

A STUDY ON MICROBIAL PROFILE AND ANTIBIOGRAM OF DIABETIC FOOT INFECTION CASES ATTENDING A TERTIARY CARE HOSPITAL WITH SPECIAL REFERENCE TO METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS(MRSA), EXTENDED SPECTRUM BETA-LACTAMASES(ESBL) AND METALLO-BETA-LACTAMASE (MBL) SCREENING

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Received : 01/03/2026
Received in revised form : 10/04/2026
Accepted : 29/04/2026

Keywords:

Diabetic foot ulcer, MRSA, Microbial Profile, Antibiogram, Beta-Lactamases, ESBL, MBL.

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DOI: 10.47009/jamp.2026.8.3.121

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (3); 661-668



ABSTRACT

Background: The worldwide prevalence rate of diabetes is increasing with leading foot complications thus raising the burden on patient and society due to poor quality of life and cost of treatment involved. According to an estimate by International Diabetes Federation (IDF), India is first among five countries from South East Asia with 77.0 million number of people with diabetes between (20–79 years), 2019 data. Diabetic foot infection cases may present clinically with various manifestations like paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis to severe lower limb complications like Diabetic microangiopathy, peripheral neuropathy, charcot's arthropathy, nonhealing foot ulcers & recurrent infections leading to lower limb amputations. The relative risk of lower extremity amputation is 15 to 46 times higher in diabetic patients compared to those who are nondiabetic. **Materials and Methods:** This was descriptive study conducted at Sri Venkateshwaraa Medical College and research centre for a period of 3 months, from Jan 2021 to March 2021. This study included Patients attending a Tertiary Care Hospital with diabetic foot ulcers to the Department of General surgery. 50 Diabetic foot ulcer cases attending as both inpatient and outpatient to the department of general surgery, irrespective of age and sex were included. Pus samples were collected from diabetic wound infection cases and samples were subjected to various standard microbiological testing protocols by following clinical laboratory standard guidelines (CLSI). Antimicrobial susceptibility testing done by performing Kirby Bauer disc diffusion technique with special reference to MRSA, ESBL and MBL screening. **Results:** Among 50 cases majority belongs to 40-60 years age group predominantly. Diabetic foot infection was more commonly seen in between 41-70 years age group about 47(94%) cases, of which 33(66%) were males and 14(28%) were females. Male to female ratio was 2.5:1. Out of 50 cases of diabetic foot ulcer studied, (45/50) 90% positive and (5/50) 10% were culture negative. Out of 45 culture positive samples a total of 60 isolates were obtained, Mono microbial flora was seen in 34 (56.6%) of cases, Polymicrobial flora in 26 (43.3%) of cases, two organisms isolated each in 14 cases and 3 organisms isolated in 4 cases with 12 isolates. Mono microbial flora 34(56.6%) slightly higher than poly microbial flora 26(43.3%) in this study. Out of 60 organisms isolated, commonest organism among gram negative isolates was *Pseudomonas* species 16 (27%) followed by *Klebsiella* species 15(25%), *Escherichia coli* 8 (13.3%), *Citrobacter* 2(3.3%) and *Proteus mirabilis* 5 (8.3%). The present study shows there is predominance of Gram negative organisms with *Pseudomonas* species followed by *Klebsiella* species and *Staphylococcus aureus* among gram positive organisms. Out of 14 isolates, 14 showing (100%) sensitivity to Vancomycin and Linezolid, 78.5% sensitivity to Amikacin, 71% sensitivity to Doxycycline and Ciprofloxacin. 57.1% sensitivity to Erythromycin and Clindamycin. Most of the Gram positive

isolates are highly susceptible to Vancomycin, Linezolid and Amikacin with least sensitivity to Pencillin and Amoxicillin + Clavulanic acid. Present study shows gram positive isolates can be effectively treated with doxycycline, amikacin, Linezolid and Vancomycin. Less effective to antibiotics like Pencillin and Amoxycylav. **Conclusion:** The present study on diabetic foot infection cases there is predominance of Gram negative organisms with monomicrobial flora slightly higher than polymicrobial flora. *Pseudomonas* species isolated in common followed by *Klebsiella* species among gram negative organisms and *Staphylococcus aureus* predominant among gram positive organisms. This study shows higher prevalence rate of multidrug resistant agents like ESBL, MBL and MRSA. Antibiotic sensitivity pattern shows overall coverage of amikacin for gram negative organisms and few gram positive organisms will be effective with Piperacillin tazobactam, imipenem coverage for ESBL and MBL strains. Doxycycline found to be effective for most of the gram positive isolates with Vancomycin or linezolid for MRSA strains.

INTRODUCTION

The worldwide prevalence rate of diabetes is increasing with leading foot complications thus raising the burden on patient and society due to poor quality of life and cost of treatment involved. According to an estimate by International Diabetes Federation (IDF), India first among five countries from South East Asia with 77.0 million number of people with diabetes between (20–79 years), 2019 data. In India diabetes prevalence rate in adults between 20-79 yrs is 8.9%, Cost per person with diabetes in USD is 91.6, 1 in 5 of the people with diabetes in the world come from South-East Asia. 1 in 6 adults with diabetes in the world come from India.^[1] In India prevalence rate of diabetes comparatively in rural area is 2.4% and 12–17% in urban population.^[2] Prevalence rate of foot ulcers in diabetes patients is 3% in India, which is lower when compared to western population.^[3] Diabetic foot infection cases may present clinically with various manifestations like paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis to severe lower limb complications like peripheral neuropathy, charcot's arthropathy, nonhealing foot ulcers & recurrent infections leading lower limb amputations.^[4] The relative risk of lower extremity amputation is 15 to 46 times higher in diabetic patients compared to those who are not diabetic.^[5,6] Most frequent reason for hospitalization in diabetes patients is due to foot complications. It is estimated that diabetes accounts for more than 50% of amputation,^[2,7] of which 85% of lower amputation in diabetes patients are preceded by foot ulcers.^[8] According to few previous studies done states that among gram positive isolates *Staphylococcus* species were the commonest organism which accounts for 18.8% of infection followed by the Gram-negative *Pseudomonas* species which constitute 18.2%. Overall Gram positive aerobic bacteria was found to be the predominant organisms involved in diabetic foot infections.^[9,10] Various studies done globally states that microbiological profile of pathogenic organisms involved in diabetic foot cases varies. Proper management of diabetic foot would need along with

clinical diagnosis an appropriate selection of antimicrobial agents followed by culture and sensitivity is essential for prevention of diabetic foot complications and emerging antibiotic resistance.

Aims and Objectives

- To study the bacteriological profile of organisms involved in diabetic foot infections
- To assess the current trend of antibiotic resistance pattern involved.
- Special screening for Methicillin resistant staphylococcus aureus, Extended spectrum betalactamases and Metallobetalactamases.

MATERIALS AND METHODS

Type of Study- A prospective study

Total Sample- 50 (sample size calculation was done by the formula- $4pq/d^2$, where

p= prevalence, q= 1-p, d= precision)

Study Duration- Three months (JANUARY 2021 to MARCH 2021)

Study Population: Patients Attending a Tertiary Care Hospital with diabetic foot ulcers to the Department of General surgery

Study site- Department of Microbiology and Central Research Laboratory, Sri Venkateshwara Medical College Hospital and Research Centre, Ariyur, Puducherry

Inclusion Criteria: Diabetic foot ulcer cases attending as both inpatient and outpatient to the department of general surgery, irrespective of age and sex were included

Exclusion Criteria: Non diabetic patients with foot ulcers are excluded from this study

Ethical consideration: The study was started after getting consent from the Scientific Research Committee and from the Institutional Ethics Committee of the Institution. (Ref:10/SVMCH/IEC/0820)

Methodology

Pus samples were collected from diabetic wound infection cases and samples were subjected to various standard microbiological testing protocols by following clinical laboratory standard guidelines (CLSI).^[13]

Two pus swabs were collected from each case, among them one subjected to direct gram's stain other to culture on plates, Nutrient agar, Blood agar and Mac Conkey agar incubated for 18-24 hrs at 37° C. After 24 hours of incubation, identification of bacteria was done by studying morphology of colony, gram stain, motility, Catalase and Oxidase tests. Single colony was taken and subjected to a battery of tests along with appropriate ATCC controls. The isolates were confirmed and speciated by adapting the standard biochemical procedures.

Antibiotic susceptibility testing

Performed by the Kirby bauer disc diffusion method on Mueller Hinton agar (Himedia) according to CLSI guidelines. The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Media and discs were tested for quality control using standard strains.

The following standard strains were used as referral strains

- Staphylococcus aureus- ATCC 25923
- Escherichia coli- ATCC 25922
- Pseudomonas aeruginosa- ATCC 27853

Antimicrobial susceptibility testing done by performing Kirby Bauer disc diffusion technique with special reference to MRSA, ESBL and MBL screening.

For ESBL detection, Two discs Ceftazidime and Ceftazidime in combination with clavulanic acid were placed. A diameter of >5mm increase in zone size for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.

Metallo β lactamase detection in gram negative bacteria isolates was done by combined disk diffusion test, using two imipenem (10µg) disks and 0.5 M 10 µL EDTA solution. If the zone of inhibition of Imipenem + EDTA disk compared to imipenem alone is >7mm, then the test organism is considered as MBL producing.

Detection of Methicillin resistance in staphylococcus aureus done by:

Cefoxitin Disc Diffusion Test: Diameters of <21mm= Resistant to oxacillin(MRSA)
>22mm = Susceptible to oxacillin (MSSA).

The plate is incubated at 37° C for 16-18 hours.

For Gram positive organisms,

Antimicrobial disc like Amoxycillin, Ampicillin, Amikacin, Erythromycin, Gentamicin, Doxycycline, Cotrimoxazole, Ciprofloxacin, Cephalexin, Ceftriaxone, Cefotaxime, Cefuroxime, Cefoxitin, Vancomycin and Linezolid.

For Gram negative organisms- Antibiotic disc like Ampicillin, Amikacin, Gentamicin, Doxycycline, Cotrimoxazole, Ciprofloxacin, Cephalexin, Ceftriaxone, Cefotaxime, Cefuroxime, Ceftazidime, Imipenem, Ceftazidime +Clavulanic acid, piperacillin + tazobactam and cefaperazone + salbactam

Zone size interpretation as per CLSI guidelines¹³

For all gram negative isolates testing for ESBL & MBL detection and for Staphylococcus aureus isolates MRSA detection to be carried out.

Statistical Analysis

Data entered will be in EXCEL and analysis will be done using statistical package SPSS version 23. Categorical variable will be expressed as proportions and continuous variables will be expressed in mean and standard deviation. The statistical probability of P<0.05 will be considered to be significant.

RESULTS

A total of 50 diabetic foot infection cases attending the surgery department as both in patient or outpatient were included for the study. Among 50 cases majority belongs to age group between 40-60 years age group predominantly, more common in males compared to females.

Table 1: Showing age and sex distribution(n=50)

Age group (in years)	Males (%)	Females (%)	Total (%)
0-10	0(0)	0(0)	0(0)
11-20	0(0)	0(0)	0(0)
21-30	0(0)	0(0)	0(0)
31-40	1(2.7)	0(0)	1(2)
41-50	10(27.7)	4(28)	14(28)
51-60	11(30.5)	7(50)	18(36)
61-70	12(33.3)	3(21)	15(30)
71 and above	2(5.5)	0(0)	2(4)
Total(%)	36(100)	14(100)	50(100)

Out of 50 cases studied, 36(72%) were males and 14(28%) were females. Among them only 1 case of age group 31-40 years, of which 1 male (2.7%) and no females affected. 14 cases (28) were of age group 41-50 years of which 10 (27.7%) males and 4(28%) females. 18(36%) cases of age group 51-60 years, of which 11(30.5%) males and 7(50%) females. 15(30) cases of age group 61-70 years of which 12(33.3%)

males and 3(21%) females. 2 cases of age group 71 and above. No cases for age groups between 0-30 years respectively.

Diabetic foot infection was more commonly seen in between 41-70 years age group about 47(94%) cases, of which 33(66%) were males and 14(28%) were females (Table-1). Male to female ratio of 2.5:1 showing male predominance. Mean age for males

56.1 ± 5.45 and for females 54.3 ±2.66 and overall mean 55.

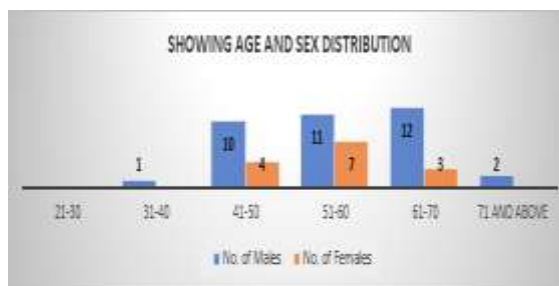


Figure 1: Showing age and sex distribution among all isolates

Table 2: Showing culture reports of the isolates (n=50)

Culture report	No of swabs studied	Percentage
Culture positive	45	90%
Culture negative	5	10%
Total	50	100%

Out of 50 cases of diabetic foot ulcer studied, (45/50) 90% positive and (5/50) 10% were culture negative (Table-2) & Figure -2.

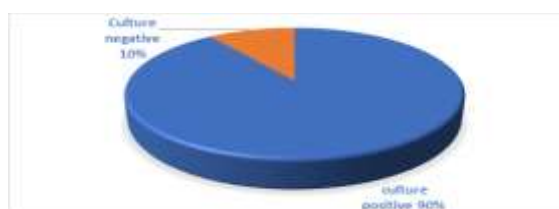


Figure 2: Culture positivity rate of diabetic foot infection cases

Table 3: Showing Details of Microbial Flora Isolated

No of Microbial flora	Total No of isolates	Percentage
Monomicrobial flora	34	56.6%
Polymicrobial with 2 organisms	14	23.3%
Polymicrobial with 3 organisms	12	20.1%
Total	60	100

Out of 45 culture positive samples a total of 60 isolates were obtained, Mono microbial flora was seen in 34 (56.6%) of cases, Polymicrobial flora in 26 (43.3%) of cases, two organisms isolated each in 14 cases and 3 organisms isolated in 4 cases with 12 isolates. Mono microbial flora 34(56.6%) slightly higher than poly microbial flora 26(43.3%) in this study. (Figure -3). [Table-3]

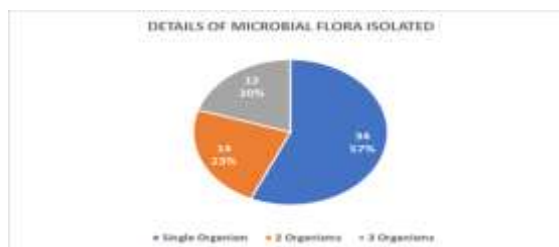


Figure 3: Showing The Details of Microbial Flora Isolated

Table 4: Showing various organisms isolated

Organisms	No. of organisms	Percentage (%)
<i>Pseudomonas</i> species	16	27%
<i>Klebsiella</i> species	15	25%
<i>Escherichia coli</i>	8	13.3%
<i>Proteus mirabilis</i>	5	8.3%
<i>Citrobacter</i>	2	3.3%
<i>Staphylococcus aureus</i>	11	18.3%
<i>Staphylococcus epidermidis</i>	2	3.3%
<i>Streptococci pyogenes</i>	1	1.5%
Total	60	100%

Out of 60 organisms isolated, commonest organism among gram negative isolates was *Pseudomonas* species 16 (27%) followed by *Klebsiella* species 15(25%), *Escherichia coli* 8 (13.3%), *Citrobacter* 2(3.3%) and *Proteus mirabilis* 5 (8.3%). (Figure -4) Among gram positive organisms *Staphylococcus aureus* 11 (18.3%) predominant, followed by *S. epidermidis* 2(3.3%) and *Streptococci pyogenes*

(1.5%) (Table – 4). The present study shows there is predominance of gram negative organisms with *Pseudomonas* species followed by *Klebsiella* species and *Staphylococcus aureus* among gram positive organisms. [Table-4] (Figure-4)

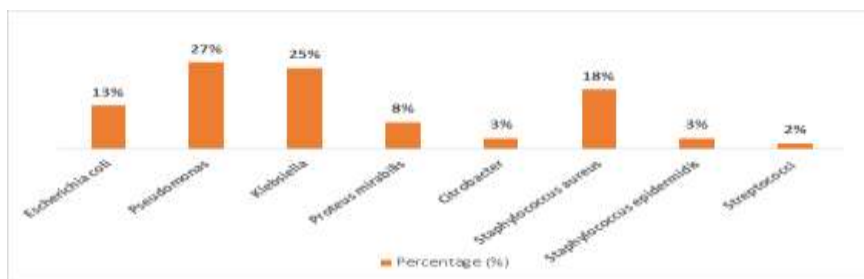


Figure 4: Showing The Percentage of Organisms Isolated

Table 5: Showing Antibiotic Susceptibility Pattern of Gram Positive Organisms Isolated

Organisms	No of isolates	ANTIBIOTICS								
		PEN (%)	ERY (%)	CD (%)	DOX (%)	VA (%)	LZ (%)	AMC (%)	AK (%)	CIP (%)
S. aureus	11	3 (27.2)	5 (45.5)	5 (45.5)	8 (72.7)	11 (100)	11 (100)	4 (36.6)	8 (72.2)	8 (72.7)
S. epidermidis	2	1 (50)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)	2 (100)	1 (50)
Streptococci	1	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)
Total	14	4 (28.5)	8 (57.1)	8 (57.1)	10 (71)	14 (100)	14 (100)	5 (35.7)	11 (78.5)	10 (71)

(PEN- Pencillin, ERY- Erythromycin, CD- Clindamycin, DOX- Doxycycline, VA- Vancomycin, LZ- Linezolid, AMC- Amoxyclav, AK- Amikacin, CIP- Ciprofloxacin)

The above table shows the antibiotic sensitivity pattern of gram positive organisms isolated from diabetic foot infection cases. Out of 14 isolates, 14 showing (100%) sensitivity to Vancomycin and Linezolid, 78.5% sensitivity to Amikacin, 71% sensitivity to Doxycycline and Ciprofloxacin. 57.1% sensitivity to Erythromycin and Clindamycin. Most

of the gram positive isolates are highly susceptible to Vancomycin, Linezolid and Amikacin with least sensitivity to Pencillin and Amoxicillin + Clavulanic acid (Table- 5). Present study shows gram positive isolates can be effectively treated with doxycycline, amikacin, Linezolid and Vancomycin. Less effective to antibiotics like Pencillin and Amoxycav.

Table 6: Showing Antibiotic Susceptibility Pattern of Gram Negative Organisms

Organisms	No of isolates	ANTIBIOTICS								
		Amp (%)	AK (%)	Gen (%)	CIP (%)	CTX (%)	CZM (%)	IPM (%)	COT (%)	PIT (%)
Pseudomonas species	16	4 (25)	11 (68.7)	7 (43.7)	2 (12.5)	5 (31.2)	7 (43.7)	11 (68.7)	7 (68.7)	14 (87.5)
Klebsiella species	15	2 (13.3)	9 (60%)	4 (27)	5 (33)	8 (53)	7 (46.6)	11 (73.3)	4 (26.6)	15 (27)
Escherichia. Coli	8	1 (0)	6 (75%)	2 (25)	3 (37.5)	4 (50)	6 (75)	7 (87.5)	5 (62.5)	8 (100)
Proteus mirabilis	5	1 (0)	4 (80%)	4 (80)	1 (20)	2 (40)	3 (60)	4 (80)	3 (60)	5 (100)
Citrobacter	2	1 (0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Total	46	9 (19.5)	32 (69.5)	19 (41)	12 (26.0)	21 (46)	25 (54)	35 (76)	21 (46)	44 (95.6)

(Amp-Ampicillin, AK- Amikacin, Gen- Gentamycin, CIP- Ciprofloxacin, CTX- Cefotaxime, CZM- Ceftazidime, IPM- Imipenem, COT- Co trimaxozole, PIT- Piperacillin and Tazobactam)

The above table shows the antibiotic sensitivity pattern of gram negative organisms isolated from diabetic foot infection cases. Out of 46 gram negative isolates, most of the isolates are highly sensitive to Piperacillin + Tazobactam (95.6%) followed by Imipenem (76%) and Amikacin (69.5%). Less

sensitivity rate to Ampicillin (19.5%) and Ciprofloxacin (26.0%) (Table- 6). This study shows most of the gram negative isolates highly susceptible to Piperacillin + Tazobactam followed by Imipenem and Amikacin and least susceptible to Ampicillin and Ciprofloxacin.

Table 7: Showing Methicillin Resistant Staphylococcus aureus (MRSA) Isolated

Type of organism	Frequency (%)
Methicillin sensitive staphylococcus aureus (MSSA)	5 (45.5%)
Methicillin resistant staphylococcus aureus (MRSA)	6 (54.5%)
Total (%)	11 (100%)

Out of 11 isolates of Staphylococcus aureus, 6 were Methicillin Resistant Staphylococcus aureus and 5 were Methicillin Sensitive staphylococcus aureus. The percentage of MRSA (54.5%) > MSSA (45.5%)

comparatively Figure-5 (Table -7). This shows predominance of MRSA isolates.

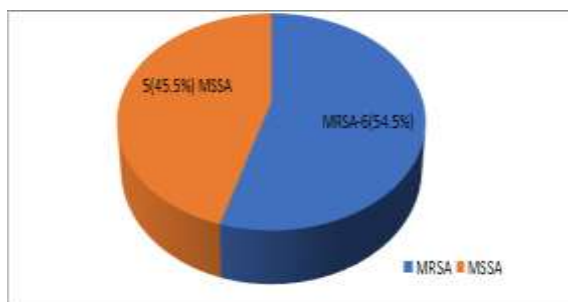


Figure 5: Distribution frequency of MRSA Isolated

Table 8: Showing Extended Spectrum β Lactamases (ESBL)

Organisms	No of isolates	ESBL producers
<i>Escherichia coli</i>	8	5 (62.5%)
<i>Klebsiella species</i>	15	7 (46.6%)
<i>Proteus mirabilis</i>	5	2 (40%)
<i>Pseudomonas species</i>	16	6 (37.5%)
<i>Citrobacter</i>	1	0 (0)
TOTAL (%)	46	20 (43.4%)

The prevalence of ESBL producers among total gram negative bacteria isolated is 43.4%, predominantly with *Escherichia coli* 5(62.5%), followed by

Klebsiella species 7(46.6%) and *Proteus* 2(40%) (Table-8)

Table 9: Showing Metallo β Lactamases (MBL) producers

Organisms	No of isolates	MBL producers
<i>Pseudomonas species</i>	16	5(31.25%)
<i>Klebsiella species</i>	15	4(26.6%)
<i>Escherichia coli</i>	8	2(25%)
<i>Proteus mirabilis</i>	5	1(20%)
<i>Citrobacter</i>	1	0(0%)
TOTAL(%)	46	12(26%)

The overall percentage of MBL producers among gram negative bacteria isolated were 12(26%). Predominantly with 5(31.2%) of *Pseudomonas aeruginosa* and 4(26.6%) for *Klebsiella pneumoniae* followed by 2(25%) of *Escherichia coli* and 20% *Proteus mirabilis*. (Table-9)

DISCUSSION

Globally the rate of diabetic patients with various foot complications are raising at an alarming level. Undiagnosed and inadequately treated diabetic cases can develop severe foot complications ended up with amputation of foot. It is causing social, economical burden on the patient and society due to late diagnosis and prolonged treatment and cost involved. Proper management of diabetic foot requires early and accurate identification of pathogens involved and appropriate antibiotic administration. Microorganisms play dominant role in the further spread of disease. The presence of multi drug resistant organisms poses high morbidity and therapeutic challenge for treating physician. Present study aimed to know the etiological agents and their susceptibility pattern involved with special focus on multidrug resistant organisms. A study done by Kow RY et al,^[11] total of 188 pathogens were isolated from 173 patients. Majority of the pathogens isolated were gram negative pathogens (73.4%). The most commonly isolated pathogens were *Staphylococcus*

aureus (17.5%) among gram positive and among gram negative organisms *Klebsiella spp* (17%) seen predominantly followed by *Pseudomonas spp* (15.4%) and *Proteus spp* (13.8%). Gram positive pathogens were sensitive to most of the antimicrobial agents tested except penicillin and fusidic acid. Gram negative pathogens were sensitive to all antimicrobial agents tested except ampicillin and amoxicillin/clavulanic acid. Amikacin provide overall coverage for all gram negative pathogens in diabetic foot infections.

In the present study diabetic foot infection was more commonly seen in between 41-70 years age group about 47(94%) cases, of which 33(66%) were males and 14(28%) were females which is similar to other studies done by Gadepalli et al,^[14] and SM Sekhar,^[12] showing in diabetic foot infections males predominantly affected compared to females. In our study, out of 50 cases of diabetic foot infection studied, 45/50(90%) culture positive and 5/50(10%) were culture negative. Mono microbial flora was predominant with 56.6% compared to Polymicrobial flora which is about 43.3%, in contrast to study done by Rama Kant et al,^[19] 66% were polymicrobial, whereas 23% were monomicrobial. Polymicrobial growth is usually seen in severe and moderate diabetic foot infections cases, whereas monomicrobial growth is seen in mild cases of

diabetic foot infection 20 also due to prior antibiotic administration before culture.

In the present study, aerobic gram negative isolates were predominant over gram positive isolates, this finding is similar to a study done by SM Sekhar et al,^[12] and also in a study done by Gadepalli et al,^[14] out of 80 ulcer specimens, recovered 183 isolates, of which 28.7% were Gram-negative and only 13.8% Gram positive. Shankar et al,^[17] also reported Gram-negative aerobes to be the most frequently isolated pathogens (51.4%), followed by Gram-positive aerobes (33.3%) and anaerobes. In contrast to studies done by western countries Dang et al,^[9] Citron et al,^[10] Goldstein et al,^[16] shows predominance of Gram-positive aerobes compared to gram negative organisms isolated. Though the reason for the predominance of gram negative organisms in developing countries compared to western countries is highly unknown, a study states that various environmental factors, health style and sanitary habits like perianal wash with water leading to contamination of hands with fecal flora this could be a reason for increased gram negative infections in developing countries.^[19]

Present study, most common organisms isolated were Pseudomonas species 16 (27%) followed by Klebsiella species 15(25%) among gram negative organisms isolated, whereas Staphylococcus aureus (18.3%) common isolate among gram positive organisms. This pattern is similar to study done by Rama Kant et al.^[19] The most common isolate was P. aeruginosa (16.9%) followed by E. coli (16.1%) and Proteus spp. (8.8%). In studies done by Shankar et al,^[17] and Bansal et al,^[18] also P. aeruginosa is the predominant organism.

In the present study done on diabetic foot infection cases. Among gram positive organisms isolated, Staphylococcus aureus was the predominant organism with percentage of MRSA being 54.5% showing highest sensitivity 100% to vancomycin and Linezolid, 78.5% sensitivity to amikacin, 71% sensitivity to doxycycline and ciprofloxacin. (Table-5). Even though few strains of MRSA show susceptibility to few beta lactams antibiotics in vitro, clinically they are ineffective⁽²³⁾ and Vancomycin though susceptible by disc diffusion method need to be confirmed with MIC method according to CLSI guidelines.

In the present study, Among gram negative isolates predominant organism is pseudomonas aeruginosa. Most of the isolates showed highest susceptibility to piperacillin / tazobactam (95.6%) followed by imipenem (76%) and amikacin (69.5%). Lowest sensitivity rate to ampicillin (19.5%) and ciprofloxacin(26.0%).

The prevalence of multidrug resistant organisms pose a therapeutic challenge for treating clinician with emergence of ESBL, Amp C, MBL and MRSA due high cost of treatment with prolonged stay at hospital and morbidity involved. The knowledge of the causative agent involved and resistant pattern involved is necessary for treatment. Present study

shows ESBL producers among organisms isolated were of 43.4%, predominantly with 62.5% of Escherichia coli isolates, 46.6% of Klebsiella pneumoniae and 40% of Proteus (Table-8). A study done by Gadepalli et al,^[14] also reported 44.7% of ESBL production. Ramanakant et al,^[19] states that various studies reported from India showed the prevalence rate of ESBL producers ranging between 55% and 70%.^[21,22] The percentage of MBL producers were 26% predominantly with 31.2% pseudomonas aeruginosa isolates (Table-9). This correlates with studies done by Ramanakant et al,^[19] and Gadepalli et al.^[14]

The reason for multidrug resistance seen in diabetic foot infection cases is due to its chronic nature of the disease which needs prolonged treatment for cure with usage of multiple antibiotics for treatment.^[24]

In the present study even though culture and sensitivity helps us to know the pattern of organism involved and its susceptibility pattern by phenotypic method. For detection of certain strains of ESBL, MRSA and MBL we need to go for genotypic methods like molecular characterization for more reliability which are expensive.^[25] Continuous surveillance of resistant pathogens involved in diabetic foot infections helps in preventing treatment failure due to multidrug pathogens involved and reduces the further spread of infection and prevents the risk of complications.

Limitation of the study

In this study only aerobic culture was done, anaerobic culture could not be performed. Detection of MRSA, ESBL, MBL performed only by phenotypic method needs to be confirmed by genotypic methods like molecular characterization which are not done due to high cost and equipment needed.

CONCLUSION

The present study on diabetic foot infection cases there is predominance of gram negative organisms with monomicrobial flora slightly higher than polymicrobial flora. Pseudomonas species isolated in common followed by Klebsiella species among gram negative organisms and staphylococcus aureus predominant among gram positive organisms. This study shows higher prevalence rate of multidrug resistant agents like ESBL, MBL and MRSA. Antibiotic sensitivity pattern shows overall coverage of amikacin for gram negative organisms and few gram positive organisms will be effective with Piperacillin tazobactam, imipenem coverage for ESBL and MBL strains. Doxycycline found to be effective for most of the gram positive isolates with Vancomycin or linezolid for MRSA strains.

Summary

Hence diabetes and its complications are increasing at an alarming level in India. Early clinical detection of diabetic foot infection and microbiological characterization along with routine antibiotic sensitivity testing of organisms testing for MRSA,

ESBL and MBL production will help the clinicians for proper management of patients also helps in preventing antibiotic resistance. With emerging antibiotic resistance throughout the worldwide, there is a need for continuous surveillance of diabetic foot infections and multidrug resistant organisms involved with antibiotic sensitivity testing thus helps clinicians in providing a basis of empirical therapy and prevention of diabetic complication.

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