ejpmr, 2016,3(7), 214-220.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA IN DIABETIC FOOT ULCER (DFU) PATIENTS

Acharya Nagarjun Pyde*

Department of Microbiology, Osmania University, Hyderabad, India.

***Corresponding Author: Acharya Nagarjun Pyde**

Department of Microbiology, Osmania University, Hyderabad, India.

Article Received on 30/04/2016 **Article Revised on 19/05/2016** **Article Accepted on 08/06/2016**

ABSTRACT

The aim of his study was to isolate and identify the pathogenic bacteria in patient suffering from Diabetic foot ulcer (DFU) and to determine the antibiotic susceptibility pattern of the isolates. Specimen was cultured using optimal aerobic and anaerobic microbiological techniques. Identification was done by using gram staining and other biochemical methods. Antibiotic susceptibility pattern was done by using Kirby Bauer Disc Diffusion method. We further detected Methicillin resistance *Staphylococcus aureus* (MRSA) by using oxacillin disc, and extended spectrum betalactamase (ESBL). Twenty five organisms were isolated aerobically, out of which 10 (40%) were gram negative and 15 (60%) were gram positive. Among the gram negative isolates Pseudomonas were 8(32%) and Klebsiella 2(8%). Clostridia species was the only anaerobic bacteria which were identified. Most of Diabetic foot infection (DFI) specimens were monomicrobial infection and Predominent bacteria were *S. aureus.* Imipenem was sensitive to most of the isolates and we recomonds to be started empirically based on the clinical sign of the infection.

KEYWORDS: Diabetic foot ulcer, Diabetic foot infection, Antibiotic susceptibility pattern, Aerobic, Anaerobic.

INTRODUCTION

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%.[1] However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria. In fact, within 8-12 years after wide-spread use, strains resistant to multiple drugs become widespread.[2-5] Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment. Antibiotic resistance in bacteria may be an inherent trait of the organism that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.[6, 7] Taxonomically, the genus Staphylococcus is in the Bacterial family Staphylococcaceae, which includes three lesser known genera, Gamella, Macrococcus and Salinicoccus.[8, 9] The best-known of its nearby phylogenetic relatives are the members of the genus Bacillus in the family Bacillaceae, which is on the same level as the family Staphylococcaceae.[10] Staphylococcus aureus forms a fairly large yellow colony on rich medium, S. epidermidis has a relatively small white colony. S. aureus is often hemolytic on blood agar; S. epidermidis is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. S. aureus can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent.[11, 12] Staphylococcus aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis.[13, 14] S. aureus is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices.[15] S. aureus causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream.

MATERIALS AND METHODS

Sample Collection

Samples collected from hospital by wearing sterile gloves. These samples were taken for serial dilutions. Grams staining, Endospore staining test, capsulated staining test, Motility test were carried out for the morphology of cell. Catalase, ONPG, Lysine decarboxylase, Ornithine, Urease, Phenyl alanine deamination, Nitrate reduction, H2S production, Citrate utilization, Voges proskaeurs, Methyl red, Indole and Malonate were suited for biochemical studies.

DISC DIFFUSION METHOD

The disk-diffusion method (Kirby-Bauer) is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. An agar plate is uniformly inoculated with the test organism and a paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations. The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism.

RESULTS AND DISCUSSION

STREAK PLATE TECHNIQUE

Color less colonies were observed over the NAM medium. Colonies of isolated organism on Nutrient agar medium. On Gram staining blue colored cocci were observed. Hence it is a Gram positive Bacterium.

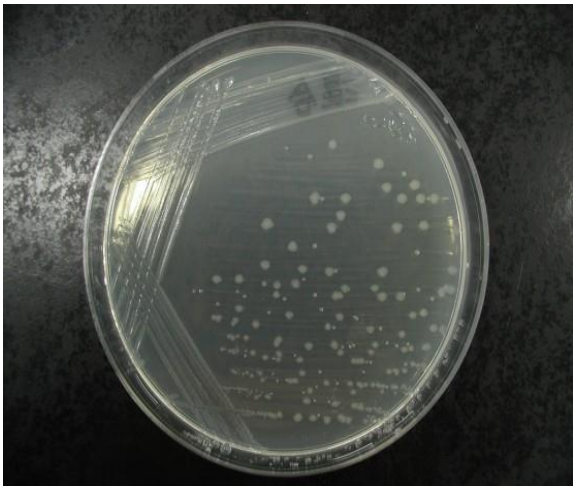


FIGURE 1: streak plate technique

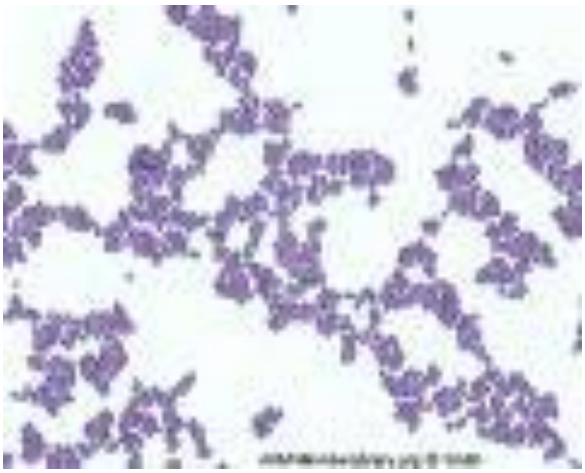


FIGURE 2: gram staining

NEGATIVE STAINING

On negative staining spherical cells occurring in clusters appear transparent (colorless) against a blue-black ground. From above observation isolated organism is capsulated.

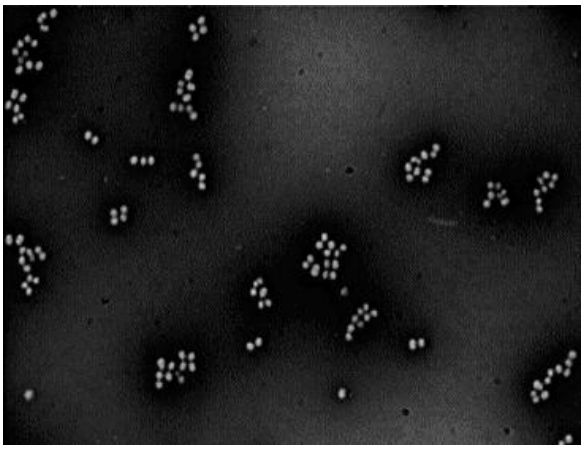


FIGURE 3: Negative staining

FERMENTATION OF CARBOHYDRATES

After 48 hrs of incubation it was observed that sugars that are glucose, sucrose and lactose were utilized by *isolated organism* acid was produced in glucose, lactose and sucrose (Figure 4). As *isolated organism* utilized all the three sugars and produced to the acid so it is positive. Where as *P.vulgarius* did not utilized the any sugars so it is negative.

Table1: Biochemical test for Gram positive cocci

a	catalase	Coagulase
MRSA	+	+
MSSA	+	+
CoNs	+	_

CATALASE ACTIVITY

After 48 hours of incubation when four drops of hydrogen peroxide was added to the slants slow appearance of gas bubbles was observed (Figure 5). After the addition of hydrogen peroxide gas bubbles were observed which the indication of positive test is. Hence *isolated organism i*s positive for catalase test.

FIGURE 4: Fermentation of sugars

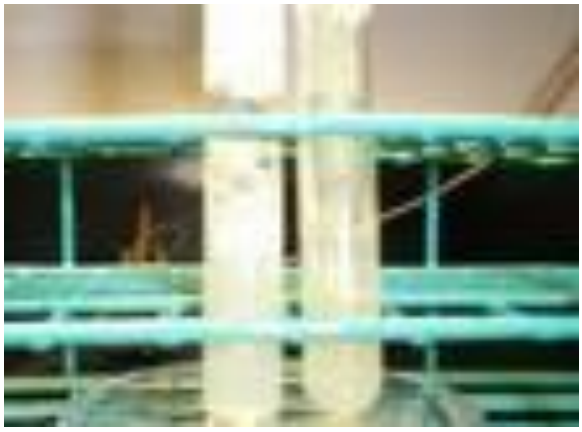


FIGURE 5: Catalase test

HYDROGEN SULPHIDE PRODUCTION TEST

No black coloration along the line of stab inoculation was observed (Figure 6). Black coloration along the line of stab inoculation was not observed. Hence the Organism may be H_2S negative.

INDOLE PRODUCTION TEST

Development of cherry (deep) red color in the top layer of the tube is not observed. Hence, *isolated organism* an indole – negative bacterium (Figure 7). As development of cherry red color is not observed in the top layer of the tube so isolated organism is negative test.

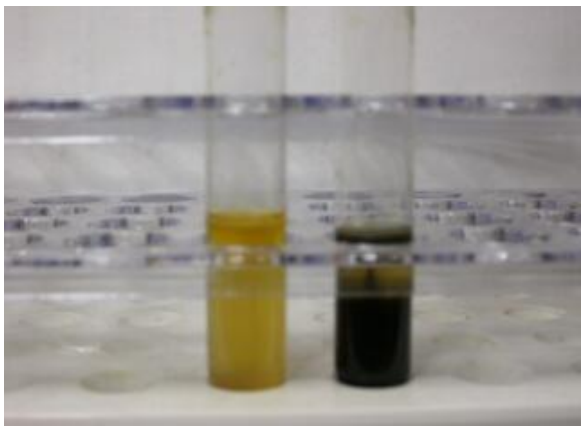


Fig 6: H_2S test

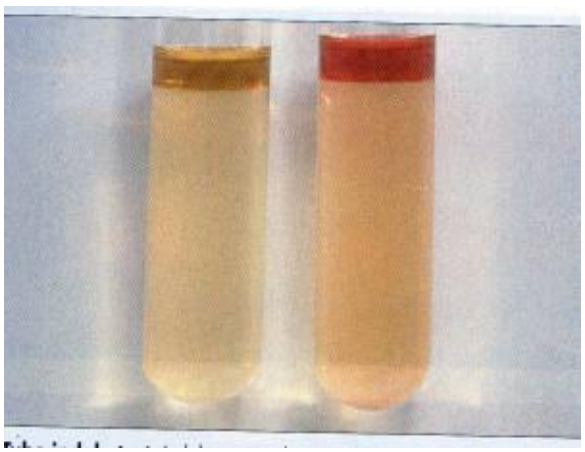


Figure 7: Indole test

METHYL-RED AND VOGES-PROSKAUER TESTS

The tubes in which methyl red was added no red color was observed in the V-P test tubes when V-P reagents I & II were added no red color was observed (Figure 8 & 9). As in the methyl red test red color is observed hence, it is positive test. In the VP test, red color is not observed hence, it is negative test.

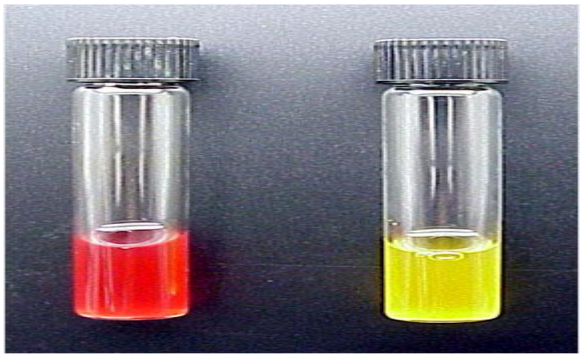


Figure 8: Methyl-Red Test

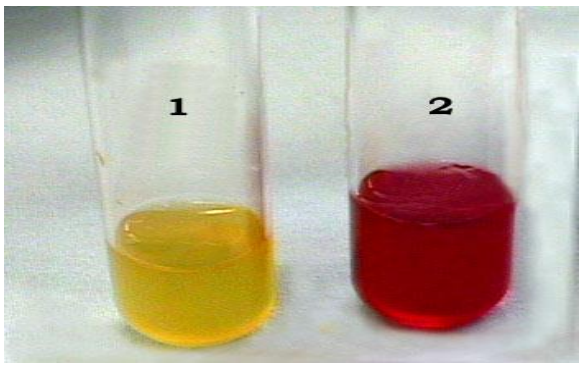


Figure 9: Voges-Proskeur Test

CITRATE UTILIZATION TEST

After 48 hours of incubation it was observed that there is no change in the medium colour. From the above observation it is said that *isolated organism* is negative to this test.

UREASE TEST

After 48 hours of incubation it was observed that there is no change in the medium. From the above observation it is said that *isolated organism* shows positive test.

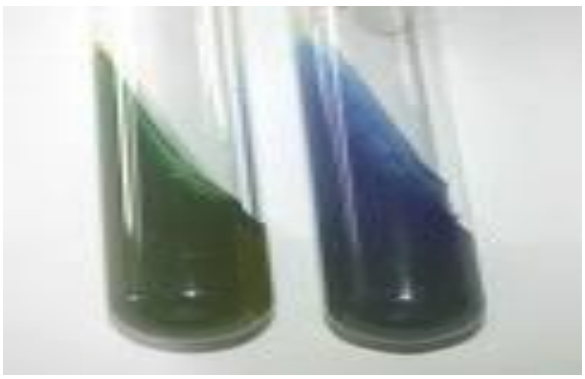


Figure 10: Citrate test.

Fig 11: Ureasse test.

Table 2: Biochemical test for Gram negative bacilli

Isolates	oxidase	Indole	citrate	urease	TSI
Pseudomonas	+	_	+	_	K/A
Klebsiella	_	_	+	+	A/A

DISC DIFFUSSION METHOD

Antibiotic susceptibility pattern was done by using Kirby Bauer Disc Diffusion method. We further detected Methicillin resistance *Staphylococcus aureus* (MRSA) by using oxacillin disc, and extended spectrum betalactamase (ESBL). Twenty five organisms were isolated aerobically, out of which 10 (40%) were gram negative and 15 (60%) were gram positive. Among the gram negative isolates Pseudomonas were 8(32%) and Klebsiella 2(8%). Clostridia species was the only anaerobic bacteria which were identified. Most of Diabetic foot infection (DFI) specimens were monomicrobial infection and Predominent bacteria were *S. aureus.* Imipenem was sensitive to most of the isolates and we recomonds to be started empirically based on the clinical sign of the infection.

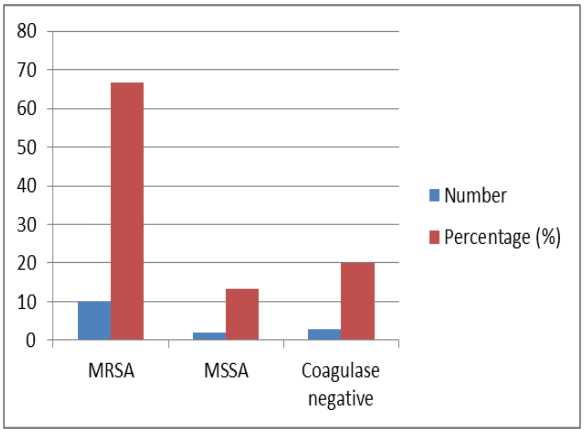
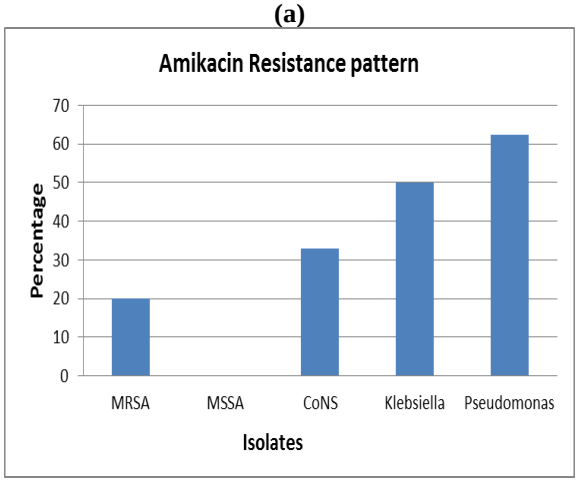


Fig 12: Number of Staphylococcus and percentage

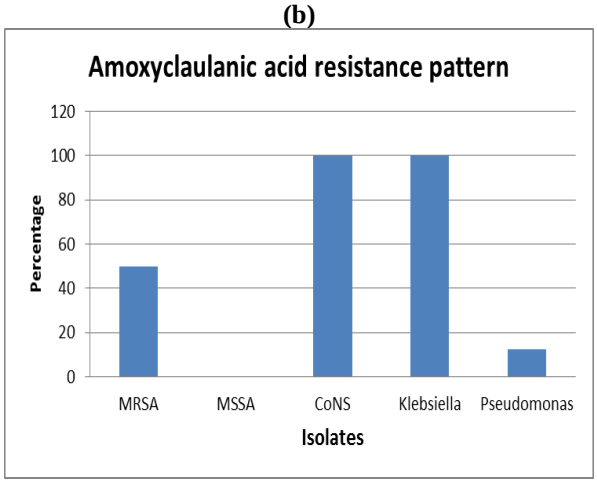
Table 3: Percentage of antibiotic resistance pattern

Antibiotic	MRSA	MSSA	CoNS	Klebsiella	Pseudomonas
Amikacin	20	0	33	50	62.5
Amoxyclaulanic acid	50	0	100	100	12.5
Ampicillin	90	50	66	100	62.5
Ceftriaxone	20	0	100	50	12.5
Cephoxitin	60	0	66	100	12.5
Erythromycin	100	0	66	100	87.5
Gentamicin	10	50	50	50	50
Imipenem	10	0	0	0	12.5
Oxacillin	70	100	33	100	100
Penicillin G	100	100	100	100	100
Teicoplanin	40	0	33	100	100
Tricarcillin clavunic acid	10	0	66	50	75
Vancomycin	10	50	33	50	37.5
Ciprofloxacin	40	0	66	50	37.5

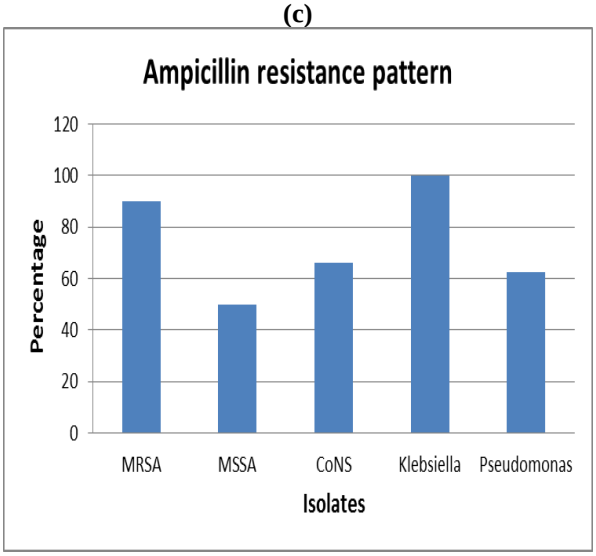
(a)



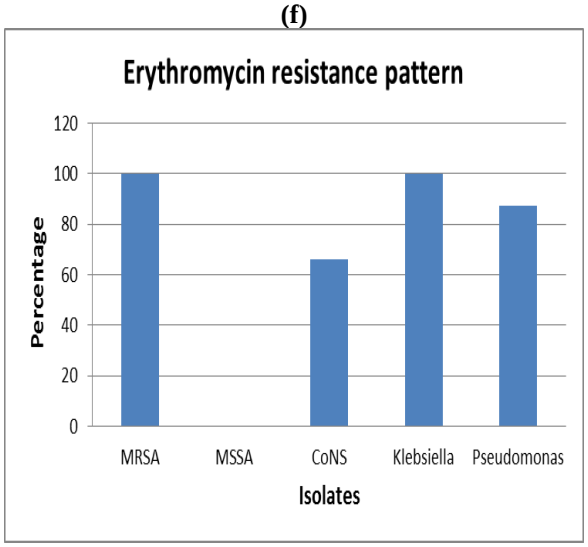
(b)



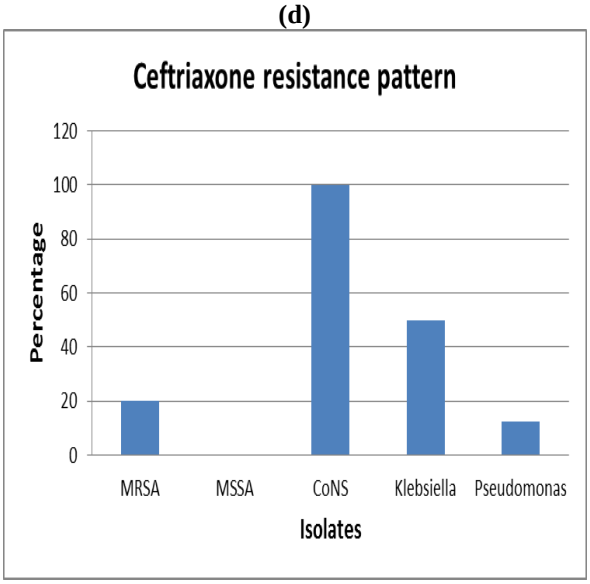
(c)


Ampicillin resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

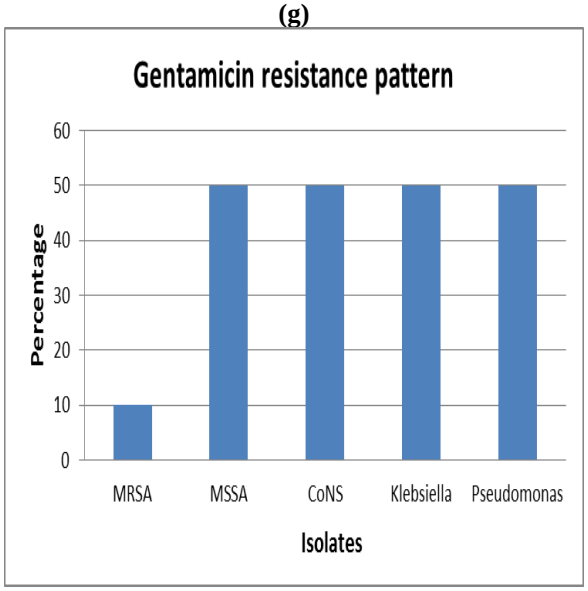
(f)


Erythromycin resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

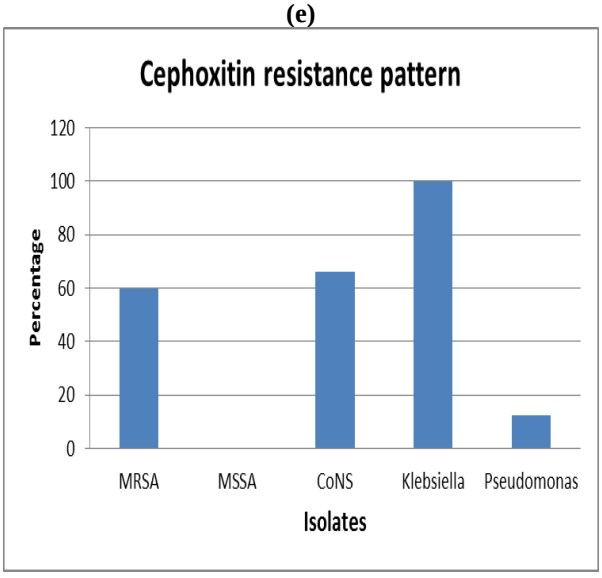
(d)


Ceftriaxone resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

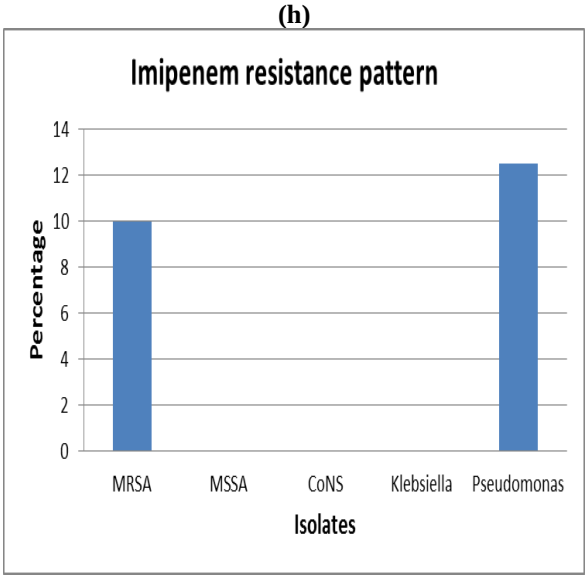
(g)


Gentamicin resistance pattern
Percentage
60
50
40
30
20
10
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

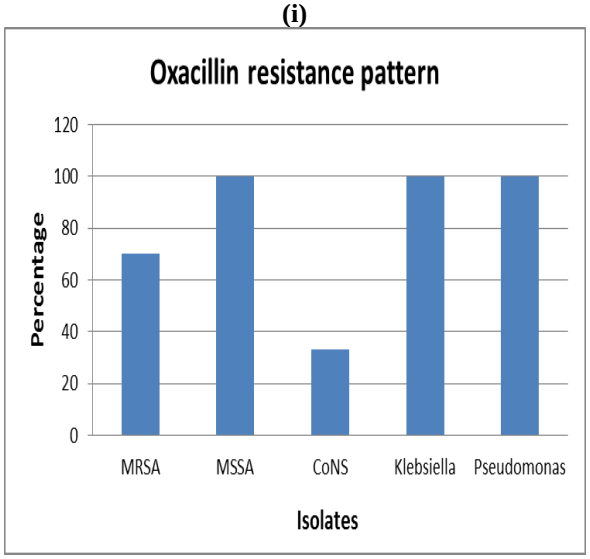
(e)


Cephoxitin resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

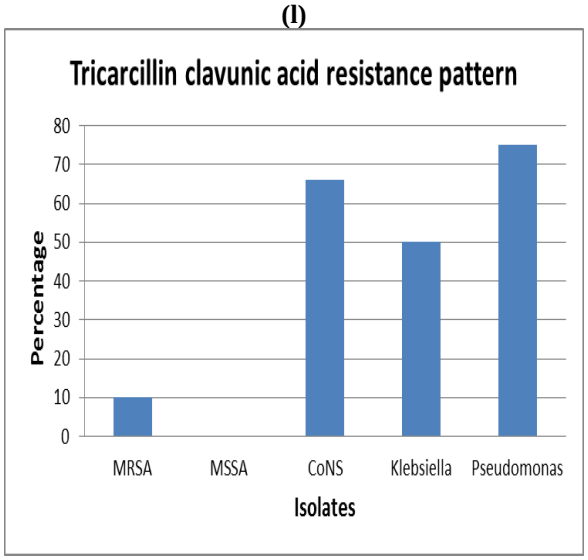
(h)


Imipenem resistance pattern
Percentage
14
12
10
8
6
4
2
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

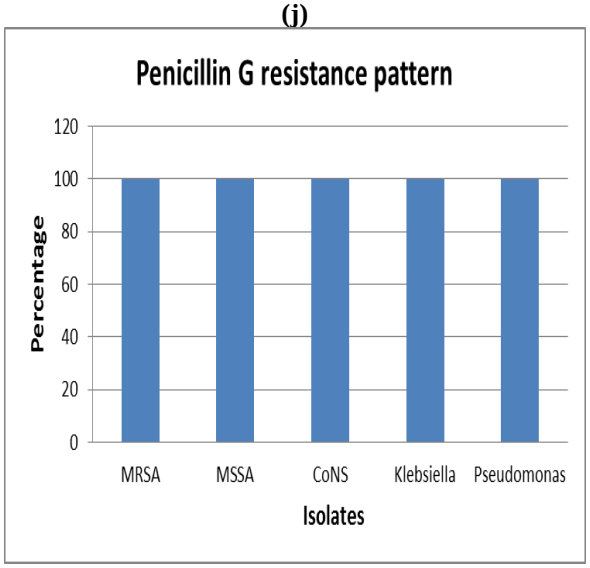
(i)


Oxacillin resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

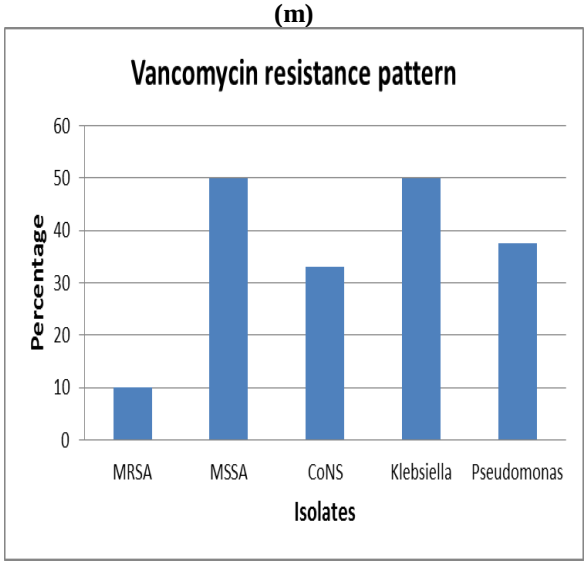
(l)


Tricarcillin clavunic acid resistance pattern
Percentage
80
70
60
50
40
30
20
10
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

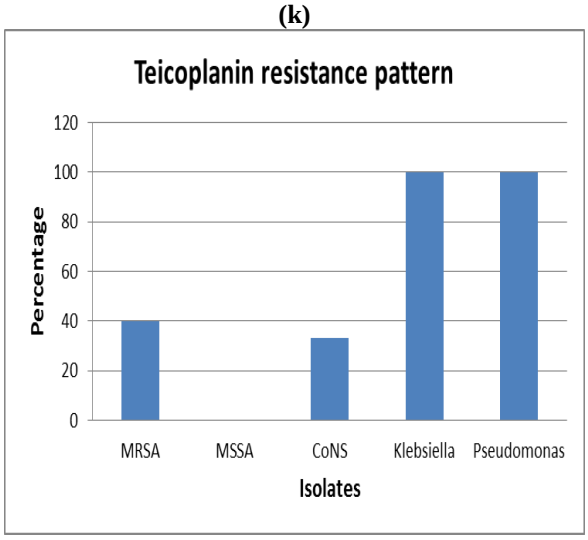
(j)


Penicillin G resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

(m)


Vancomycin resistance pattern
Percentage
60
50
40
30
20
10
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

(k)


Teicoplanin resistance pattern
Percentage
120
100
80
60
40
20
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

(n)

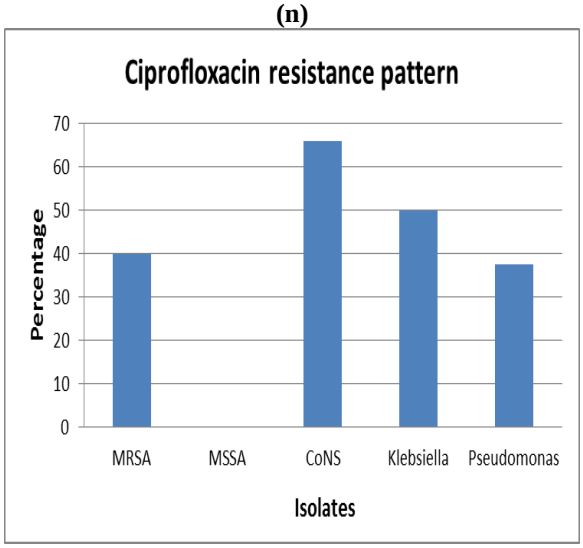

Ciprofloxacin resistance pattern
Percentage
70
60
50
40
30
20
10
0
MRSA
MSSA
CoNS
Klebsiella
Pseudomonas
Isolates

Table 4: Susceptibility pattern of (anaerobes) clostridia species

Antibiotics	Concentration (µg/ml)	sensitive	resistance
Penicillin G	10	+	
Ampicillin	10	+	
Tricarcillin clav	75/10		_
Oxacillin	1		_
Erythromycin	1		_
Vancomycin	30	+	
Ciprofloxacin	5	+	
Imipenem	10	+	
Ceftriaxone	30		_
Amikacin	30		_
Cloxacillin	1		_

CONCLUSION

The prevalence of Gram-positive infection was higher in diabetic foot patients from our study. There were rare cases of poly-microbial infection. Imipenem showed the highest sensitivity and it may be started empirically based on the clinical characteristics of infection, and can be changed subsequent to learning the results from a definitive bacteriological study. Sometimes culture reports are negative despite the deteriorating condition of the wound and other clinical findings. In such cases, application of molecular techniques may help to identify microorganisms in the diabetic foot wound and to choose suitable antibiotics against them.

REFERENCES

1. Zighelboim S, Tomasz A. Penicillin-binding proteins of multiply antibiotic-resistant South African strains of Streptococcus pneumoniae. Antimicrob Agents Chemother. 1980 Penicillin-binding proteins of multiply antibiotic-resistant South African strains of Streptococcus pneumoniae.
2. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science., 1988 Jan 29.
3. Dougherty TJ, Koller AE, Tomasz A. Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant Neisseria gonorrhoeae. Antimicrob Agents Chemother. 1980 Nov; 18.
4. Parr TR Jr, Bryan LE. Mechanism of resistance of an ampicillin-resistant, beta-lactamase-negative clinical isolate of Haemophilus influenzae type b to beta-lactam antibiotics. Antimicrob Agents Chemother., 1984 Jun; 25.
5. Latorre C, Juncosa T, Sanfeliu I. Antibiotic resistance and serotypes of 100 Streptococcus pneumoniae strains isolated in a children's hospital in Barcelona, Spain. Antimicrob Agents Chemother., 1985 Aug; 28.
6. Song MD, Wachi M, Doi M, Ishino F, Matsuhashi M. Evolution of an inducible penicillin-target protein in methicillin-resistant Staphylococcus aureus by gene fusion. FEBS Lett., 1987 Aug; 31
7. Brakstad OG, Aasbakk K, Maeland JA. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol., 1992 Jul;30
8. Henze, UU, Berger-Bachi, B. Staphylococcus aureus penicillin-binding protein 4 and intrinsic beta-lactam resistance. Antimicrob Agents Chemother., 1995; 39: 2415.
9. Tomasz, A, Drugeon, HB, de Lencastre, HM, et al. New mechanism for methicillin resistance in Staphylococcus aureus: clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. Antimicrob Agents Chemother., 1989; 33: 1869.
10. Chambers, HF, Sachdeva, M, Kennedy, S. Binding affinity for penicillin-binding protein 2a correlates with in vivo activity ofß-lactam antibiotics against methicillin-resistant Staphylococcus aureus. J Infect Dis., 1990; 162: 705.
11. Bush LM, Calmon J, Cherney CL, et al. High-level penicillin resistance among isolates of enterococci: implications for treatment of enterococcal infections. Ann Intern Med., 1989; 110: 515–20.
12. Hartman BM, Tomaz A. Low-affinity penicillin binding protein associated with beta-lactam resistance inStaphylococcus aureus. J Bacteriol., 1984; 158: 513.
13. Voyich, J. M., Braughton, K. R., Sturdevant, D. E., Whitney, A. R., Said-Salim, B., Porcella, S. F., Long, R. D., Dorward, D. W., Gardner, D. J., Kreiswirth, B. N., Musser, J. M., DeLeo, F. R. Insights into Mechanisms Used by Staphylococcus aureus to Avoid Destruction by Human Neutrophils. *J. Immunol.*, 2005; 175: 3907-3919
14. Amon RB, Dimond RL. Toxic epidermal necrolysis. Rapid differentiation between staphylococcal- and drug-induced disease. Arch Dermatol., 1975 Nov; 111(11): 1433–1437.
15. de Azavedo J, Arbuthnott JP. Prevalence of epidermolytic toxin in clinical isolates of Staphylococcus aureus. J Med Microbiol., 1981 Aug; 14(3): 341–344.