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MICROBIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY TESTING OF DIABETIC FOOT INFECTIONS AT TERTIARY CARE CENTRE

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ABSTRACT
Background: Diabetes mellitus (DM) refers to a group of common metabolic disorder and trio problem leading onto Diabetic foot and ulcers are neuropathy, vascular changes and infection. Despite of its common prevalence, Diabetic foot infections are often mismanaged, particularly in regard to antibiotics which has led to development of Multi Drug Resistant Organisms (MDRO).
Objectives:
To Isolate and evaluate the aerobic Bacteriological profile in cases of Diabetic foot.
To study the Antimicrobial susceptibility pattern with special reference to detection of MRSA (Methicillin resistant *Staphylococcus aureus) and* ESBL (Extended Spectrum Beta Lactamases).
Materials and Methods: Pus and wound swabs will be collected from patients with Type 2 DM with history of ulcer on the foot, subjected to aerobic culture, identification and antibiotic susceptibility by Modified Kirby Bauer Disc Diffusion method. Potential ESBL producers were screened and subjected to PCDDT. Cefoxitin disc (30µg) was used to identify MRSA.
Results: Of the 200 pus/wound swabs, 180 showed aerobic growth. Klebsiella *spp was* the highest isolate at 23% followed by *E.coli* at 22%. 58 % of *Klebsiella* spp. and 56% of *E.coli* were ESBL producers. Imipenem(100%) and combination agents such as Cefoperazone/Sulbactam(55-60%) retained good sensitivities against the ESBL producers. *Staphylococcus aureus* was seen the most amongst the Gram-positive cocci, 59% were MRSA isolates. Vancomycin and Linezolid were 100% sensitive.
Conclusions: Resistance is now a global problem and more so in developing countries like India due to high prevalence of infectious diseases, weakly monitored healthcare and laboratory facilities, easy accessibility to drugs without prescription, lack of guidelines for infectious disease therapy leading to indiscriminate use of antibiotics and poor facilities for infection control. ESBL and MRSA are associated with increased morbidity and mortality in patients with Diabetes. Thus early appropriate and judicious use of selective antibiotics can prevent complications such as amputations and other co-morbidities associated with diabetic foot infections

KEYWORDS: Diabetic Foot; aerobic organism; ESBL; PCDDT; MRSA

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by elevated blood glucose levels which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades the prevalence of type 2 Diabetes has risen dramatically in countries of all income levels.1.5 million deaths are directly attributed to Diabetes each year. 9% of adults in the world have Diabetes. 90% of people with diabetes in the world have type 2 Diabetes. WHO projects that Diabetes will be the 7th leading cause of death in 2030 [1, 2]. According to the International Diabetes Federation (IDF), an estimated 588.7 million adults (aged 20-79 years) worldwide were living with diabetes as of 2024. This number is projected to increase to 852.5 million by 2050, highlighting a significant global rise in diabetes prevalence [3]. Infections in Diabetic patients are mostly polymicrobial in nature, comprising of both aerobic and anaerobic microorganisms. Amongst the Gram positive organisms *Staphylococcus aureus* and *Enterococcus* species predominate. The Gram negative comprise mainly of microorganisms belonging to Enterobacteriaceae family such as *Escherichia coli, Klebsiella* species, *Proteus* species etc. Nonfermenters such as *Pseudomonas* spp. and *Acinetobacter* spp. have been isolated as well.

Objectives

1. To Isolate Bacterial organisms from Diabetic foot cases.
2. To evaluate the aerobic bacteriological profile in cases of Diabetic foot.
3. To study the Antimicrobial susceptibility pattern of bacterial isolates with special reference to detection of MRSA (Methicillin resistant *Staphylococcus aureus) and* ESBL (Extended Spectrum Beta Lactamases).

MATERIALS & METHODS

- **Study Design and Settings:**

This Prospective Cross sectional study was conducted in the Department of Microbiology, at Navodaya Medical College & Hospital, Raichur over a period of 1 year between July 2025 and June 2026.

- **Inclusion Criteria:**
- Individuals with established type 2 Diabetes mellitus.
- Age above 45years.
- Male and Female patients.
- Diabetic foot ulcer patients including all Grades of Wagner's classification.

- **Exclusion Criteria:**
- Individuals with type1 Diabetes Mellitus.
- Ageless than 45years.

METHODOLOGY:

- **Sample collection and Processing:** Specimens such as wound exudates and pus were obtained after the surface of the wound was washed with sterile saline followed by debridement of superficial granulation tissue with two sterile cotton swabs, which was immediately transported in Stuarts Transport medium (HiMedia laboratories ltd) without delay to the Microbiology laboratory. Direct smears were made from one swab and stained with Grams stain. The smear was screened for the presence of inflammatory cells and the type of microbial flora under oil immersion.
- **Characterization and Identification of Isolates:** The second swab was cultured onto MacConkey agar and Blood agar and were incubated aerobically at 37°C for 24 hours and then subjected to preliminary and biochemical tests confirming them. For fungal isolation it was plated onto Sabourauds Dextrose agar.
- **Antimicrobial Susceptibility testing:** Susceptibility testing was performed by standard disc diffusion method as per CLSI guidelines by Kirby Bauer disc diffusion method .All the discs were commercial, procured from Hi-media Laboratories Limited. All *Staphylococcal* strains were tested for methicillin resistance by using oxacillin [1 µg] disc, Cefoxitin [30 µg]. All the Gram-negative bacteria identified as *E.coli* or *Klebsiella* spp. were subjected to a screening test for detection of probable ESBL producers using ceftazidime (30µg) and cefotaxime discs (30µg) ,Phenotypic Confirmatory Disc Diffusion Test (PCDDT) as confirmatory test.

- **Statistical analysis:**

Data were analyzed using SPSS software. Chi-square test was applied and odds ratio with 95% Confidence intervals were calculated. A p value of < 0.05 was considered statistically significant.

- **Ethical Approval :**

Approval was obtained from the ethical committee of the institute for this study.

RESULTS

During the study period between July 2025 and June 2026, 200 pus samples were collected from foot ulcers of Diabetic patients. The ulcers were assessed according to Wagner's grading of Diabetic Foot Ulcers.54%of the patients belonged to Grade 2 of Wagner's classification system. There were 132 male patients and 68 female patients. The male to female ratio was1.9: 1. Age group Distribution shows 39% of the patients were between 61-70 years followed by 51-60 years (26%) the least being between 45-50 years (7%).Out of the 200 samples, 180 yielded aerobic bacterial growths, and 20 samples did not yield any growth. Poly microbial growth was found in 94 (52%) of the 180 samples and mono microbial pattern of growth in 86 (48%). Of the 94 samples, which belonged to isolates from Enterobacteriaceae family, 30 had pure growth of members of this family while 64 had growth of an Enterobacteriaceae along with other bacteria such as *Staphylococci, Streptococci, Pseudomonas, Acinetobacter, Candida spp* etc. The remaining 86 samples grew bacteria belonging to other families. 70 had pure growth and 16 had mixed isolates shown below in Table 1-2.

Table 1: Growth of samples:

Aerobic Growth	180	90%

No Growth	20	10%
Total	200	100%

Table 2: Pattern Distribution of samples:

A total of 296 organisms were isolated of which 166 (56%) were Gram-negative bacilli, 118 Gram-positive cocci (40%) and 12(4%) Gram-positive yeast like budding.

Organisms	Number	Percentage (%)
Pure Enterobacteriaceae	30	16.60
Mixed Enterobacteriaceae	64	35.50
Pure Others	70	38.90
Mixed Others	16	9
Total	180	100

Table 3, Figure 1 shows Distribution of Organisms.

Table 3:

Organism	Number	Percentage (%)
Gram Negative Bacilli	166	56.10
Gram Positive Cocci	118	39.90
Gram Positive Yeast Like Budding Cells	12	4.00
Total	296	100

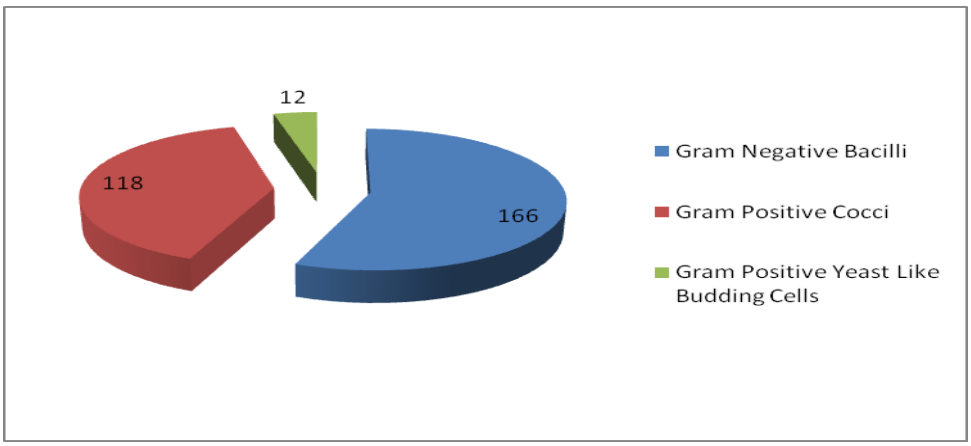


Figure1: 116 of the 166 gram negative bacilli belonged to the family Enterobacteriaceae. Others include non-enterobacteriaceae, such as *Pseudomonas* spp , *Acinetobacter* spp. etc. shown in Table 4,Figure 2.

Table 4:

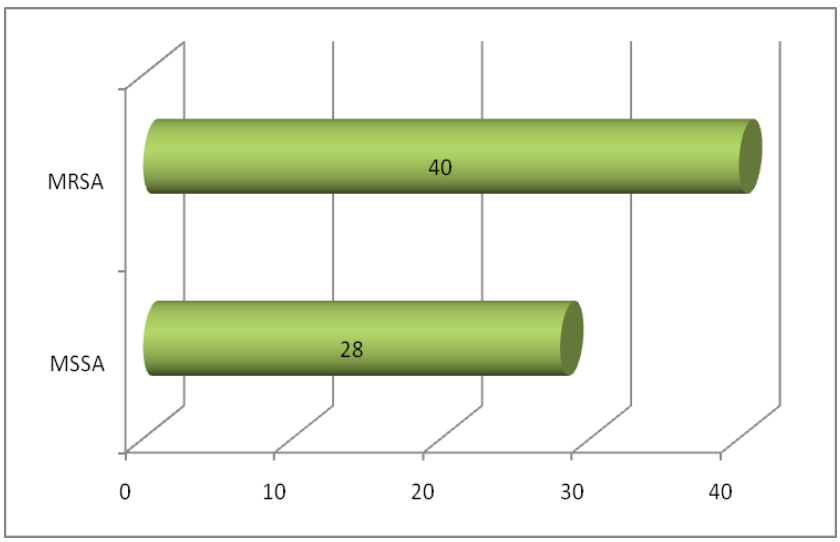
Particulars	Number	Percentage (%)
Klebsiella spp.	38	22.89
E.coli	36	21.68
*Pseudomonas*spp.	30	18.07
Proteus spp.	26	15.66
*Acinetobacter*spp.	20	12.04
*Citrobacter*spp.	10	6.02
*Enterobacter*spp.	6	3.61
Total	166	100

Figure 2:

- Amongst the Gram-positive cocci, 68 were *Staphylococcus aureus,* 34 Coagulase negative *Staphylococci*, 10 belonged to *Enterococcus* species and 6 to *Streptococcus* species.58.82% of the *Staphylococcus aureus* isolates were MRSA while 41.18 were MSSA, MRSA as 58.82% shown in Table 5, Figure 3-4.

- Table 5:

GramPositiveCocci	Number	Percentage (%)
Staphylococcus aureus	68	57.60
CoagulaseNegative Staphylococci	34	28.80
*Enterococcus*spp.	10	8.50
*Streptococcus*spp.	6	5.10
Total	118	100



- Figure 3-4 Amongst Enterobacteriaceae *Klebsiella spp.*was isolated the highest followed by *E.coli*. The percentage of ESBL producers among *Klebsiella* spp was 38(57.89%) shown in Fig 5, *E.coli* was 36(55.56%) shown in figure 6.

Figure 5

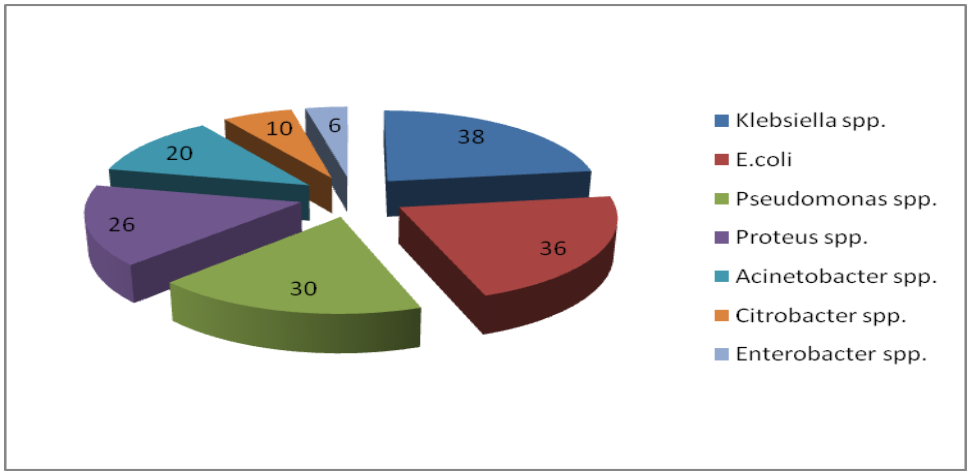
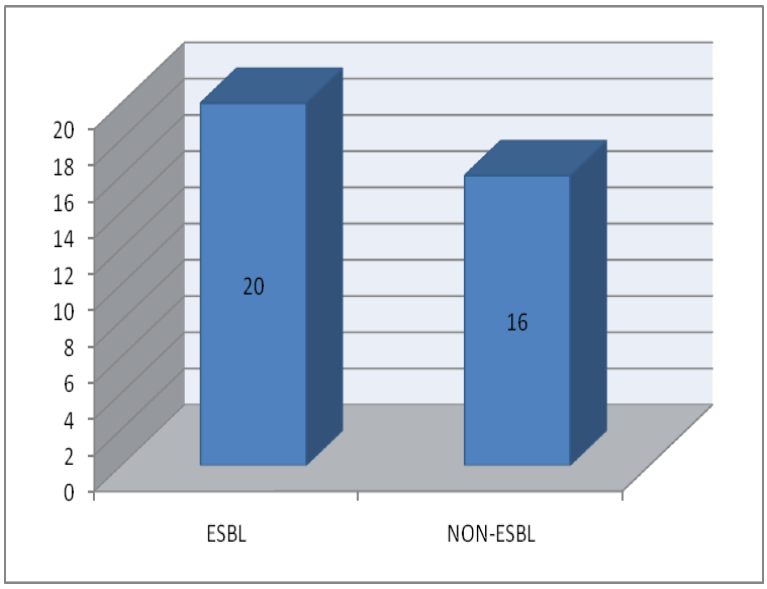
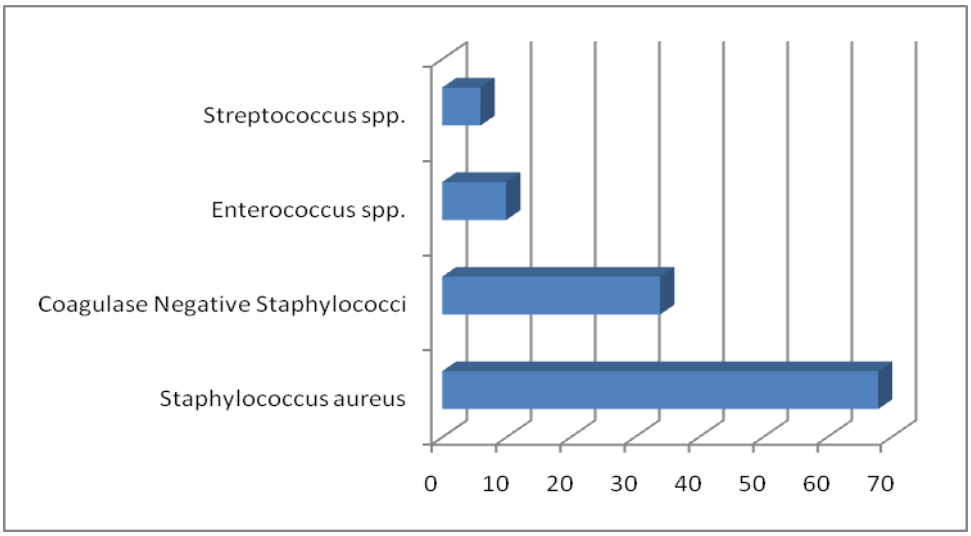
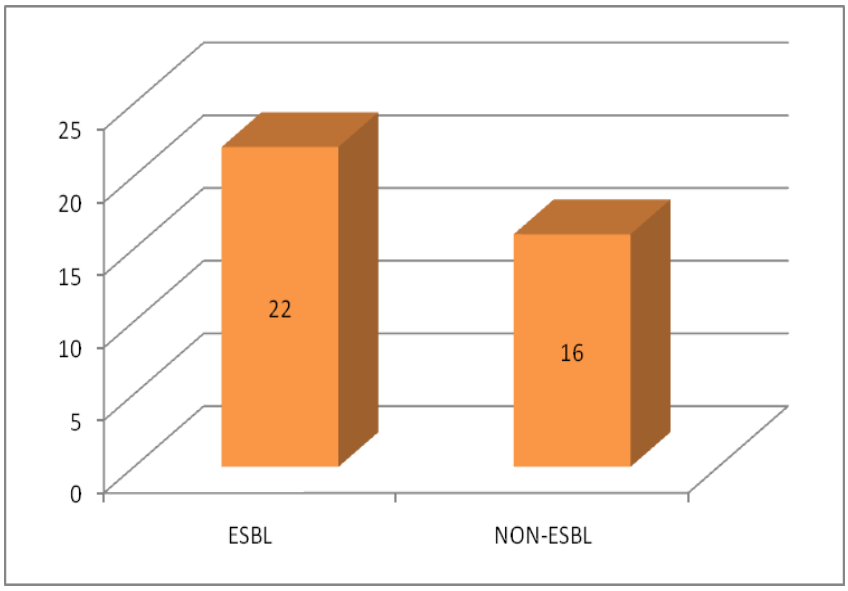


Figure 6



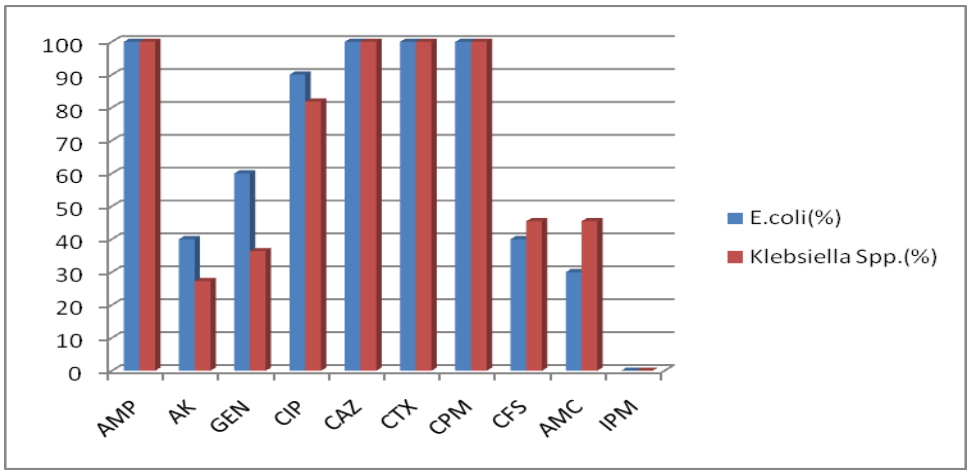




- The Resistance to the third and fourth generation cephalosporins ranged between 42-61 % for *E. coli* and *Klebsiella* spp. with resistance to fourth generation cephalosporin, cefepime being 50% for *E. coli* and 42% for *Klebsiella* spp. All the members were less resistant to beta-lactam/beta-lactamase combination drugs such as cefoperazone/sulbactam and amoxyclav (0-16%). Resistance patterns of ESBL Producers shown in below

- **Table 6,**

Antibiotic	E.coli(%)	Klebsiella spp.(%)
AMP	100	100
AK	40	27.27
GEN	60	36.36
CIP	90	81.81
CAZ	100	100
CTX	100	100
CPM	100	100
CFS	40	45.45
AMC	30	45.45
IPM	0	0

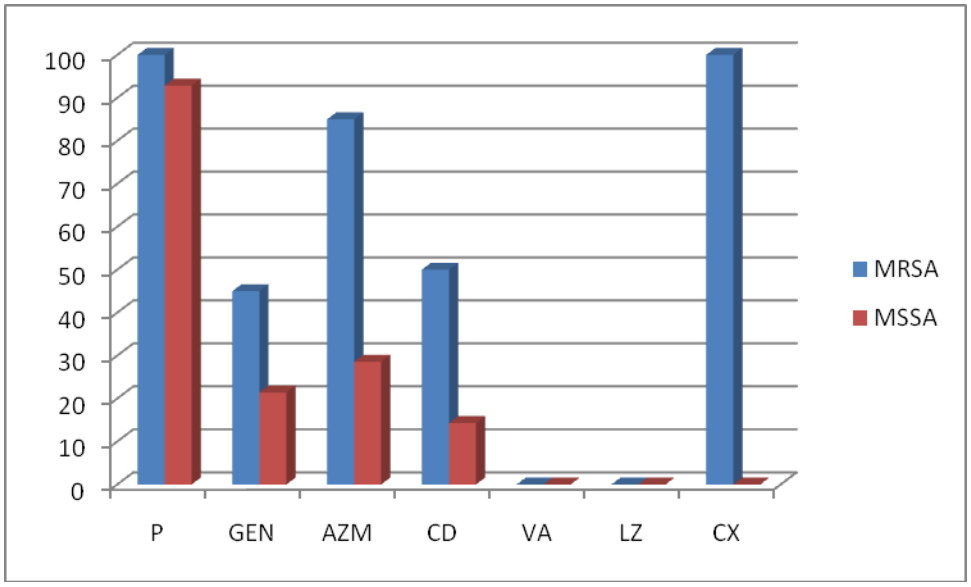


- **Figure 7**

• The MRSA isolates showed 85% resistance to erythromycin and 50% resistance to clindamycin. They were 100% sensitive to vancomycin and linezolid. The resistance of MSSA to the above drugs varied between 14% and 28%.Resistance pattern of MSSA & MRSA shown in below

• **Table 7**

Orga- nism	No.	P		GEN		AZM		CD		VA		LZ		CX	
		No	%	N	%	N	%	N	%	N	%	N	%	N	%
MRSA	40	40	100	18	45	34	85	20	50	0	0	0	0	40	10
MSSA	28	26	92.8	6	21.4	8	28.5	4	14.2	0	0	0	0	0	0



• **Figure 8**

Discussion

• 200 pus and wound samples were collected from patients above 45 years with known history of Type 2 Diabetes Mellitus. Most of the patients were in the age range of 61-70 years (24%) followed by 51-60years(21%) the least being between 45-50years.This was similar to the study by Y. Kavitha et al [4] , which showed the mean age to be 60-70 years and study by Govindaswamy et al[5] which shows mean age as 60.82 yrs. The male to female ratio was found to be 1.9:1 which correlates to a study conducted by V.Vijay et al [6], the male to female ratio was 1.54:1 and study by Govindaswamy et al[5] as 2.9:1 shows slightly higher ratio. Comorbid features such as Hypertension, peripheral neuropathy and vascular insufficiency were present in varying proportions in all the patients, especially in individuals with higher grades of ulcer. Our study showed a slightly higher presence of polymicrobial infection over monomicrobial whereas studies by Govindaswamy et al [5], Kavitha et al [4] shows monomicrobial higher predominance over polymicrobial.

• In present study, A total of 166(56%) were Gram-negative bacilli, 118 (40%) Gram-positive cocci and 12(4%) Gram-positive yeast like budding cells which shows Gram negative predominance over Gram positive bacteria that correlates with studies by Govindaswamy et al [5],Boya et al [7] .69.8 % of the Gram Negative isolates belonged to family Enterobacteriaceae while 30% belonged to other families and , *Klebsiella* spp, the highest isolate at 23%, followed by *E.coli* at 22%, *Pseudomonas* spp at 18% and *Acinetobacter*spp.at 13% which correlates to study by Govindaswamy et al[5], whereas studies by Boya et al [7] , Kavitha et al [4]

• The emergence of ESBLs as enzymes, which hydrolyze β-lactams antibiotics and MRSA render them

ineffective, has posed a significant problem in clinical therapeutics.In our present study ESBL MRSA production shows slight high percentage compared to study by Y. Kavitha et al [4]. The observed discrepancy may be attributed to the characteristics of the sampled population

- The resistance to the third and fourth generation cephalosporin's ranged between 42-61% for *E.coli* and *Klebsiella* spp. with resistance to fourth generation cephalosporin, cefepime being 50% for *E.coli* and 42% for *Klebsiella* spp. All the members were less resistant to beta-lactam/beta-lactamase combination drugs such as cefoperazone-sulbactam and amoxyclav (0-16%). All were sensitive to carbepenem drug Imipenem. In our study, all the ESBL strains were 100% susceptible to Carbapenems and MRSA were 100% sensitivity to linezolid similar to study by Y. Kavitha et al [4].

CONCLUSION

The excitement with the discovery of antibiotics to tackle and subdue organisms was short-lived, with the rampant emergence of antibiotic resistance to various classes of agents. This has in turn contributed to increase in hospital stay, which has adverse effect on the patient, by increasing the morbidity, mortality as well as the financial burden. The study emphasizes the need for all diagnostic laboratories to screen for ESBL detection among the Enterobacteriaceae and MRSA screening for *Staphylococcus aureus* as a routine practice, especially in Diabetic Foot Infections. Combined effort of the Microbiologists, clinicians and infection control team are needed to tackle the problem of increasing multidrug resistance particularly the MRSA and ESBL producing strains.

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