Original article:

Bacteriological profile and antibiotic resistance patterns of Podiatric infections in Diabetic patients - A Prospective study

Dr.Sripriya.C.S*., Arockia Doss. S. Dr.Shanthi.B, Dr.Vijayapriya., Durai murugan

Department of Microbiology, Anderson Diagnostics and labs, Chennai, 2016.

*Corresponding author

Abstract:

The mono microbial and polymicrobial populations in diabetic foot infections are developing much resistance to antibiotics and they are hard to treat. The emergence of ESBL producers, carbapenemase producers among Enterobacteriacae, methicillin resistance and Inducible clindamycin resistance in *Staphylococcus aureus*, Multidrug resistance A*cinetobater baumannii* are commonly associated with morbidity and mortality in many cases. This prospective study aims in determining the multi drug resistance of microbial populations among diabetics over a period of time. The study was conducted for a period of one year and 575 patients were included in the study, out of which 76% were males and 24% were females. Among 417 patients with bacterial infections, 79.13% had Mono microbial infection and 20.87% had poly microbial infection, with 82.49% Gram negative bacteria and 17.50% Gram positive bacteria. Patients with repeated infections had

To Conclude, Gram Negative bacteria were predominant in our study with more ESBL producers (18.89%) and carbapenemase produces (28.48%). Among Gram positive bacteria, methicillin resistant S*taphylococcus aureus* and Inducible clindamycin resistant S*taphylococcus aureus* were reported more .Organisms have developed resistance to most of the second & third generation cephalosporin. Aminoglycosides resitance was also seen in many cases. Carbapenemase once being choice of physicians in treating severe infection have also gained resistance.

Keywords: Type 2 Diabetes, ESBL, Carbapenamase producers, MRSA, MDR *Acinetobacter baumannii.*

INTRODUCTION:

A diabetic foot infection is most simply defined as any inframalleolar infection in a person with diabetes mellitus. These include paronychia, cellulites, and myositis , abscesses, necrotizing fasciitis, septic arthritis, tendonitis and osteomyelitis. The most common and classical lesion however is the infected diabetic (mal perforans) foot ulcer (Caputo *et al.*, 1994; Frykberg., 1998).Foot infection in patient with diabetes causes substantial morbidity and frequent visits to health care professions and may lead to amputation of a lower extremity. Diabetic foot infection requires attention to local (foot) and systemic (metabolic) issues and coordinated management, preferably by a multi disciplinary foot care wound infection must be diagnosed clinically on the basis of local (and occasionally systemic) signs and symptoms of inflammation. Tissue specimen is obtained by biopsy, ulcer curettage or aspiration and is preferred over wound swab specimens (Lipsky *et al.*, 2014) .Optimal management of diabetes foot infections can potentially reduce the incidence of infection related morbidities , the need for and duration of hospitalization and the incidence of major limb amputation (Armstrong *et al.*, 1995 ; Calhoun *et al*., 1988)**.**

It was also documented that patient undergoing podiatric surgery established a infection rate of 1.3% and over a due course of time the complications were reported high. The patient responded positively to a short course of oral antibiotics where few required readmission to hospital for intravenous antibiotic therapy (Butterworth *et al*., 2010).Severe diabetic foot infections usually yield poly microbial isolates whereas mild infections are frequently mono microbial (Frykberg., 2003).Several studies have also described a high prevalence rate (80% to 87.2%) of polymicrobial infections in diabetic foot patients (Wright-Pascoe *et al*., 2000 ; Loan *et al*.,2005).Reports suggest that S*taphylococcus aureus* and ß-hemolytic S*treptococci* are the common causative pathogens, (Ramakant *et al*., 2011)and *E.coli*, *Proteus* species, P*seudomonas* species, *Staphylococcus* aureus and *Enterococcus* species are the most frequent pathogens contributing to progressive and widespread tissue destruction (Anandi *et al*., 2004; Gadepalli *et al*., 2006)**.**Extended spectrum beta- lactamases (ESBL) are defined as beta lactamases capable of hydrolyzing oxyimino-cephalosporin's and are inhibited by beta lactamase inhibitors and they are found in a variety of Enterobacteriacae species. The majority of ESBL producing stains are *Klebsiella pneumoniae*, *Klebsiella oxytoca* , *E. coli*. In recent years there has been increase in the incidence and prevalence of ESBL and currently there is a paucity of data on ESBL producers and Carbapenamase producers from diabetic foot infections (Frykberg ., 1998). Being plasmid mediated they are easily transmitted among members of Enterobacteriae. The chromosomally mediated beta-lactamases production is mainly through expression of Ampc gene (Babypadmini and Appalaraju., 2004 ; Rodrigues *et al*., 2004).Multidrug resistance seen in *Acinetobacter baumannii* is an important cause of hospital acquired infection and has been shown in some studies to increase mortality and length of stay.

Materials & Methods:

Study setting:-

The study was conducted for a period of one year (Jan 2016 to Dec2016) at a standalone lab in Chennai. Demographical data that included age, sex and location of foot ulcer were recorded. Tissue samples were collected aseptically and transported in a sterile container immediately after collection.

Tissue processing isolation &Identification:

The tissue sample were weighed in a sterile tube, homogenized thoroughly and 0.1ml of original homogenate tissue sample placed in Blood agar plate, Chocolate agar plate, MacConkey agar plate and labeled as 10^1 (1-5)

Then serially dilute the homogenate using 0.5ul aliquot and 4.5ml of sterile 0.85% Nacl and place 0.1ml of 10^4 dilutions into Blood agar plate, Chocolate agar plate, MacConkey agar plate. Evenly distribute inoculums with sterile rod and incubate at 37^{o}C for 18-24 hours.

The results are reported quantitatively by using the formula.

Colony count x5(homogenate dilution)x10^1(plate dilution)

Weight of the tissue

- Pure culture for mixture of organisms is obtained by isolation on Blood agar plate.
- Identification was performed by Gram staining and Biochemical characteristics using standard methods as well as using automated vitek 2 system.

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Antibiotic sensitivity testing:

Antibiotic sensitivity test for the isolated bacteria were performed by Kirby Bauer disc diffusion method (CLSI guidelines, 26th Edition) the isolate was interpreted and MIC for multi drug resistant strains were done with automated vitek 2 systems. ESBL producers, carbapenemase producers, Inducible clindamycin resistance and methicillin resistance were tested and reported as per CLSI guidelines.

ESBL producers:

While performing antibiotic testing ,ceftazidime (30mg) and ceftazidime/clavulanic acid (30/10mg) discs were placed on MHA plate on which a 0.5 McFarland standard of the organism was swabbed and was considered an ESBL produce if there was >5mm increase in zone diameter of ceftazidime /clavulanic acid disc and that of ceftazidime disc alone (CLSI guidelines., 26th Edition).

Carbapenemase producers:

Modified Hodge test were performed for Enterobacteriaceae, with suspected carbapenemase production. 0.5 McFarland suspension of ATCC E.coli 25922 was diluted 1:10 in sterile saline. This was inoculated on a Muller Hinton agar plate as for the routine disc diffusion testing. The plate was dried and a disc of meropenem 10ul was placed in the centre of the agar plate, colonies of the test organism were picked and inoculated in a straight line, from the edge of the disc upto a distance of at test 20-25mm in length. The plates were incubated at 37°C overnight and they were examined next day. They were checked for enhanced growth around the test organism at the intersection of the streak and zone of inhibition. The presence of an enhanced growth indicated carbapenemase production and the absence of an enhanced growth meant that the test isolate did not produce carbapenemase (CLSI guidelines, 26th Edition).

Inducible clindamycin resistance:

The detection of inducible clindamycin resistance in S*taphylococcus aureus*, S*treptococcus species* were done on MHA agar plate on which, 15mg erythromycin and 2mg clindamycin disc spaced 15-26mm were placed and incubated at 37°c for 24 hours. Positive result shows flattening of the zone of inhibition of clindamycin disc adjacent to the erythromycin disc (referred to as D-zone) (CLSI guidelines, 26th Edition).

MRSA Detection:

The MRSA detection was done using cefoxitin10ul disc. A zone of inhibition which was equal to or more than 22mm was considered as susceptible to cefoxitin and the organisms were reported as methicilin sensitive *Staphylococcus aureus* (MSSA). Those isolates which had zone of inhibition less than equal to 21 were considered as methicillin resistant *Staphylococcus aureus* (MRSA) (CLSI guidelines., 26th Edition)

RESULTS:

Tissue samples collected from 575 diabetic patients, 437 (76%) males and 138 (24%) females were included in the study. The ages ranged from 16 to 80 years. (Fig.1.)

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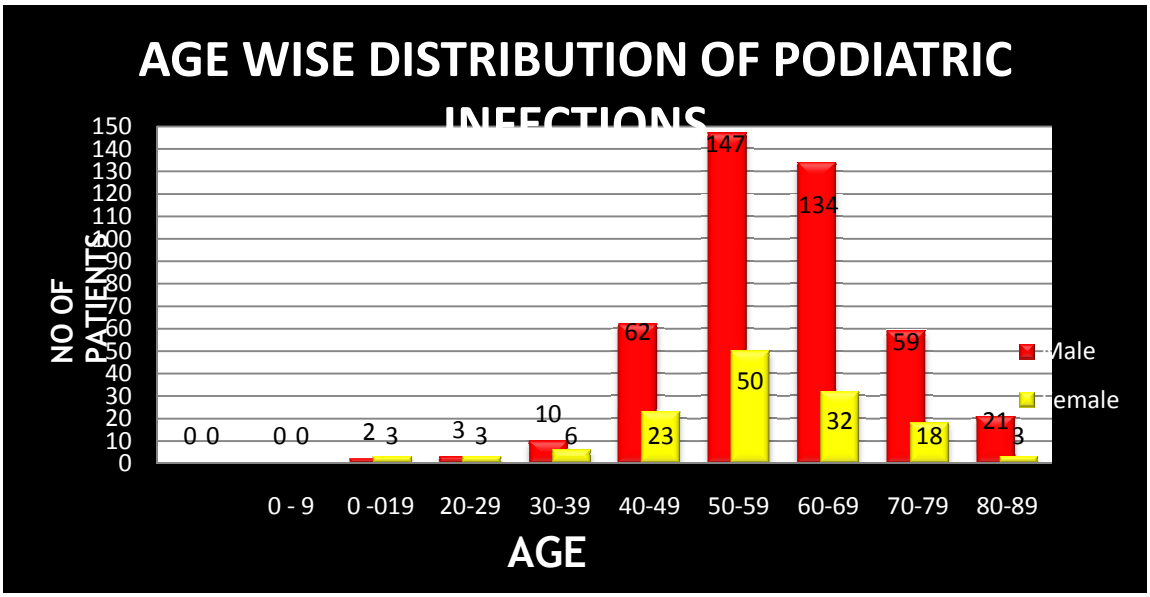
Fig. 1. Agewise distribution of podiatric infections.



A total of 417 Bacteria were isolated from the tissue samples of diabetic patients out of which 344 (82.49%) were Gram negative bacterial pathogens (Table.1.) and 73 (17.50%) were Gram positive bacteria. A total of 330 (79.13%) had mono microbial infection and 87(20.87%) had poly microbial infection (Table.2.). The most common Gram negative isolate was *Pseudomonas aeruginosa* (38.60) (41.61% were carbapenemase producers), followed by *Klebsiella pneumoniae* (18.46) (37.66% ESBL, 19.5% Carbapenemase producers) and *E.coli* (9.59%) (54.54% ESBL, 1.5% carbapenemase producers) Fig.2. Other Gram negative organisms isolated include, *Klebsiella oxytoca* , *Enterobacter aerogenes, Enterobacter cloacae, Citrobacter koseri, Citrobacter freundii, Providentia rettgeri, Providentia stuarttii, Morganella morganii, Acinetobacter baumannii, Proteus vulgaris, Proteus mirabilis*(Fig.3.) . Gram positive organisms include *Staphylococcus aureus, Streptococcus* species and *Enterococcus faecalis.*

Citrobacter species were isolated in very few numbers and they also exhibited ESBL (1.19%). Incidence of *E.coli* was less compared to *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. 55% of E.coli was ESBL producers and 15.1% were carbapenemase producers. Among the Quinolone group of antibiotics, ciprofloxacin was found to be more resistant while levofloxacin was found to be susceptible in most of the isolates. None of the *Staphylococcus aureus* isolate was susceptible to penicillin. Enterococcus species showed susceptibility to most of the antibiotics. Follow up cases were also observed and tested every time and there was no much change in antibiotic patterns over a short duration but some developed multidrug resistance in due course of time.

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Table1. Gram negative profile of Podiatric infections

GRAM NEGATIVE ORGANISMS	TOTAL NO OF ISOLATES	ESBL PRODUCERS	CARBAPENASE PRODUCERS
Escherichia coli	33	18	5
Klebsiella pneumoniae	77	33	15
Klebsiella oxytoca	2	2	0
Enterobacter aerogenes	7	7	0
Enterobacter cloacae	11	3	2
Cirobacter freundii	1	0	0
Citrobacter koseri	4	2	2
Providentia rettgeri	2	0	0
Providentia stuartii	2	0	0
Morganella morganii	7	0	1
Acinetobacter baumannii	7	0	6
Pseudomonas aeruginosa	161	0	67
Proteus vulgaris	7	0	0
proteus mirabilis	23	0	0

Fig.2. Incidence of ESBL & Carbapenamase producers in commonly isolated GNB

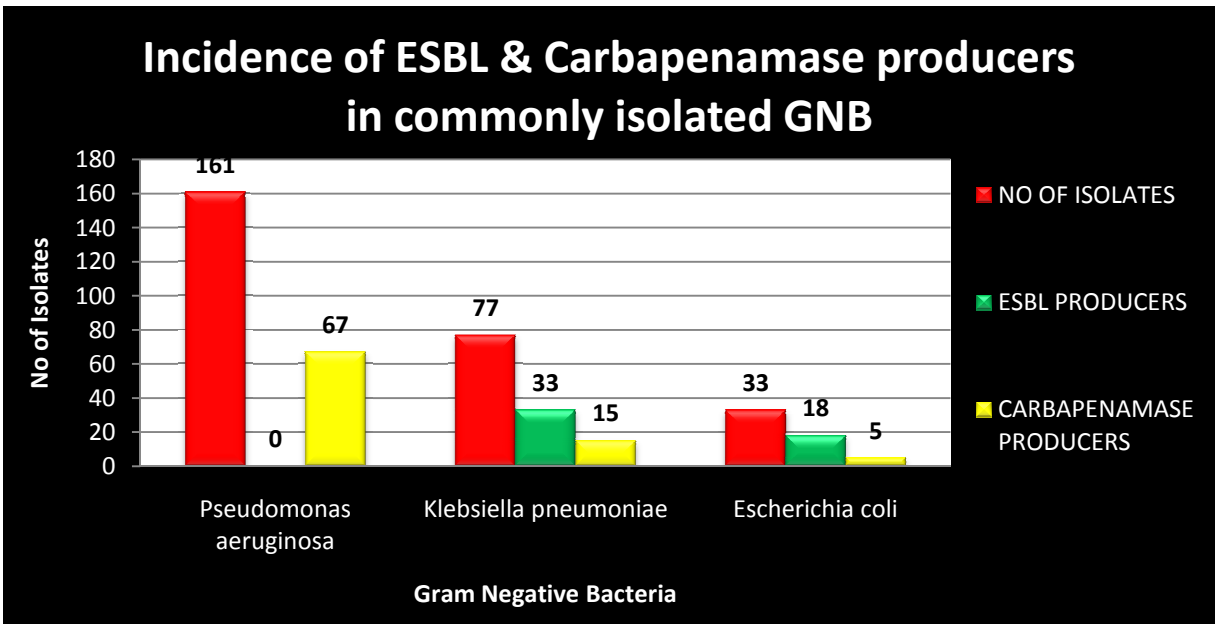

Fig.3. Incidence of ESBL & Carbapenamase producers in other GNB

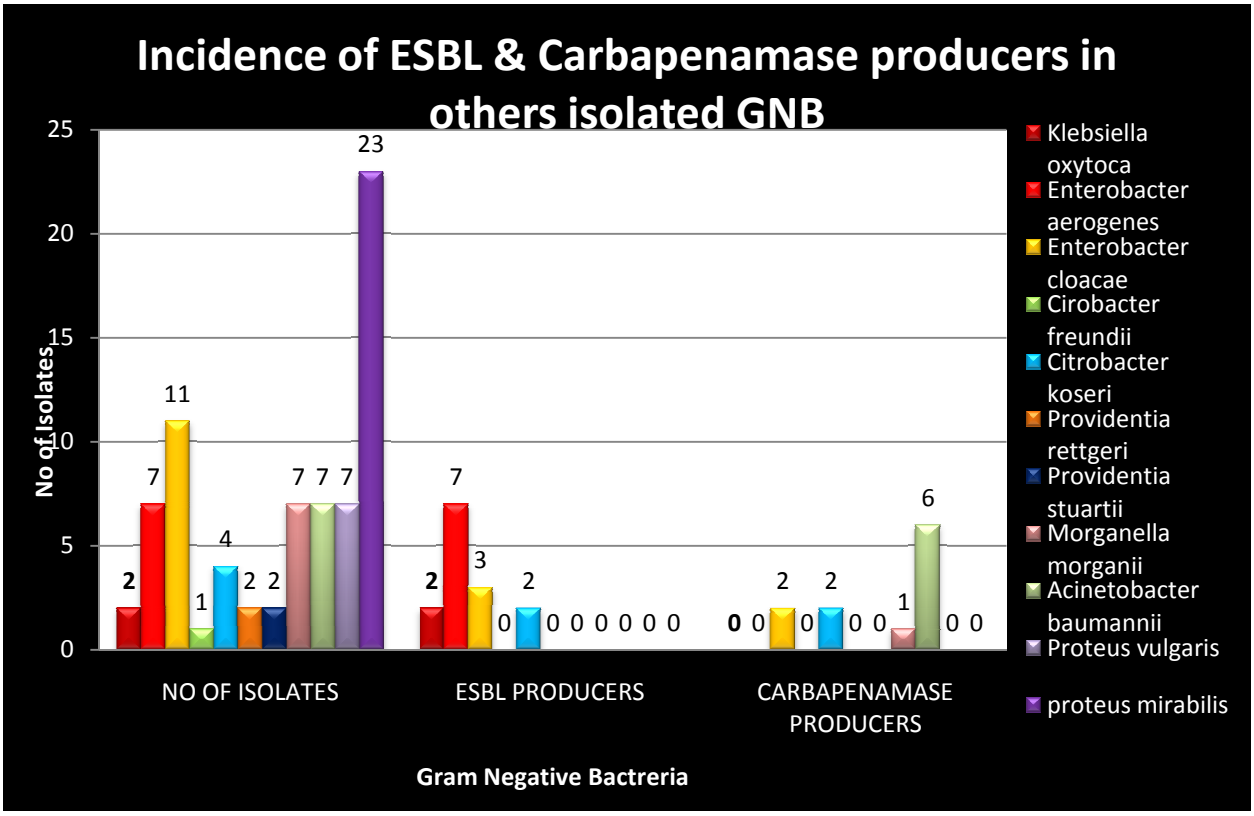

Table.2. Polymicrobial isolates in Podiatric infections

POLYMICROBIAL ORGANISMS	NO OF ISOLATES
Klebsiella pneumoniae/Pseudomonas aeruginosa	34
Enterobacter cloacae/Pseudomonas aeruginosa	6
Pseudomonas aeruginosa/E.coli	8
Pseudomonas aeruginosa/Providencia stuartii	2
Klebsiella pneumoniae/Morganella morganii	2
Klebsiella oxytoca/Enterococcus faecalis	1
Proteus mirabilis/Enterococcus faecalis	7
Enterobacter cloacae/Staphylococcus aureus	2
Pseudomonas aeruginosa/Acinetobacter baumannii	2
Citrobacter koseri/Morganella morganii	1
Pseudomonas aeruginosa/Citrobacter koseri	5
Klebsiella Pneumoniae/Proteus mirabilis	5
E.coli/Enterobacter cloacae	1

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Pseudomonas aeruginosa/Staphylococcus aureus	2
E.coli/Morganella morganii	2
E.coli/Staphylococcus aureus	1
Klebsiella pnuemoniae/Staphylococcus aureus	1
Proteus mirabilis/Staphyloccus aureus	2
E.coli/Proteus mirabilis	1
Proteus mirabilis/Peudomonas aeruginosa	2

73 Gram positive organisms were isolated, (Fig.4.) out of which 54 were *Staphylococcus aureus* isolates, 9 (16.66%) was methicillin resistant and 11.11 % (6) had Inducible clindamycin resistance. Other gram positive infections such as *Streptococcus* species and *Enterococcus faecalis* were seen in less numbers 19 (3.30%) (Fig.5.)

Fig.4. Gram positive profile of Podiatric infections

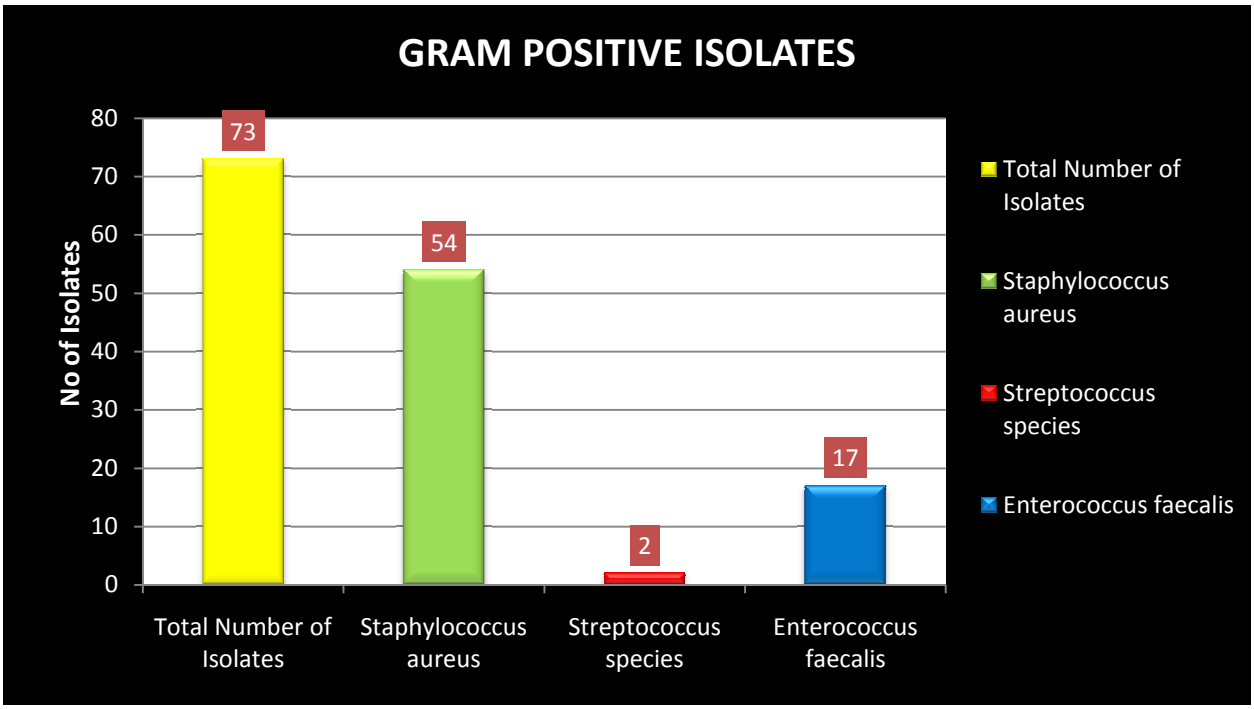
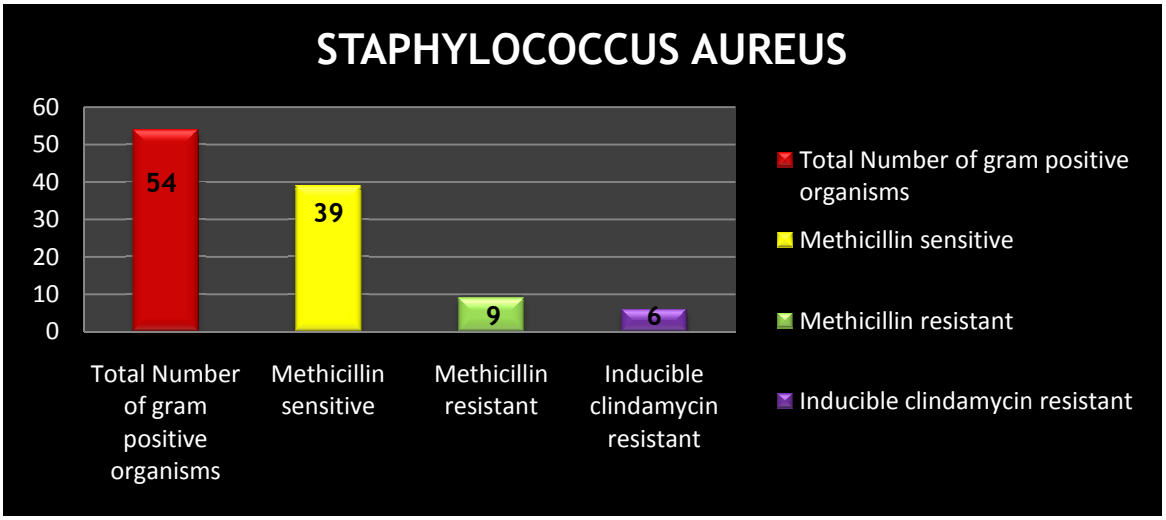


Fig.5. Antibiotic resistance pattern of Staphylococcus aureus



DISCUSSION:

In Podiatric infections, Multi drug resistance is on an increase among isolates of Enterobacteriacae family in our study, which is also evident from the studies of Andrew s powlson and (Anthony p.coll., 2010) , that Enterobacteriacae developed resistance to third-generation cephalosporins. Also the presence of MDR organisms was associated with a higher incidence of lower-limb amputation (35-6% versus 11-2% in non MDR infection) with the majority (87.5%) of their amputations being minor. However multivariate analysis indicated that the presence of MDR bacteria did not affect healing time, whereas Game *et al., (*2003) found no evidence for the significant difference in healing time percentage of healed wound in the presence of MRSA. Amoxycilin was found to be resistant in most of the cases but when given with clavulanic acid , found to be effective which was also evident with (Goldstein *et al.,* 1996).

Most of the cases in diabetic foot infections had mono microbial infection (57.39) and less percentage (15-13) had polymicrobial infection with two or more isolates in our study. This is seen in contrast to the work of (Ramakant *et al.,* 2011) were diabetic foot infection usually had polymicrobial infection where mild diabetic foot Infection had mono microbial. Studies from western countries show that Gram positive aerobes are predominant organisms isolated from diabetic foot infections whereas (Gadepalli *et al*., 2006) in their study isolated 28.7% of Gram negative and 13.8% of Gram positive.

(Shankar *et al*., 2005) also reported Gram negative aerobes to be the most frequently isolated pathogens (51.4%) followed by Gram positive aerobes (33.3%). It was also evident from our study that Gram negative bacteria were predominant in podiatric infections, especially *Pseudomonas aeruginosa, Klebsiella pneumoniae and E.coli.*

Pseudomonas aeruginosa was the predominant Gram negative bacteria bacteria isolated in our study and most of the isolates were carbapenemase producers (41.6%).This was also seen in study by (Jain *et al*., 2015) where P*seudomonas* was the predominant pathogen (23%).

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Inducible clindamycin resistance was seen in 11.11% of *Staphylococcus aureus* isolates .Most of the *Enterococcus* species in our study were susceptible to all antibiotic in our study including penicillin which was in contrast to the study of (Gadepalli *et al.,* 2006).

Most of the Enterobacteriacae were found to be sensitive to amikacin, Beta lactams, Beta lactam inhibitors like piperacillin / tazobactum ticarcillin / clavulamic acid, cefeperazon/ sulbactum and imipenem, Game *et al*., (2003).The Emergence of plasmid mediated ESBL among Enterobacteriacae has increased worldwide as 68% of the Enterobacteriacae are ESBL producers. (Baby padmini *et al.,* 2004) have shown that 40% of *Klebsiella pneumoniae* and 41% of *E.coli* isolated to be ESBL producers in their study, which is also evident from our study. *Acinetobacter baumannii* an important pathogen in recent years had developed multi drug resistance and 58% were resistant to imipenem, amikacin and ampicillin sulbactum, thereby increasing the morbidity and mortality. Nearly 46% of all isolates are resistant to all commonly used antibiotics including aminoglycosides, cephalosporins, carbapenemase extended spectrum penicillin and quinolones which was also seen from our study, (Dent *et al*., 2010).

CONCLUSION:

From the present study it is more evident that Diabetic foot infections are more predominant in male patients when compared to females. An increase in occurance of Multi drug resistant organisms has been observed, which is going to be a threat to physicians treating podiatric infections. *Pseudomonas aeruginosa* was the predominant gram negative bacteria isolated in our study and most of the isolates were carbapenemase producers (41.6%). ESBL producers and carbapenemase producers are seen more among Enterobacteriacae isolates, especially *Klebsiella pneumoniae and E.coli*.

Finally, this era is facing a major threat especially in this part of the world where India having more diabetic patients, need for newer antibiotics and curtailing the use of available antibiotics is much needed. This is an alarm and implementation of more Antimicrobial stewardship programmes and infection control measures are the need of the hour.

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