l imiti ng the access of phagocyti c cells and thus leading to i nfecti ons in diabeti c foot (**Pradeep M. S. S *et al .*, 2017³⁵**). Common method of checking uncont roll ed diabet es is to check the value of Hb A 1c. After goi ng through various art icl es and consul ti ng with physi cians a cut off value of more than 8 was consi dered as un cont rol led diabet es. Out of the 136 patients who had diabeti c foot infecti ons (we were unable to get the Hb A 1c value of 2 pati ent s),

92 had an Hb A1c value more than 8(68%). The rem ai ni ng 44 pati ent s had Hba1C less than 8. Thus our study shows higher rate of diabeti c foot infect ion in those who have uncont rol led diabet es from those who have their diabetes in control. It is well document ed that di abeti c foot infecti on are frequentl y pol ymi crobi al in those who have uncont roll ed diabet es. But

contradictory to such study our study showed more monomicrobial growth in those who had uncontrolled diabetes. Similar findings were seen in a study by (Shajahan *et al.*, 2018)⁶ But unlike other studies, those who had higher Hb A1c they showed more monomicrobial (47/68 - 69%) and in those who had their diabetes in control the growth was polymicrobial. In uncontrolled diabetes the predominant organisms were Gram negative bacilli of which the highest rate of isolation were *Escherichia coli* and *Klebsiella pneumoniae* followed by pseudomonas species (10.5%, 10.3% 10%).

Over the years studies have reported variations in the type of isolates in diabetic foot infections. Organisms such as Gram negative bacilli, Gram positive cocci, fungi and anaerobes have been isolated. Most studies show a predominance of either Gram negative bacilli (Khallifa Al Benwan *et al.*, 2012², Haji Khan Khoharo *et al.*, 2009²⁵ Ekta Bansal *et al.*, 2008¹²

P. S. Gangania *et al.*, 2016³²) or Gram positive cocci (Mojtaba Anvarin ejad *et al.*, 2015⁶ Mohammad Taghi Akhi *et al.*, 2015¹) Our study (which included only aerobes) in Diabetic foot ulcer infections showed Gram negative bacteria 57.3% (128/223) to be more predominant than gram positive bacteria 42.6% (95/223).

Mojtaba Anvarin ejad *et al.*, 2015⁶, in their study on diabetic foot infections, isolated Enterococcus species as the second most frequent microorganism and in their discussion mention that the prevalence of enterococci has emerged as a public health concern. Now five years down the

line, in our study out of the 223 spectra of isolates, *Enterococcus faecalis* was the predominant bacteria (24% n= 51) followed by the Gram negative bacteria *Escherichia coli* (17% n= 37) and *Staphylococcus aureus* (17% n= 39) with equal percentage. We did not find any other study which had similar findings where *E. faecalis* being the predominant isolate but studies such as (P. S. Gangania *et al.*, 2016³² Javedh Shareef *et al.*, 2018⁴²) had a high rate of Enterococcus species isolation.

Antibiogram susceptibility pattern for Gram positive isolates shows *Enterococcus faecalis*, which was our predominant isolate to be 100% susceptible to vancomycin and Linezolid. 36% were susceptible to Erythromycin, 97% susceptible to Gentamycin and 55% susceptible to Ciprofloxacin. Similarly *Staphylococcus* species showed 100% susceptibility to Vancomycin and Linezolid. 11 isolates out of 29 were resistant to cefoxitin. Bringing the MRSA rate to 23.07%. Studies such as done by Ekta Bansal *et al.*, 2008¹² Pradeep M. S. S *et al.*, 2017³⁵ Sarita Otta *et al.*, 2019³⁴ showed a range of MRSA isolation rate to be in the range of 5.7 to 77%. In our study the MRSA isolates showed 100% resistance to ciprofloxacin and Ampicillin. 55% were resistant to Clindamycin and 66% showed resistance to Erythromycin. *Streptococcus* species showed 100% susceptibility to ampicillin, Cefotaxime and Ciprofloxacin.

MDR or Multi drug resistance was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (CDC) In our study out of 223 isolates 27 were multidrug resistant 12.1%. Unlike our study Mojtaba Anvarin ejad *et al.*, 2015⁶ in their study had MDR isolation rate of 91%. Ranjini Chittur Yerat *et al.*, 2015⁴⁷ had an MDR rate of 28.26%. Even though in our study, *Acinetobacter* only had an isolation rate of less than 10% yet it showed the highest rate of multidrug resistance (44.5%). Amikacin susceptibility was only 33.3%, ciprofloxacin susceptibility (22.3), and third generation cefotaxime was 100% resistant. It showed only 55.6% susceptibility to imipenem and 66.7% susceptibility to meropenem In a same study by Ranjini Chittur Yerat *et al.*, 2015⁴⁷ also demonstrated the predominant MDR organism isolated from Diabetic foot infections to be *Acinetobacter* 100%. Multidrug resistance rate in pseudomonas was 38.8%. showing 100% resistance to amikacin and levofloxacin and 38% resistance to both Cefoperazone / Sulbactam and Tazobactam/ Piperacillin. It showed a

resistance rate of 50% to ceftazidime and 36% resistance rate for Imipenem and Meropenem. Out of 27 multidrug resistant organisms 16 were part of polymicrobial growth (59%) and 11 had monomicrobial growth (40%). In these 27 multidrug resistant organisms 15 (56%) had an Hb A1c value of >8. Thus our study indicates that multidrug resistant organisms are more associated with higher Hb A1c values and polymicrobial growth of microorganisms in DFU. Our most common isolate of Gram negative bacteria, *Escherichia coli* had a multidrug resistance rate of 8.1%. In our study we had zero percent isolation of pan drug resistant isolates.

CONCLUSION

1. 193 diabetic foot ulcer samples were taken for this study out of which 138 were culture positive. Bringing the rate of diabetic foot infection in our study to 72%.
2. The total number of isolates in our study were 223.
3. 51% of the cultures were polymicrobial (70/138) and 49% were monomicrobial (68/138).
4. Our study shows diabetic foot ulcer infections are more common in males (67%) in comparison to females (33%).
5. This study shows the highest rate of infection of diabetic foot is in the age group of 40 - 50 years (31%).
6. Our study shows higher rate of diabetic foot infection in those who have uncontrolled diabetes from those who have their diabetes in control.
7. In patients with high Hb A1c the growth was more monomicrobial in nature (47/68) (69%) with the predominant organism being *Escherichia coli*.
8. Our study in Diabetic foot ulcer infections showed Gram negative bacteria 57.3% (128/223) to be more predominant than gram positive bacteria 42.6% (95/223).
9. Of the 223 spectra of isolates, *Enterococcus faecalis* was the predominant bacteria (47/223) 21%.
10. Our study showed an MRSA rate of 23.07%.
11. 12.1% was the overall multidrug resistance rate. Acinetobacter showed the highest rate of multidrug resistance (44.5%).
12. Multidrug resistant isolates had a growth which was more polymicrobial in nature (59%) and they were isolated more from patients who had Hb A1c value of >8 (56%).

To conclude, our study reinforced the fact that diabetic foot infections have a diverse bacterial population. In our study the highest isolation rate was of Gram-negative bacilli but the most common organism isolated was Gram positive cocci, *Enterococcus faecalis*. Thus, stressing the importance of both these groups of organisms in diabetic foot infections uniformly. As it also can be highlighted from our study the importance of the clinical factors such as Hb A1c level, the polymicrobial nature of organism and multidrug resistance of bacteria such as Acinetobacter before choosing an empirical antibiotic for patients with diabetic foot infections.

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