A STUDY ON MULTIPLE DRUG RESISTANT BACTERIAL ISOLATES IN FOOT ULCERS IN DIABETES WITH SPECIAL REFERENCE TO *bla*$_{KPC}$ and *bla*$_{NDM}$

Dissertation Submitted to
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M.D.(MICROBIOLOGY)
BRANCH –IV

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY
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CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON THE MULTIPLE DRUG RESISTANT BACTERIAL ISOLATES IN FOOT ULCERS IN DIABETES WITH SPECIAL REFERENCE TO DETECTION OF *bla*$_{KPC}$ AND *bla*$_{NDM}$ "** is the bonafide original work done by **DR. VANDANA VIJAYETA KIRO**, M.D. Postgraduate in Microbiology (2014-2017), under my overall supervision and guidance in the Department of Microbiology , Stanley Medical College , Chennai in partial fulfillment of the regulations of the The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D. Degree in Microbiology (Branch IV).**

DR.ISSAC CHRISTIAN MOSES.M.D., FICP, FACP
DEAN, GOVT.STANLEY S MEDICAL COLLEGE,
CHENNAI -600001

DR.ROSY VENNILA
PROFESSOR & HOD,
DEPARTMENT OF MICROBIOLOGY
CHENNAI-600001

DECLARATION

I solemnly declare that this dissertation **" A STUDY ON THE MULTIPLE DRUG RESISTANT BACTERIAL ISOLATES IN FOOT ULCERS IN DIABETES WITH SPECIAL REFERENCE TO DETECTION OF *bla*$_{KPC}$AND *bla*$_{NDM}$"** is the bonafide work done by me during my post graduate course in M.D.Microbiology (2014-2017) at the Department of Microbiology , Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr. SELVI,M.D**., Professor of Microbiology, Govt. Stanley Medical College , Chennai, 600001. The dissertation is submitted to **The Tamil Nadu Dr.M.G.R. Medical University**, Chennai in partial fulfilment of the University regulations for the award of degree of **M.D. Branch IV Microbiology** examinations to be held in April 2017.

Place: Chennai **Dr. VandanaVijayeta Kiro**

Date:

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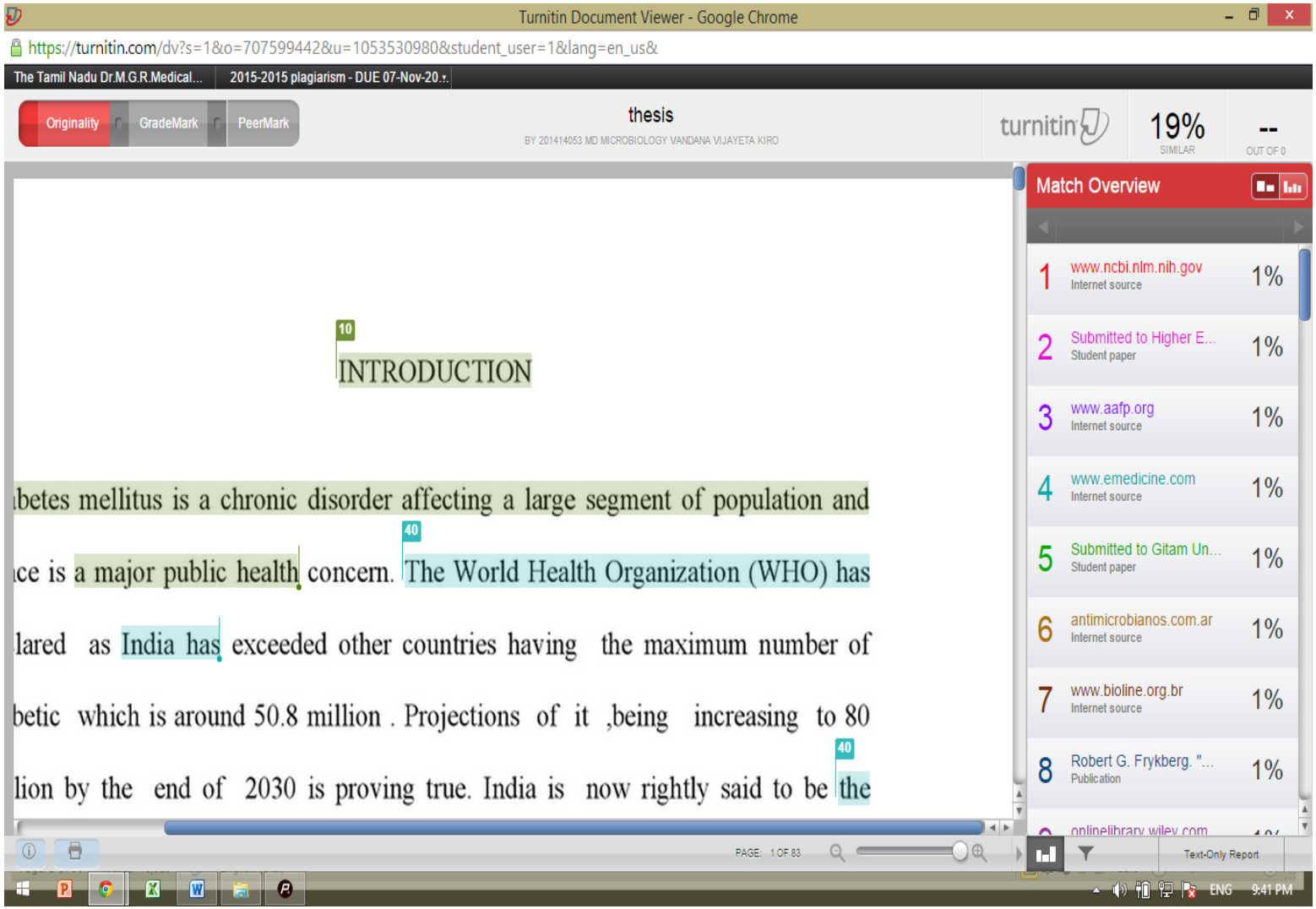
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INTRODUCTION
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lion by the end of 2030 is proving true. India is now rightly said to be the
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LIST OF ABBREVIATIONS USED

AFB	Acid fast bacilli
AGEs	Advanced glycation end products
ATCC	American type culture collection
ATP	Adenosine triphosphate
BKA	Below knee amputation
bla	Beta-lactamase gene
CLSI	Clinical and laboratory standards institute
DHP	Dehydropeptidase
ESBL	Extended spectrum beta- lactamase
HbA_1c	Glycosylated hemoglobin
HLGR	High level gentamicin resistance
IP	In-Patient
KPC	Klebsiella pneumoniae carbapenemase
MBL	Metallo beta lactamase
MDROs	Multiple drug resistance organisms
MIC	Minimum inhibitory concentration
NDM	New Delhi metallo –beta- lactamase
OGTT	Oral glucose tolerance test
OP	Out patient
PBP	Penicillin binding proteins
PHC	Primary health care
PCR	Polymerase chain reaction

CONTENTS

INTRODUCTION

1. INTRODUCTION

Diabetes mellitus is a chronic disorder affecting a large segment of population and hence is a major public health concern. The World Health Organization (WHO) has declared that India has exceeded other countries in having the maximum number of diabeticswhich is around 62 million.[78]It has been projected to increase to 80 million by the end of 2030 thus India is rightly said to be the "Diabetic Capital of the World".[1]There has been a gradual increase in the prevalence of diabetes mellitus in rural areas of India since 1975 withsome studies pointing to low birth weight as one of the contributory factors..[2]WHO Expert committee on diabetes has issued a clarion to workers around the World to carry out epidemiological survey of diabetes with a view to identify ,before its too late ;the cultural, social and other factors which may contribute to diabetes and its complications.

The longevity of the diabetic population is increasing and has lead todiscovery of novel complications related to this chronic ailment**.**[4,5]Among the complications of Diabetes ,foot problems are the most common cause of non –traumatic limb amputation.[4] There is a 10-fold greater risk of hospitalization for soft tissue and bone infections in diabetics than non-diabetics.

Several studies have been reported on the bacteriology of bacteriology of Diabetic Foot Ulcers(DFU s) over the past 30 years but results have variations and are often contradictory.[6] In some studies Diabetic foot infections are primarily polymicrobial,and rationale management of these require an appropriate antibiotic selection ,on the basis of culture and antimicrobial susceptibility testing results.The

patterns of the infection too is not constant in patients and therefore repeated evaluation of microbial characteristics and their antibiotic sensitivity is necessary for providing appropriate antibiotics. Studies which were done in 1970s demonstrated Gram positive cocci with Staphlylococcus aureus as the main pathogen and aerobic Gram negative bacilli in chronic and previously treated wounds.In recent years ESBLs and MRSA are causing substantial problem. [7] Infectionprogression in diabetic foot is a result of suppressed immune status ,metabolic derrangements,delayed diagnosis ,underestimation of the extent of infection ,or suboptimal (if not ;inappropriate) antimicrobial therapy.

Antibiotic resistance in diabetic foots has become a major concern as there are very few or no alternatives left for the treatment.Infections with MDROs has lead to inadequate or delayed antimicrobial therapy and are also associated with poorer patient outcomes.[8-11]It increases the duration of hospital stay,cost of management as well as morbidity and mortality.There is a dearth of data on MDROs and especially evolving carbapenemase resistance; if any from this part of the world.

This study is mainly focused on resistance in carbapenems in pathogens associatedwith Diabetic foot ulcers in our tertiary care centre .Resistance in carbapenems is mainly mediated by Metallo-β-lactamases.Around ten years back the genes encoding

MBL were mainly present in the non-fermenting Gram negative bacterias likePseudomonas aeruginosa and Acinetobacter species.Now several studies done on this

suggests that MBLs have disseminated at an alarming rates to members of family Enterobacteriaceae which has been seen in epidemics of *bla*$_{KPC}$ clones in USA , Europe and the worldwide epidemic with *bla*$_{NDM}$ producing Gram negative bacterias.It's from North Carolina in 2001 that KPC producing enterobacteriacea was first reported.Further outbreaks related to this organism was reported from North Eastern United States.Nine out of 602 Klebsiella pneumoniae isolates were found to have the KPC gene in a study in New York between 2002-2003.In the following year 20 isolates producing KPC were identified.Since then KPC producing isolates have become more widespread in United States.Now KPC producing enterobacteriacea have been reported from other parts of the world- probably associated with receipt of medical care in United States indicating intercontinental spread of these organisms.

In 2008,NDM -1 was first detected from a Swedish patient of Indian origin in Klebsiella pneumoniae.Later theses were reported from India, Pakistan,United Kingdom,United States,Canada and Japan. The *bla*NDM-1 produces NDM-1gene that hydrolyses and inactivates carbapenems.

Though initial antibiotic therapy for most patients must be selected empirically,it should be largely based on the assessment of severity and knowledge of the local microbial epidemiology.

AIM AND OBJECTIVES

2.AIM AND OBJECTIVES

AIM

To determine the bacterial profile and antimicrobial resistance pattern of infected diabetic foot ulcers in a tertiary care centre and focussing on carbapenem resistant isolates .

OBJECTIVES

- To isolate the disease causing aerobic bacteria from infected foot ulcers of diabetic patients.
- To determine the monomicrobial or polymicrobial nature of the disease.
- To perform antibiotic susceptibility tests and further confirmatory tests for MRSA, ESBL ,Amp C and Carbapenem resistance.
- To perform Polymerase Chain Reaction for Carbapenem resistant isolates to detect KPC and NDM genes.

To guide the clinicians in the appropriate usage of the antibiotics and primarily in Empirical Therapy.

REVIEW OF LITERATURE

3.REVIEW OF LITERATURE

HISTORY OF DIABETES

It was in 1550 BC , diabetes like illness characterized by polyuria was described in Egyptian Papyrus .Charaka Samhita which was written in the pre Buddhist period (600 BC) contains a graphic description of the disorder .[12]

By 400 BC ,Sushruta , an astute clinician and a deft surgeon supplemented the earlier information and presented a comprehensive picture of diabetes, its possible predisposing factors; clinical features, course and complication along with principles of medical care and surgical intervention wherever necessary.[12] This disorder was called MADHUMEHA (rain of honey) because of sweet taste of urine attracting ants and insects.[12]Chinese physician Neizling (400 BC) described the important clinical features of Diabetes and Celsius of Greece(30 BC-50AD) further described in greater detail.[12]

Although Demetrios (several century BC) has been known to have first linked the excessive water drinking and polyuria of the disorder to “flowing through a siphon”; Arateus ,first centuary AD according to some historians of Cappedocia is generally believed to have coined the term “DIABETES” conveying the same meaning.[12]

During Medieval Period(600-1500BC)progress on diabetes was nominal.

With the advent of modern era Thomas Wills (1674-75 AD) rediscovered the

sweetness of urine of patients with diabetes.Matthew Dobson (1776) evaporatedsamples of urine and observed that the residue looked and tasted like brown sugar.Further he realized that serum from patients of Diabetes was also sweet.[12]Cullen (1710-90) added "mellitus"(mel-honey) to diabetes to constitute the full name that the disorder bears till far. [12] More detailed knowledge of the metabolism of sugar had to wait the studies of Claude Bernard.

The discovery of insulin in 1921 ranks with the discovery of penicillin and streptomycin .Following the introduction of insulin when death in coma became a less frequent outcome of disease, attention began to be turned to the long term complications and it was recognized that deaths from vascular disease had become common.

John Rollo (1798) first recognized an association between diabetes and symptoms in the limbs. His patient had pain and paraesthesia and described as "lumbago and sciatica is so great a degree as to nearly deprived of the use of the lower limbs."[13]

Between 1850-1870 both gangrene and plantar ulcers were recognized as complications of diabetes.In 1888 Hunt collected 72 cases of diabetic gangrene and concluded that "gangrene in diabetics is something more than coincidence".[13]

In 1887, Pyrce; for the very first time reported on the association of neuropathy and vascular disease with foot ulcer.[13]

EPIDEMIOLOGY

The burden of diabetes mellitus is increasing worldwide in both the developing and developed world. Increasing urbanization and longevity of diabetic patients is one of the contributory factors of the burden of disease.People of all age groups and races are affected by Diabetes.

The estimated number of people with diabetes worldwide is expected to double from 171 million in 2000 to 366 million in 2030.[1] In the population of adults older than 20 years of age ,the prevalence was higher (6.3%) in the developed compared to the developing world(4.1%) in 2000. Although the developing world will experience the fastest increase in numbers of people with diabetes, the prevalence will continue to rise at 8.4% in developed countries until 2030 compared to the 6.0% rate of rise for developing countries. It has been estimated that 45 to 65 years of age of population will be affected in developing countries compared to > 65 years aged in developed countries.[1] ·India at present has the largest number of diabetic population approximately 62 million and it is expected to explode beyond 83 million by the year 2030.[14]

DEFINITION AND CLASSIFICATION

DIABETES MELLITUS is a metabolic disorder where persistent hyperglycemia is observed resulting from defects in pancreatic activity. Either the insulin secretion is depleted or action of insulin is hampered.

Type 1 diabetes

In this condition there is absolute deficiency of insulin due to autoimmune destruction of beta cells. The main genetic abnormality is in the major histocompatibility complex on chromosome 6. This usually presents at younger age.

Type 2 Diabetes

This occurs as a result of insulin resistance or insensitivity of tissues to insulin and relative insulin deficiency and may later lead to absolute insulin deficiency. Obesity is a major risk factor. Insulin resistance seems to be caused by the toxic effects of increased lipid accumulation, which interferes with insulin signaling processes between receptor activation and cellular effects.

Adverse effects of Disturbed Carbohydrate Metabolism

Disturbance in carbohydrate metabolism leads to hyperglycemia, ketosis ,polyuria, polydipsia, polyphagia, glycosuria, polyuria and polydipsia.

Adverse effects of Disturbed lipid metabolism

Increased fat breakdown leads to increased formation of ketone bodies,which leads to ketosis and acidosis. The ketone bodies include aceto acetate, acetone and β-hydroxybutyrate.The pH drop due to acidosis and the increased hydrogen ion concentration stimulates the respiratory centre causing the characteristic rapid and regular deep breathing called kussmaul breathing. Acidosis and dehydration can lead to coma and death .The hormone sensitive lipase converts triglycerides to FFA and

glycerol.Insulin has an inhibitory effect on this hormone.In the absence of insulin, FFA levels greatly increase .The FFA is catabolized to acetyl CoA in the liver and other tissues and the excess acetyl CoA is converted to form ketone bodies.

Adverse effects of disturbed protein metabolism

There is decreased protein synthesis and increased protein breakdown leading to protein catabolism and muscle wasting .There is increased plasma amino acids and nitrogen loss in urine. All leads to negative nitrogen balance and protein depletion. Protein depletion causes poor resistance to infections. There is increased gluconeogenesis in the liver since amino acids are converted to glucose.

Adverse effects of Disturbed Cholesterol metabolism

Diabetics are more prone for myocardial infarction and stroke because the cholesterol levels are elevated causing atherosclerosis .Augmented production of VLDL in the liver or decreased removal of LDL and VLDL from the blood stream leads to the increased level of LDL and VLDL in plasma supposedly causes the above phenomenon.

Further Complications

In course of time; prolonged hyperglycemia and associated metabolic aberrations result in tissue toxicity manifested as accelerated atherosclerosis, reno retinal microangiopathy and neuropathy leading to a variety of vascular ,neurological and focal complications.

Duration of diabetes and uncontrolled plasma glucose levels are related to microvascular comlications in diabetics. Increased intracellular glucose levels cause formation of sorbitol due to activation of the enzyme aldose reductase .Sorbitol decreases sodium potassium ATPase activity .The increased intracellular glucose also non enzymatically attaches to protein amino groups to form amadori products. The amadori products form advanced glycosylation end products (AGE s) which cause cross linkage of matrix proteins thus, causing damage to blood vessels. There isalso increased accumulation of sorbitol and fructose in Schwann cells due to hyperglycemia .This can interfere with its structure and function.

Criteria for diagnosis of Diabetes mellitus[15]

(1) HbA1c $\geq$ 6.5%.

OR

(2) Fasting Level ≥126 mg/dl. Patient advised for fasting for 8 hours before the test.

OR

(3) 2- Hour plasma glucose ≥200mg/dl during an OGTT. The test needs to be performed using a glucose load containing the equivalent of 75 gm anhydrous glucose dissolved in water.

OR

(4) Random plasma glucose ≥200mg/dl associated with symptoms of hyperglycemia.

Random is defined as any time of the day irrespective of the time since last meal.The classic symptoms of hyperglycemia include polyuria ,polydipsia and unexplained weight loss.

Diabetic foot ulcers:

Foot ulcer: A full thickness wound below the ankle, irrespective of duration.

Ulcer Episode: The interval from ulcer identification to healing.

Non-traumatic lower limb amputation:The removal of a terminal ,non viable portion of a limb.

Aetiology of foot ulceration:

It is usually multifactorial. Majority of the diabetic foot ulceration are due to clinical triad of peripheral sensory neuropathy, trauma and deformity. Infection,callus formation and edema are the other factors implicated in the causation. Infection plays a major role in countries like India where bare foot walking is prevalent. Most of the risk factors for foot ulcer are also predisposing factors for amputation, because ulcers are the primary causes leading to amputation.

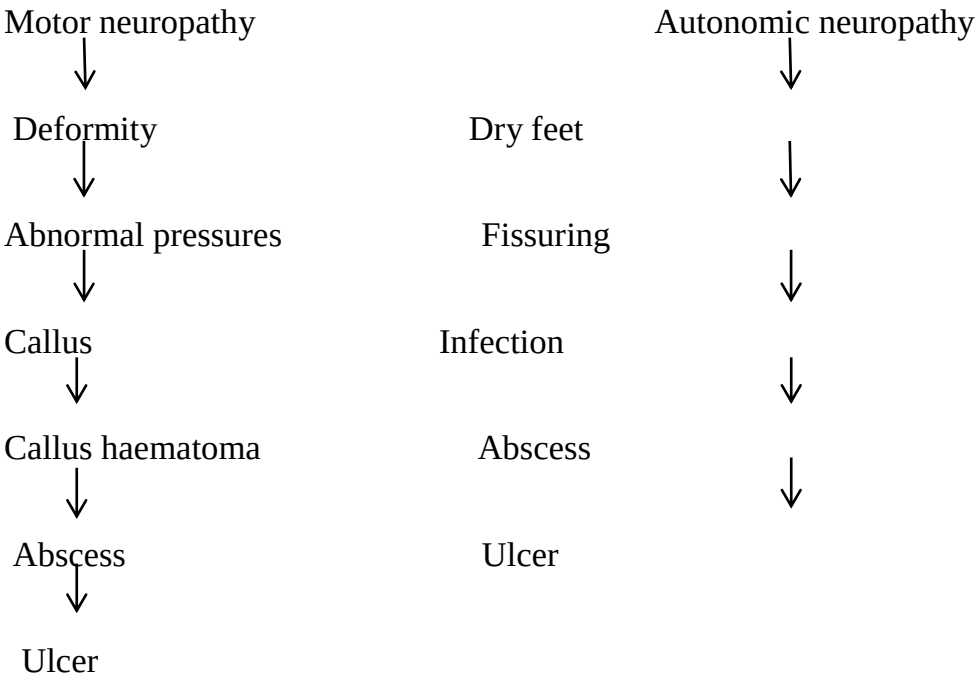
Recognized risk factors for diabetic foot ulceration are as follows:[16]

Absence of protective sensation due to peripheral neuropathy ; arterial insufficiency ; foot deformity and callus formation resulting in focal areas of high pressure ; autonomic neuropathy causing decreased sweating and dry, fissured skin; Limited joint mobility ; Obesity ; Impaired vision ; poor glucose control leading to impaired wound healing ; poor footwear that causes skin breakdown or inadequate protection form high pressure and shear forces ;history of foot ulcer or amputation.

Patho-biology of Ulceration in foot

Neuropathy whether sensory, motor or autonomic may lead to ulceration through various mechanisms:

Diabetic foot ulceration occurs through either of the two pathways:[17]



Origin: MOTOROrigin:DYSAUTONOMIA

Foot ulcer evaluation:

To plan a proper management a thorough evaluation of any ulcer is important.An adequate description of ulcer characteristics like appearance ,location ,size ,depth are important for observing the progress during treatment.

Appearance and dimensions of ulcer are noted and it is probed with a sterile probe. Gentle and cautious probing detects sinus formation ,undermining of ulcer margins and dissection of the ulcer into tendon sheaths ,bone or joints .A positive probe to probe finding suggests highly of steomyelitis. Failure in diagnosing underlying osteomyelitis often leads to failure of wound healing. The odour and exudates; if present and the extension of cellulitis must be noted.

Adverse signs leading to amputation are i)Cellulitis which extends beyond 2 cm from ulcer perimeter ii)deep abscess iii)osteomyelitis iv)critical ishchemia.[18]

Aerobic and anaerobic cultures should be taken when signs of infection e.g. purulence and inflammation are present. Cultures are best taken from purulent drainage or curetted material from deep within the wound.

Classification of Diabetic Foot Ulcers: The classification system to quantify the ulcer severity was developed by Wagner–Meggit.(fivegrades).[19]

GRADES	LESIONS
GRADE 0	Foot symptoms like pain,only
GRADE 1	Superficial ulcers
GRADE 2	Deep ulcers
GREDE 3	Ulcers with bone involvement
GRADE 4	Forefoot gangrene
GRADE 5	Whole foot gangrene

Infections in Diabetic Foot Ulcer:

Though infection is usually not the direct cause of ulceration; it is certainly a major determinant of both treatment and prognosis for any foot lesion.

The inherent susceptibility of the diabetic foot infection is directly attributable to neuropathy or vascular disease acting alone or in combination. Additionally certain impairments in leukocyte function and wound repair is implicated in the contribution of the altered host response to injury seen in patients with diabetes.[20]

Studies have revealed that there was a significant reduction in leucocytes phagocytosis in poorly controlled diabetes and that with correction of hyperglycemia the microbicidal rates were improved although less than normal.[21]

Diabetic patients tolerate infection poorly, infection adversely affects diabetes control, and uncontrolled diabetes adversely affects the infection. [22]

Worldwide it has been accepted that the Diabetic foot infections are polymicrobial and frequently harbours anaerobes.[22-27] The usual organisms isolated are the aerobic Gram positive cocci including Staphylococcus ,Streptococcus species and Enterococci.Gram Negative pathogenscommonly encountered include Proteus species, Escherichia coli,and other variousspecies of Enterobacteriaciae .Pseudomonas; interestingly is encountered much less often than these gram negative bacilli.Anaerobes ,although rarely occurring in pure culture ,can be recovered in over 80 percent of these patients and will often exhibit heavier growth than the accompanying aerobic pathogens.[27]

Bacteroides species are the most important organisms of this group and have been the only anaerobes cultured from blood in diabetic patients developing bacteremia subsequent to foot infections.[28]

Deep tissue specimens should be obtained by avoiding draining sinuses to provide reliable culture indicative of the true underlying pathogens.Empiric antibiotic selection need to be based upon the severity of infection ,status ofthe patient and the knowledge that these infections are polymicrobial in over 70 percent of patients cultured.[29,30] Hospitalisation and parenteral antibiotics are necessary for moderate or serious infections involving deeper structures when there are systemic signs of toxicity or fever ,or when the infection progresses despite appropriate out–patient therapy.[31] Vascular reconstruction if indicated ,is done following the initial drainage procedures after infection has been brought under control.[22]

Treatment:

(1)Debridement [32]: Debridement of all necrotic tissues, calluses and fibrous tissue is the basic treatment to be followed. Soaking ulcers need to be avoided as the neuropathic patient can easily get scalded by hot water. Although numerous topical medications and gels are available for ulcer care relatively very few have proved to be more efficacious than saline wet-to dry dressings. Semipermeable films,foams and hydrocolloids provide a warm and moist environment that is most conducive to wound healing.

(2)Antibiotic therapy:

In most instances the initial choice of antimicrobial agents is made prior to knowledge of microbiologic culture results. The decision will therefore have to be based on the most likely probabilities, in turn based on what previous studies have yielded in the past, and on the prevailing microbial flora seen in one's particular institution. The severity of the illness may also play a role in the choice of antimicrobial therapy and combination therapy is most commonly utilized in the presence of systemic manifestations of sepsis and of severe local infections. The antimicrobials may be changed later, depending on the clinical response and the culture and sensitivity reports. If the infection is relatively mild (as in case of minimal cellulitis ,mild purulence in a pre-existent ulcer ,or an infected blister) and no clinical manisfestations of sepsis is present ; antibiotic therapy with single antibiotic can be administered before culture and sensitivity results are received.

Anaerobic infection is usually indicated by presence of necrotic tissue ,foul smellingdischarge ,and frank gangrene .If those signs are not present; Staphylococcus aureus as well as coagulase –negative staphylococci are dominant organisms.Non-group D Streptococci (including Group B streptococci as well as enterococci may also be isolated .First generation cephalosporins will cover the first two microorganisms but will be inactive against the third.Ticaracillin –clavulanate and imipenem inhibits mostcoagulase positive and negative staphylococci ,but the former may not be active against several strains of enterococci and the latter is inactive against the Enterococcus. Ampicillin–sulbactam will provide more coverage for Gram –positive organisms but somewhat limited in its coverage of Gram- negative bacilli. The first generation cephalosporins are not as active as the newer agents.In the presence of more severe infection with tissue necrosis, gangrene and sepsis ;high broad spectrum empiric antimicrobial therapy is administered before culture and sensitivity reports are received.

The rationale approach to the empiric choice of antimicrobial agents in these polymicrobial infection include a good knowledge of the susceptibility patterns of the organisms most likely to be isolated in such settings.It is also important to be familiar with the antibiotic susceptibility patterns in one's individual institution. Metronidazole is excellent antibiotic for the Gram negative anaerobic bacilli but not advised for Gram positive anaerobic bacilli and microaerophilic cocci .Imipenem ,Ticaracillin-clavulanate and Ampicillin-sulbactam is effectiveagainst almost all anaerobic bacteria.[33,34]

Due to development of nephrotoxicity; use of aminoglycosides is discouraged in patients with preexisting impairment in renal function.The anti –pseudomonals include the older agents(carbenicillin and ticarcillin),the newer ones (piperacillin,mezlocillin,azlocillin), the third generation cephalosporins (ceftazidime and cefoperazone) and monobactams (aztreonam).Oral ciprofloxacin are more appropriate in long term out-patient therapy, particularly when bone infection is present.

In severe infections and/or in the presence of sepsis and toxemia ,better to administer combination of two antimicrobial agents as preliminary empiric therapy while deep tissue culture and sensitivity report is awaited.The combination should be effectiveagainst the most commonly isolated microorganisms.Thus the regimen should include coverage of S.aureus ,anaerobic bacteria including B.fragilis ,Gram negative enteric bacilli and aerobic streptococci ,including enterococci . A reasonable combination is ;example :Ampiciilin –sulbactam plus Aztreonam or ceftazidime (or an aminoglycoside ,if renal function is unimpaired).This regimen will cover almost all of the microorganisms most likely to be encountered in this setting.

Care must be exercised to adjust β -lactams dosages in the presence of renal insufficiency, since combinations of multiple β-lactams agents can potentially predispose to seizures .The combination of ampicillin, metronidazole and gentamicin or aztreonam can provide reasonably wide coverage. Ceftazidime or cefoperazone plus metronidazole may be suboptimal for staphylococcal as well as enterococcal coverage. The second generation cephalosporin may not provide optimal coverage for S.aureus, Enterococccus spps. And other aerobic Streptococci, P.aeruginosa and

possibly other enteric aerobic gram-negative bacilli as well as some bacteroides spps. belonging to the B.fragilis group.Again it is of utmost importance to be familiar with the antimicrobial susceptibility patterns of certain microorganisms in one's particular institution.This is of particular concern with microorganisms such as B.fragilis ,S.aureus, P.aeruginosa as well as other gram-negative aerobic bacilli.In less severe infections ,however when one can afford to wait for culture and sensitivity results single drug therapy with ticarcillin-clavulanate ,ampicillin-sulbactam ,or imipenem may be adequate.

In a polymicrobial infection it has not been totally clarified whether one needsantibiotic coverage for all the organisms present in the infected tissue. The duration of the antimicrobial has to be individualized according to the severity of infection, the presence or absence of septic complications and the presence or absence of bone involvement.

Causes of delay in wound healing and gangrene in diabetes include: Circulatory insufficiency due to atherosclerosis, Neuropathy, Protein depletion causing poor resistance to infection andAGEs cause a decrease in leukocyte response to infection.

(3)Role of Amputation:[35]

The concept of function–preserving amputation surgery is vital to diabetic foot management. Partial or Whole foot amputations frequently are necessary as treatment for infection or gangrene. The goal of treatment is preservation of function ,not just preservation of tissue. Amputation surgery should be the first step in rehabilitation of

the patient. A direct construction of a residual limb for weight bearing with prosthesis, when performing debridement or partial foot amputation should be employed.

The major value of partial foot amputation is the potential for retention of plantar load bearing tissues, which are uniquely capable of tolerating the forces involved in weight bearing.

The use of split thickness skin grafts in load bearing areas should be avoided Deformity should be avoided as much as possible. Tendo–Achilles lengthening to avoid equinus deformity and increased loading of the residual forefoot in partial foot amputations is beneficial. Retention of a deformed foot with exposed bony prominence leads only to decreased walking ability and recurrent ulceration.

Antibiotic Susceptibility testing

There is a need of rationale antibiotic therapy administration in the light of infection control. The choice of antibiotics need to be made according to the sensitivity profile of the pathogen ,pharmacology of antibiotic ,need of the antibiotic therapy and its affordability.

Various methods are available for susceptibility testing which includes Disc Diffusion,Broth microdilution and Broth macrodilution ,antimicrobial gradient and automated instrument methods.

‘Susceptible’ means an infection caused by the tested pathogen may be appropriately treated with the usually recommended regimen of the antibiotic agent. ‘Intermediate’ indicates that the pathogen may be inhibited by attainable concentration of certain

antibiotics if higher dosages or prolonged infusions are used safely. ‘Susceptible-dose dependent’ focuses specifically on those agents that can be safely administered in higher doses than those used to set the susceptible breakpoint or by prolonged infusions to increase exposure times at the site of infection. ‘Resistant’ organisms do not show inhibition by the concentration of antimicrobial agent normally achievable with the recommended dose and/or yield results that fall within a range suggesting that the specific resistant mechanisms are likely to be present.[36]

The microdilution and agar gradient diffusion method are advantageous over disc diffusion assay as they generate a quantitative result (MIC) rather than a category result. Rapid commercial PCR technologies are emerging which are capable of detecting critical resistances within the same day. Then also there is an ongoing interest in the disk diffusion test because of its inherent flexibility in drug selection ,its ability to respond quickly to the introduction of new agents or changes in interpretive breakpoints, and its low cost.[37]

Breakpoints (or interpretive criteria) are the values that determine the categories of susceptible, intermediate and resistant. The approach to setting breakpoints varies by organization or regulatory body. Depending on the approach taken, upto four sources of data can be examined in establishing breakpoints.[38]

MIC or minimum inhibitory concentration is defined as the lowest concentration of drug that will inhibit the growth of a test organism over a defined interval related to organism’s growth rate, commonly 18 to 24 hours. The MIC is the fundamental measurement that forms the basis of most susceptibility testing methods and against

which the levels of drug achieved in human body fluids may be compared to determine breakpoints for defining susceptibility.[38]

When a laboratory's routine susceptibility testing batteries are determined, several principles should be followed:

1. The antimicrobial agents that are included in the institution's formulary and that physicians prescribe on a daily basis should be tested.

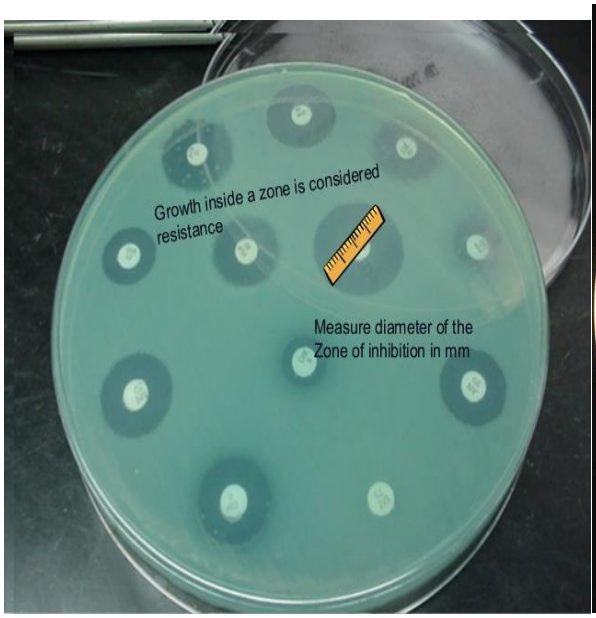
2. The species tested strongly influences the choice of antimicrobial agents for testing.The CLSI publishes tables that list the antimicrobial agents appropriate for testing various group of aerobic and fastidious bacteria.

This initial list of antibiotics must be tailored to an individual institution's specific needs through discussion with infectious disease physicians ,pharmacists,and committees concerned with infection control and the institutional formulary.

Gradient diffusion method:

The E-Test or Epsilometer test (bioMérieux) is a commercial method for quantitative antimicrobial susceptibility testing that involves a preformed antimicrobial gradient applied to one side of plastic strip to provide drug diffusion into an agar medium .MIC determined by this method generally agree well with MICs generated by standard broth or agar dilution methods.[39,40]

The next page shows pictures of various methods of antibiotic susceptibility testing.



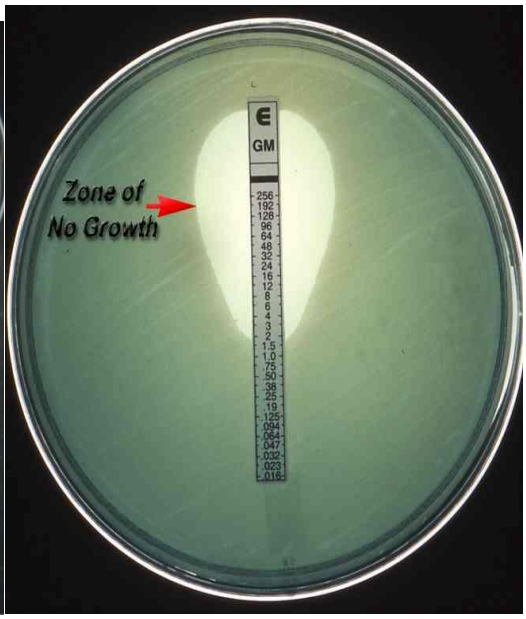
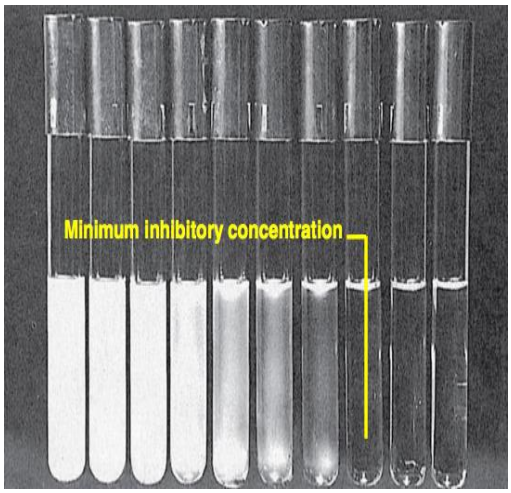


Fig.1 Disk Diffusion Antibiotic susceptibility test. Fig.2. E-Test.



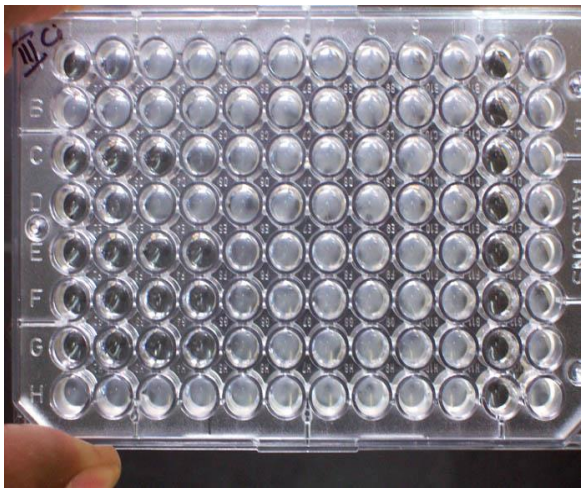


Fig.3. Broth Macrodilution

Fig.4. Broth Microdilution

Antibiotic Resistance

Antibiotic resistance is an issue of great concern for public health for both at International and national level.Though antibiotics have been held high as wonder drugs,its irrational use and inappropriate prescription sometimes makes it an abused drug. *I*neffective and inefficient use of these antibiotics has jeopardized the quality of care and has wasted limited resources in various parts of the world by the development of anibiotic resistance.

Even though antibiotic policies and programmes are being implemented at different countries in various stages ;sustainable effective interventions are lacking and not much significant impact is seen in many of the countries including some parts of India. It has been reported that 30-60% of PHC patients receive antibiotics which may be twice as high as it is clinically required.[41]

Multidrug resistant organisms(MDROs) have been labelled as those that are in-vitro resistant to more than one antimicrobial agent.Infections with MDROs usually lead to inadequate or delayed antimicrobial therapy and have been associated with poorer outcomes.The problem of increasing antimicrobial resistance is of great concern as very limited number of new antimicrobial agents are in development.European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC),held a meeting at Stockholm in January 2008 and created definitions for highly resistant ,multi resistant bacteria associated with healthcare-associated infections.Only acquired antimicrobial resistance was taken into account

in creating definitions for MDR,XDR and EDR.Intrinsic resistance was not considered. After proper evaluation of the draft manuscript majority defined MDR as resistant to ≥ 1 drug in ≥ 3 different classes of drugs. In the next page table on definition of MDROs is given.

Intrinsic resistance is defined as inherent or innate antimicrobial resistance which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species.[42]

Drug Resistance in India

India lacks in data on the use of antimicrobial agent at the population evel as we do not have any database for the consumption of antimicrobials in the community.The reason is ;prescription are kept by the patient and not with the pharmacist and antibioticsare allowed to be bought with or without a prescription.Therefore to determine the consumption of antibiotic use or monitor trends in antibiotic use is problematic.

Following table defines MDRO accordingly:

TYPE	DEFINITION SOURCE	DEFINITION
Methicillin-resistance Staphylococcus aureus	CDC, NHSN†	Includes S. aureus cultured from any specimen that tests oxacillin-resistant, cefoxitin-resistant, or methicillin-resistant by standard susceptibility testing methods, or by a laboratory test that is FDA-approved for MRSA detection from isolated colonies; these methods may also include a postiive result by any FDA-approved test for MRSA detection from specific sources.
Vancomycin – resistant Enterococcus species	CDC, NHSN†	Any Enterococcus spp. (regardless of whether identified to the species level), that is resistant to vancomycin, by standard susceptibility testing methods or by results from any FDA-approved test for VRE detection from specific specimen sources.
Carbapenemase resistant Enterobacteriaceae	Oregon	Any Enterobacteriaceae spp. testing non-susceptible (i.e., resistant or intermediate) to imipenem, meropenem, or doripenem, by standard susceptibility testing methods or by a positive result for any method FDA-approved for carbapenemase detection from specific specimen sources; AND resistant to all third-generation cephalosporins tested.
MDR-Acinetobacter	CDC, NHSN†	Non-susceptibility (i.e., resistant or intermediate) to at least one agent in at least 3 antimicrobial classes of the following 6 classes: • Ampicillin/sulbactam • Cephalosporins (cefepime, ceftazidime) • β-lactam/β-lactam β-lactamase inhibitor combination (piperacillin, piperacillin/tazobactam) • Carbapenems (imipenem,meropenem, doripenem) • Fluoroquinolones (ciprofloxacin or levofloxacin) • Aminoglycosides (gentamicin, tobramycin, or amikacin)

MDR-Pseudomonas	CDC, NHSN†	Non-susceptibility (i.e., resistant or intermediate) to at least one agent in at least 3 antimicrobial classes of the following 5 classes: • Cephalosporins (cefepime, ceftazidime) • β-lactam/β-lactam β-lactamase inhibitor combination(piperacillin, piperacillin/tazobactam) • Carbapenems (imipenem, meropenem, doripenem) • Fluoroquinolones (ciprofloxacin or levofloxacin) • Aminoglycosides (gentamicin, tobramycin, or amikacin)
Extended-spectrum beta-lactamase Gram negatives	CDC, NHSN†	• Enterobacteriaceae spp. non-susceptible (i.e., resistant or intermediate) to ceftazidime, cefepime, ceftriaxone, or cefotaxime. • Pseudomonas aeruginosa non-susceptible (i.e., resistant or intermediate) to ceftazidime or cefepime.
Clostridium difficile	CDC, NHSN†	A positive laboratory test result for C. difficile toxin A or B, (includes molecular assays [PCR] or toxin assays) OR A toxin-producing C. difficile organism detected by culture or other laboratory means performed on a stool sample.
Drug-Resistant Streptococcus pneumoniae	CDC, Emerging Infections Program	S. pneumoniae isolated from a sterile site and nonsusceptible to "at least one antimicrobial agent currently approved for use in treating pneumococcal infection." (NO LONGER INCLUDED IN LIST)
†CDC. Multidrug-Resistant Organism & Clostridium difficile Infection (MDRO/CDI) Module. January 2014. http://www.cdc.gov/nhsn/PDFs/pscManual/12pscMDRO_CDADcurrent.pdf §Oregon Public Health Division, OAR 333-019-0015 ‡ Sievert DM et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. ICHE 2013;34:1–14. Multidrug-Resistant Organism (MDRO) Definitions		

Carbapenem Resistance

Emergence of β-lactamases and threatened use of penicillin emerged in 1960s.It gave an alarm for the search of β-lactamase inhibitors.[43,44] The first β-lactamase inhibitors was discovered in 1976 called olivanic acids.They were produced from gram positive bacterium called Streptomyces clavuligerus.As it was chemically instable and did not penetrate well into the bacterial cell their use was not encouraged further.[45] Following this two superiorβ- lactamase inhibitors were discovered:(i)Clavulanic acid from Streptomyces clavuligerus ;(ii)Thienamycin from Streptomyces cattleya was the first carbapenem that serves as parent compound for carbapenems.

Carbapenem is defined as 4:5 fused green lactem of penicillins with a doublebond between C-2 andC-3 but with substituition of carbon for sulphur atC-1.The stereochemistry of hydroxyl ethyl side chain in Thienamycin is important for the activity in carbapenems.They showed broad spectrum antibacterial β-lactamase inhibitory activity. Unfortunately their unstability in aqueous solution and led to search for other derivatives with increased stability. The first to develop was Imipenem ;N-Formimidoyl derivative .It was found to be more stable than Thienamycin and less sensitive to base hydrolysis in solution.[46] Imipenem(MK0787) became available for treatment of complex microbial infections in 1985 with advice on co-administration with an inhibitor Cilastatin or Betamipron as Imipenem was susceptible to deactivation by dehydropeptidase-1(DHP-1).[4]

Mechanism of action:

Carbapenems after entering cell wall through outer membrane proteins(OMPs) called porins in gram negative bacteria ; traverses the periplasmic space and permanently acylate the PBPs.[48,49] PBPs are enzymes that catalyse the formation of peptidoglycan in the cell wall of bacteria.They not only inhibit the peptidase domain of PBPs,but also inhibits cross linking of peptide and other peptidase reactions. Their unique feature is that they can bind to multiple different PBPs leading to bursting of the cell due to osmotic pressure.[48]

Antibactericidal activity:

Carbapenems have broader antimicrobial activity in vitro than presently available penicillins,Cephalosporins and β-lactem / β-lactamase inhibitor combinations.[50]Doripenem,Pamipenem and Imipenem are useful for bactericidal activity against gram positive bacteria .[50,51]Biapenem , Doripenem ,Meropenem and Ertapenem are more effective against gram negative.[50,52]

Pharmacology and Clinical use:

As carbapenems have low oral bioavailability ,they do not cross gastrointestinal membranes readily and so administered intravenously.Then too intramuscularly ; Ertapenem and Imipenem-cilastatin can be given.[50,51]

Mechanisms of resistance against Carbapenem:

Resistance in Carbapenems is conferred through production of β-lactamases, efflux pumps and mutations that alter the expression and / or funtions of porins and

PBPs. Carbapenem resistance in Gram positive cocci is the result of substitutions in aminoacid sequences of PBPs or acquisition of/ production of new carbapenem resistant PBPs[53,54,55] while in Gram positive bacilli expression of β-lactamases and efflux pumps,porin loss and alterations in PBPs, all are associated with the resistance.[56,57,58]

B-lactamases are enzymes produced by bacteria that hydrolyse β-lactem antibiotics.β-lactamases are classified into four classes based on structural similarities(classes-A,B,C and D) or four groups based on hydrolytic and inhibitor profiles (1-4).[59,60]Class A,C and D β- lactamases use serine as a nucleophile to hydrolyse the β-lactam bond whereas class B β-lactamases uses Zn^{2+} to inactivate β-lactamases.

Carbapenemases are specific β-lactamases that hydrolyses the Carbapenems.According to various documentations, the production of β-lactamases has been found to be the most widespread cause of resistance.There has been an increase in number of class A carbapenemases(KPC and GES enzymes), class B metallo β-lactamases(VIM,IMP and NDM)and class D carbapenemases(OXA-23,-24/40,48,51,55,58 and 143).[61,62,63,64,65] The resistant genes that code for carbapenemases can be exchanged between different gram negative bacteria through genetic packets called transposons or plasmids (jumping genes).Enterobacteriace possessing this carbapenemase genes(CP-CRE) are of great public health concern as their resistance has spread around the globe.The exchange of resistant genes between the bacterial chromosomes and the plasmids and their integration into particular

genetic elements; integrons are found to play a major role in acquisition and dissemination of resistance gene.

Molecular Detection in Antibiotic Resistance

Rationale for detection of antibacterial resistance genes in bacterial isolates.

1.Speed: Detection of resistance organisms allows rapid administration of appropriate antibiotic therapy and control of hospital borne infections.[66,67]

2.Genetic methods can settle MIC results that are at or near breakpoint for resistance for bacterial species.

3.Accuracy of genetic tests have proved superior to phenotypic assays for monitoring the epidemiological spread of a particular resistant gene in hospital or community setting. [68,69]

4.Genetic tests can be employed as "gold standard" test for resistance tests in evaluating the accuracy of new susceptibility testing methods.[70]

Pitfalls associated with genetic detection of resistant genes:

1.Lack of expression of resistant genes

2.Mixed flora containing resistant gene may contain resistant or mutant genes that can alter sequences used for PCR primers and lead to false negative results.[71]

3.The resistance phenotype may be the result of multiple genes encoding antimicrobial resistance mechanisms and not just presence of single gene.

4.The presence of PCR inhibitors ,inefficient extraction of nucleic acids and other technical issues may also interfere with detection.

5.High cost has limited the use of these molecular assays ;limiting the detection of resistance to few common and well- defined resistance mechanisms.

METHODOLOGY

4.METHODOLOGY

Infection in diabetic foot often plays a significant role in causing amputations.It is important to have a knowledge of the principle bacteria,their local antibiotic sensitivities and the prevalence of resistant organisms .

Ethical Clearance

Ethical clearance was obtained by the Institutional Ethical Committee(IEC) at Stanley Medical College,Chennai.Permission was granted by the respective departments of the hospital . Patient'sconsent was obtained for those who were involved in the study.

Inclusion criteria

Diabetic patients with infected foot ulcers either out patients or in-patients above 18 years of age.

Exclusion criteria

Non diabetic foot ulcers.

Study design: Cross-sectional study

Study Period: January 2016 to August 2016

Place of study: Stanley Medical College.

Sample size: 49

Sample Size for Frequency in a Population

Population size(for finite population correction factor or fpc)(*N*):	50
Hypothesized % frequency of outcome factor in the population (*p*):	65.1%+/-5
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

ConfidenceLevel(%)	Sample Size
95%	44
80%	38
90%	42
97%	45
99%	47
99.9%	48
99.99%	49

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

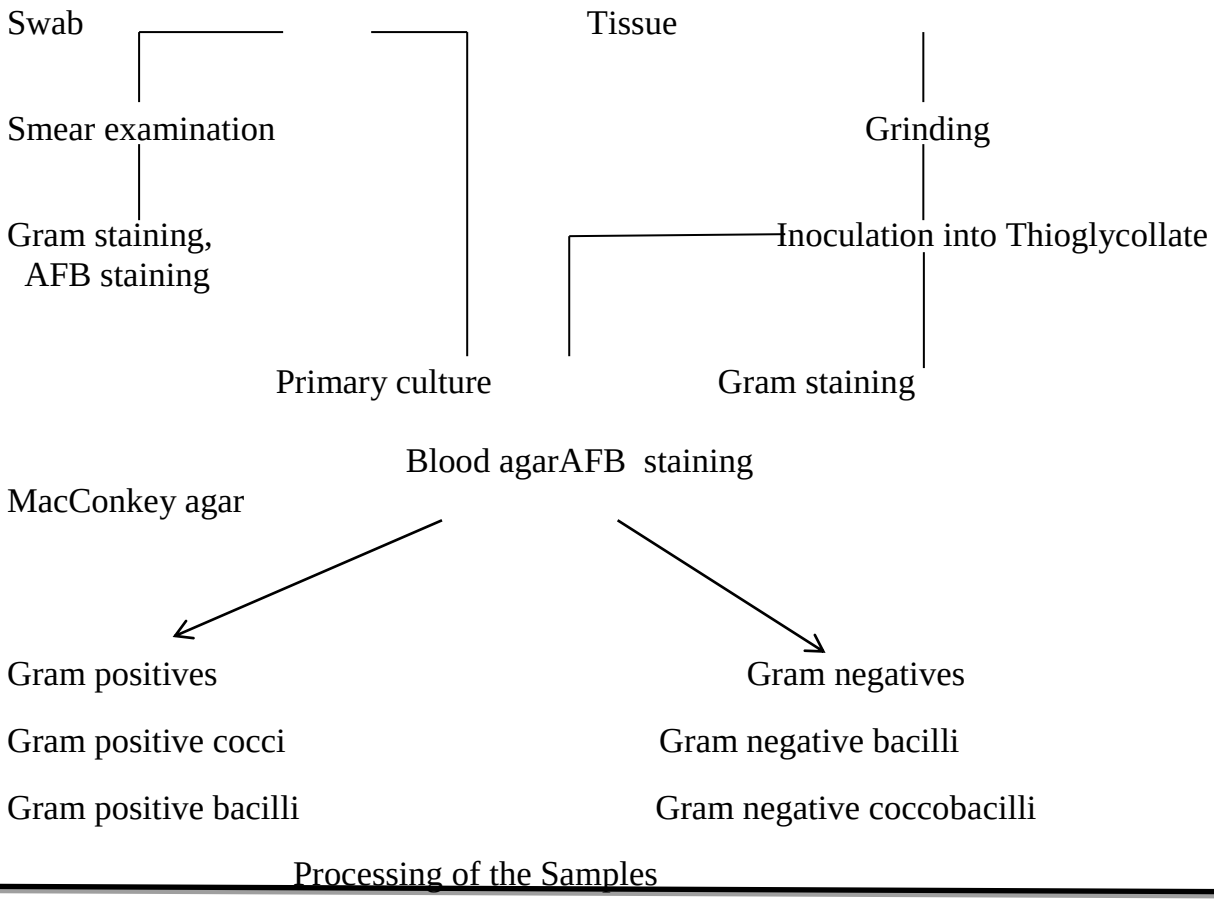
Sample Collection:

Patient having infected ulcers were taken into consideration and only from those cases samples were collected .The infected area was cleaned thoroughly with normal saline while debridement and sample was collected . For tissues the area was thoroughly cleaned with normal saline and bits of tissue was taken as sample. Aspirated pus in sterile syringe / pus collected using two swabs in a sterile test tube from abscesses and infected wounds are taken .Tissue samples were collected in sterile normal saline then grinded in a sterile mortor and inoculated into thioglycollate broth.

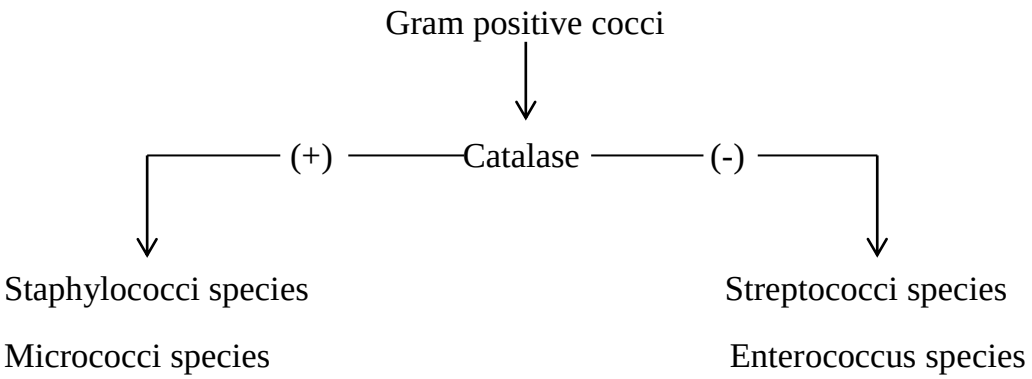
One swab is used for making smear for direct gram staining and another for culture.Pus sample was used for direct gram staining and culture.Samples were also used for making smear for AFB staining. AFB staining was done by Kinyouns method.Turbidity if observed in thioglycollate broth was processed .

Primary culture plates used were Blood agar and MacConkey agar . Growths were then further processed for biochemical reactions and antibiotic susceptibility testing was done.The pathogens were identified and accordingly antibiotics were tested and reported.

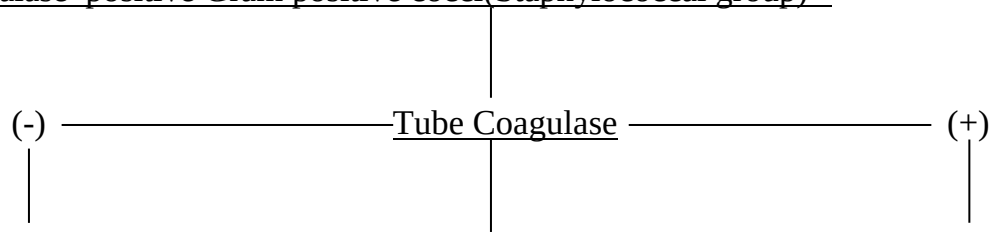
Resistant pathogens were confirmed by the phenotypic confirmation methods mentioned in CLSI. Pathogens which were resistant to carbapenems were further subjected to genotypic study for ruling out any incidence ofbla_{KPC} and bla_{NDM} .

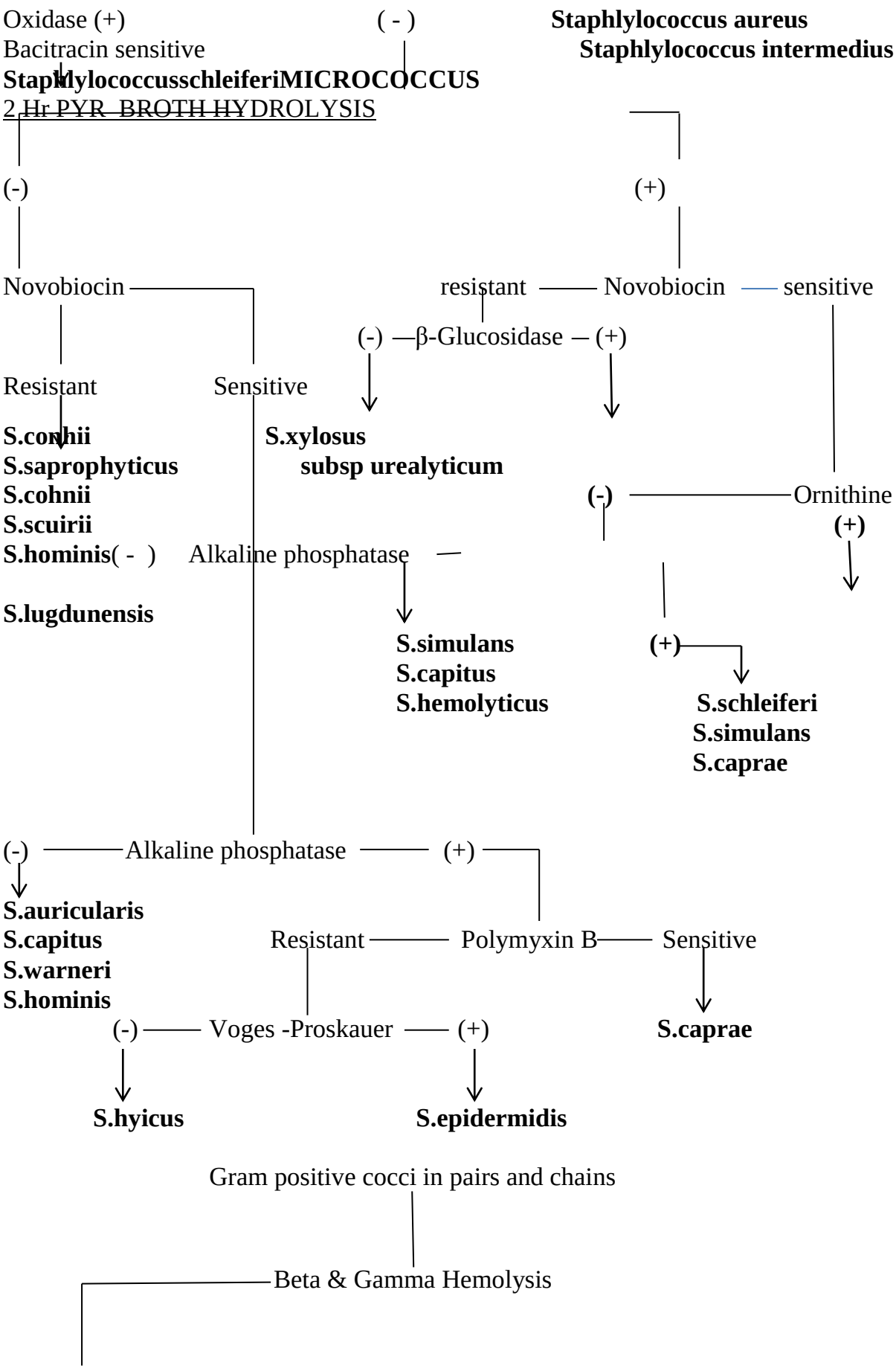


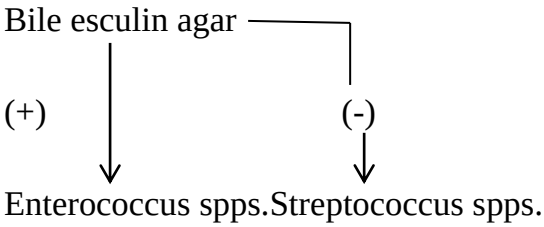
Processing of the Samples

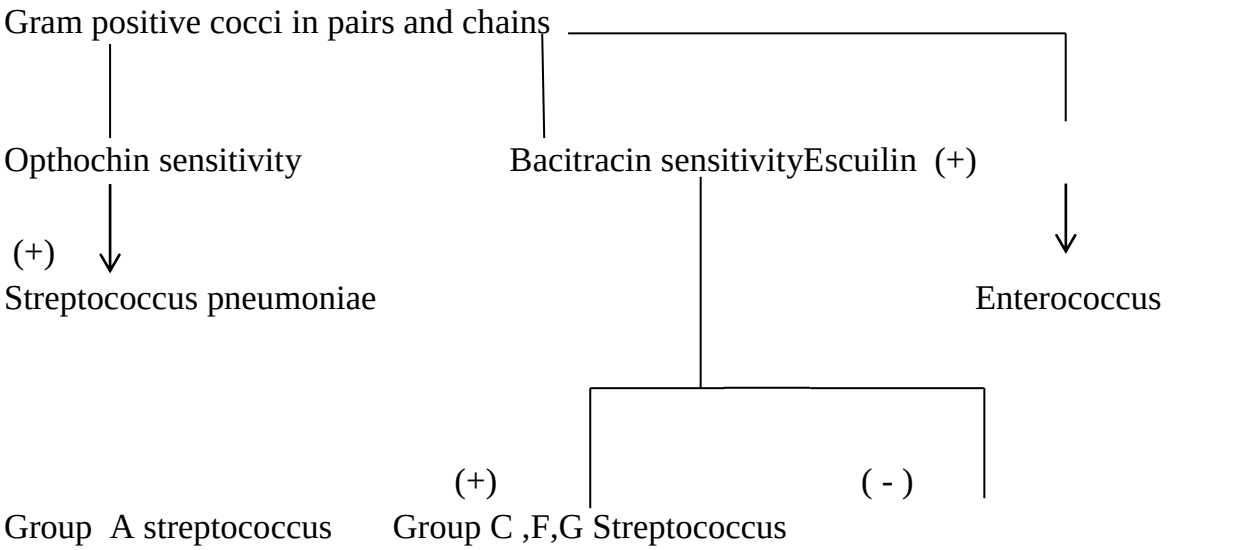


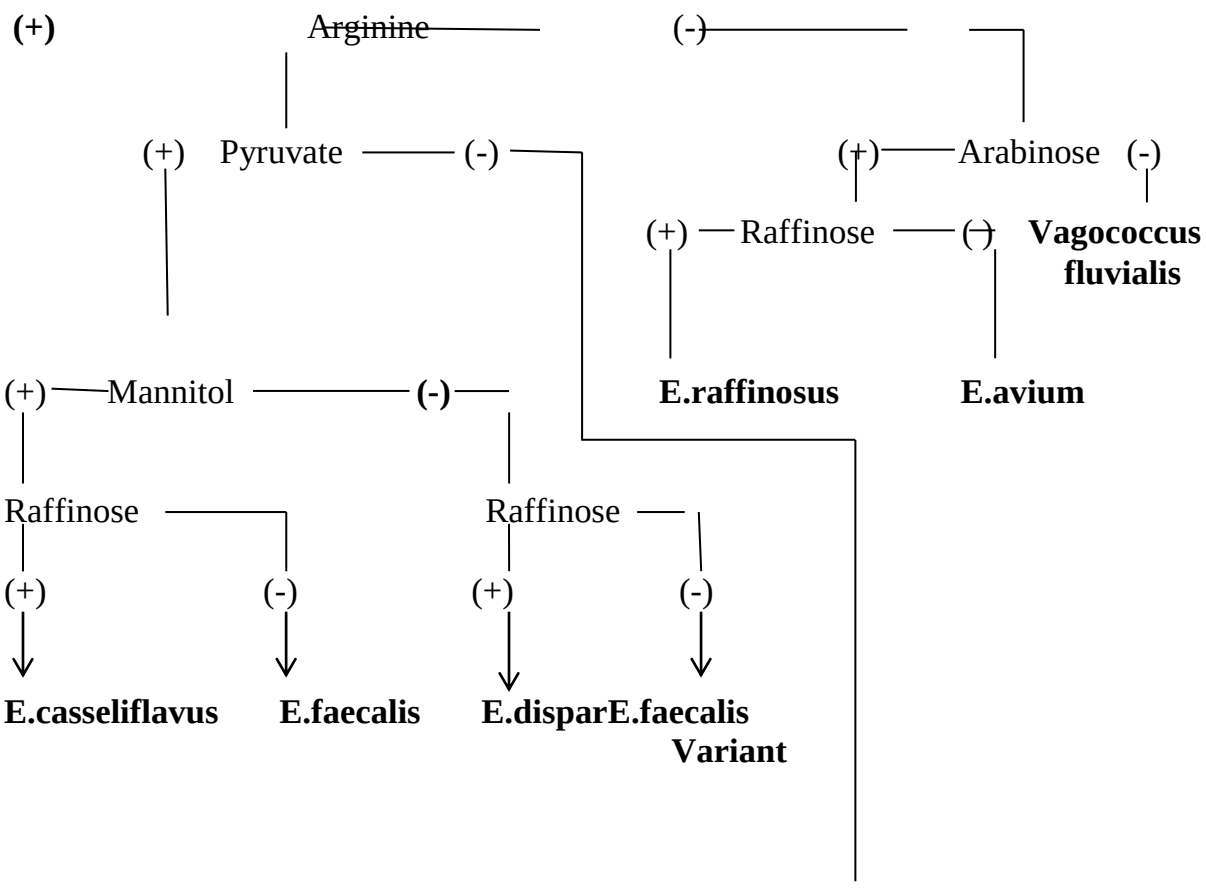
Catalase positive Gram positive cocci(Staphylococcal group)[72]

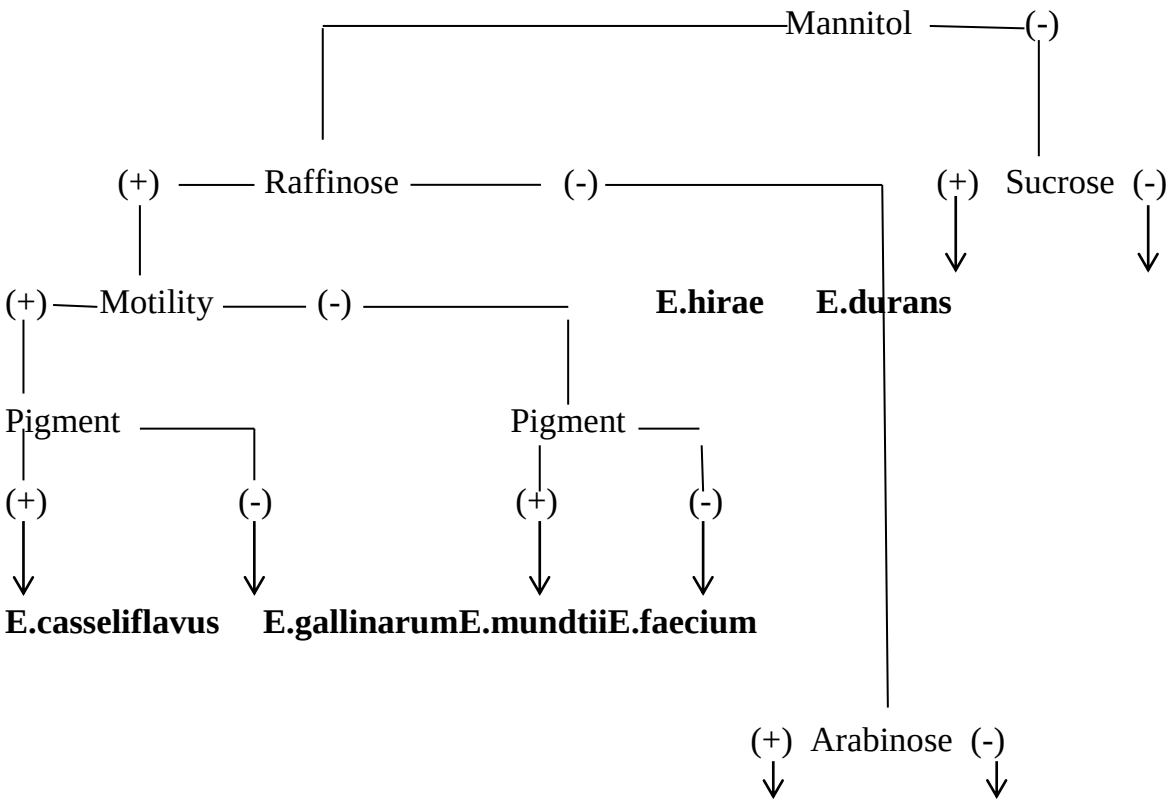



Oxidase (+)
(-)
Staphlylococcus aureus
Bacitracin sensitive
Staphlylococcus intermedius
StaphylococcusschleiferiMICROCOCCUS
2 Hr PYR BROTH HYDROLYSIS
(-)
(+)
Novobiocin
resistant
Novobiocin
sensitive
(-) β-Glucosidase (+)
Resistant
Sensitive
S.cohnii
S.saprophyticus
S.cohnii
S.scuirii
S.hominis
S.xylosus
subsp urealyticum
(-)
Ornithine
(+)
(-)
Alkaline phosphatase
S.lugdunensis
S.simulans
S.capitus
S.hemolyticus
(+)
S.schleiferi
S.simulans
S.caprae
(-)
Alkaline phosphatase
(+)
S.auricularis
S.capitus
S.warneri
S.hominis
Resistant
Polymyxin B
Sensitive
(-)
Voges -Proskauer
(+)
S.caprae
S.hyicus
S.epidermidis
Gram positive cocci in pairs and chains
Beta & Gamma Hemolysis


Bile esculin agar
(+)
(-)
Enterococcus spps.
Streptococcus spps.


Gram positive cocci in pairs and chains
Opthochin sensitivity
Bacitracin sensitivity
Escuilin (+)
(+)
Streptococcus pneumoniae
Enterococcus
(+)
(-)
Group A streptococcus
Group C ,F,G Streptococcus


(+)
Arginine
(-)
(+)
Pyruvate
(-)
(+)
Arabinose
(-)
(+)
Raffinose
(-)
Vagococcus fluvialis
E.raffinosus
E.avium
(+)
Mannitol
(-)
Raffinose
Raffinose
(+)
(-)
(+)
(-)
E.casseliflavus
E.faecalis
E.dispar
E.faecalis Variant



E. faeciumLactococcus spps.

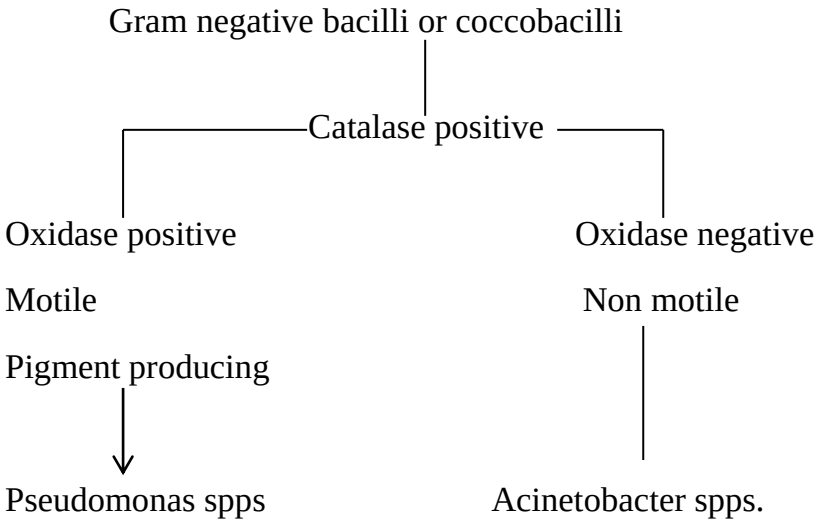
<u>Species Identification of clinically relevant Enterococcus and Enterococci like isolates[73]</u>

<table>
<tr><td colspan="2">Lactose fermenters:E.coli, Klebsiella spps.,Citrobacter spps.,Enterobacter spps.</td><td colspan="3">Non Lactose or late lactose fermenters:Citrobacter spps,Morganella,Proteus spps,Edwardiella spps.</td></tr>
<tr><td>Indole Positive</td><td>Indole Negative</td><td colspan="2">PPA positive</td><td>PPA negative</td></tr>
<tr><td>E.coli,Citrobacter spps.,klebsiella oxytoca</td><td>Citrobacter fruendii,Klebsiella pneumoniae,Enterobacter spps.</td><td colspan="2">Proteus spps.,Providencia spps,Morganella morganii</td><td>Citrobacter Edwardsiella E.coli(inactive)</td></tr>
<tr><td rowspan="2">Biochemical division
Escherichia.coli
I+U-C-
C.koseriI+U+/-C+(Motile)
MR Positive
K.oxytocaI+C+U+
(NonMotile)</td><td rowspan="2">C.freundii
I-/+C+/-U-/+
H_2S+(TSI)
Enterobacter.aerogenes
I-U-C+
A/Awith gas.
OD (+)
Enterobacter.cloacae
I-U+/-C+</td><td>H_2S positive</td><td>H_2S Negative</td><td>Mannitol positive</td><td>Mannitol negative</td></tr>
<tr><td>P.mirabilis
P.vulgaris
P.penneri(30%)
M.morganii
(20%)</td><td>P.rettgeri
P.stuartii
P.penneri
(60%)
M.morganii
(80%)</td><td>Citrobacter spps.
E.coli inactive</td><td>Edwardsiella
I+H_2S</td></tr>
</table>

MR Negative	A/A with gas(TSI) AD (+) **K.pneumoniae** I-U+C+ A/A with gas(TSI)	Biochemical reactions **P.mirabilis** I-U+C+/- **P.vulgaris** I+U+C-/+ **P.penneri** I-U+C- **M.morganii** I+U+C-	Biochemical reactions **P.rettgeri** M+,no gas I+U+C+ K/K(TSI) **P.stuartii** M-with gas I+C+U-/+ K/A(TSI) **P.penneri** M-with gas I+C-U+ K/A(TSI) **M.morganii** M- with gas I+U+C- K/A(TSI)	H_2S positive-**Citrobacter freundii**	

Above table shows:

Algorithm for identification of Enterobacteriaceae.C (in biochemical reaction growth :Simmons citrate agar;U:Urease reaction;I :Indole reaction;MR:Methyl Red;TSI:Triple sugar iron ;OD:Ornithine decarboxylase;AD:Arginine decarboxylase;PPA:Phenylalanine deamination to phenylpyruvic acid;M:Mannitol fermentation;(+) positive >90%; (-) negative<10% ; (+/-) >50% positive ; (-/+)less than 5%positive. (Algorithm modified from Gould LH et al:Recommendations for diagnosis of Shiga toxin –producingEscherichia coli in clinical laboratories,MMR 58 (RR12);1,2009)[74]



Antibiotic susceptibility testing

After identification of pathogenic growths as GPC ,GNB and GNCB antibiotic susceptibility testing was done by Kirby- Bauer's Disc Diffusion method .The medium (Meuller –Hinton Agar) was prepared and sterilized and dispensed into flat bottomed 9cm glass petri-dishes .The pH of the medium was checked while preparation.Inoculum was prepared by picking 3-4 morphologically similar colonies from the culture plates and inoculated into a test tube containing peptone water. The tubes are then incubated for 2 hrs at 37˚C to produce a bacterial suspension .The inoculum size was adjusted by comparison with a barium sulphate standard ,0.5 Mc Farland standard units .

A sterile swab was introduced into the inoculum and excess was removed by pressing and rotating the swab firmly against the side of the tube ,above the level of the liquid .Swab was streaked all over the surface of the medium three times ,rotating the plate through an angle of 60˚C after each application .The plate was then left to dry for few minutes at RT with lid closed .The antibiotic discs were placed on the inoculated plates using a pair of sterile forceps. The plates were then incubated at 37˚C for 18 to 24 hours .[75] Next day clearance of growth around each disc(including the disc) was measured and recorded in mm. The size of the zone of inhibition is an indication of the susceptibility of pathogen .The zone sizes were compared with the standard chart and reported accordingly. Staphylococcus aureus ATCC 25923 ,Escherichia coli ATCC25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.Thefollowing drugs discs purchased from HiMedia,Mumbai were used:

Gram positive cocci: Erythromycin(15µg),Clindamycin(2µg),Penicillin(10 units) Ampicillin(10µg) ,Trimethoprim-sulfamethoxazole(1.25/23.75 µg),Linezolid(30 µg), Doxycycline(30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg),Gentamicin(10 µg), Amikacin(30µg), High Level Gentamicin (120µg), Vancomycin (30µg). Teicoplanin(30µg)

Gram Negative Bacilli and Coccobacilli: Ampicillin(10µg),Gentamicin(10µg), Amikacin(30µg), Piperacillin-Tazobactam(100/10µg), cefepime(30 µg), Cefotaxime (30µg), Ceftazidime(30µg), Ciprofloxacin(5µg), Imipenem (10µg) ,Meropenem(10µg) Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Doxycycline (30 µg), Ampicillin-Sulbactum(10/10µg), Colistin(10µg), Polymixin B(300 units)

E –Test:

The test was performed in a manner similar to disk diffusion testing ,in that a 0.5 McFarland standard suspension of an isolate was swabbed onto the agar surface for inoculation.Following inoculation and incubation for 18 to 24 hours ,the MIC is noted directly from the scale on the top of the strip at the point where the ellipse of organism growth inhibition intercepts the strip.MIC determined with this technique generally agree well with MICs generated by standard broth or agar dilution methods.[39,40]Two pathogens which were found to be resistant to Imipenem and Meropenem in the disk diffusion test were subjected to the E-Test.

Testing for Extended Spectrum β-Lactamase

CLSI has given guidelines for the screening test of ESBLs for Klebsiella pneumonia,Klebsiella oxytoca ,Escherichia coli and Proteus mirabilis.Both disk diffusion and broth microdilution methods are available.In disk diffusion method for K.pneumonia,K. oxytoca ,E.coli drugs which can be used are Cepodoxime(10µg), Ceftazidime (30µg),Aztreonam(30µg),cefotaxime(30µg)and Ceftriaxone(30µg).For Proteus mirabilis Cepodoxime,ceftazidime and cefotaxime can be used. Inoculum is prepared using 0.5 Mc Farland standard suspension in broth or saline and lawn culture prepared in MHA .The use of more than one antimicrobial agent improves the sensitivity of ESBL detection.

The disc diffusion confirmatory test necessitates the use of both cefotaxime and ceftazidime alone or in combination with clavulanate.Cefotaxime/Ceftazidime(30µg) and Cefotaxime/Ceftazidime – clavulanate 30/10µg).A ≥5 – mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate VS the zone diameter of the agent when tested alone = ESBL.[76]

Amp C Disc Test

A lawn culture of E.coli ATCC 25922 was made in a MHA plate ; from an overnight culture suspension adjusted to 0.5 Mcfarland standard .30µg cefoxitin disc was kept on the surface of the agar .A blank disc (6mm diameter ,Whatman filter paper no.1) dipped in sterile saline inoculated with a few colonies of the test strain was then placed beside the cefoxitin disc almost touching it.The plate was then incubated at 37˚C .A flattening or indentation of the cefoxitin inhibiton zone in the

vicinity of the disk with test strain was interpreted as positive for the production of the AmpC βlactamases. An undistorted zone was considered as negative.[77]

Modified Hodge test

It's a simple test for identification of β- lactamase producing Enterobacteriaceae that are non susceptible to one or more carbapenems.In this test we prepared a 0.5 Mc Farland standard suspension of E.coli ATCC 25922 in broth and diluted 1:10 in broth .Then we inoculated an MHA plate as we do for the routine disc diffusion procedure .Thereafter the plate was left to dry for 3-10 minutes.Then meropenem disc was placed on the plate . Using a 10µl loop or swab ,3-5 colonies of test strain and QC organisms grown overnight on a nutrient agar plate were inoculated in a straight line out from the edge of the disc. The plate was then incubated at 35˚C ±2˚C and incubated for 16-20 hours. Following incubation we examined the plate for enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition.

Enhanced growth = positive for carbapenemase production.

No enhanced growth= negative for carbapenemase production.[78]

Detection of carbapenemase encoding genes :bla KPC and bla NDM by PCR. Polymerase chain reaction was performed for bla_{KPC} and bla_{NDM} using PureFast® Bacterial DNA minispin purification kit purchased from Helini Biomelecules,

Chennai,India.

Primers used were :

HELINI Ready to use blaKPC gene Primer mix - 5µl/reaction PCR Product: 215bp HELINI-KPC-F: CGGCAGCAGTTTGTTGATTG HELINI-KPC-R: CGCTGTGCTTGTCATCCTTG
HELINI Ready to use blaNDM1 gene Primer mix - 5µl/reaction PCR Product: 215bp HELINI-NDM1-F:GCAGCACACTTCCTATCTCG HELINI-NDM1-R:GTCCATACCGCCCATCTTGT

Procedure

Bacterial DNA purification:

1ml of overnight culture is centrifuged at 6000rpm for 5minSupernatant is discarded Pellet is suspended in 0.2ml PBS → 180µl of Lysozyme digestion buffer and 20µl of Lysozyme [10mg/ml] is added → Incubated at 37C for 15min → 400µl of Binding buffer, 5µl of internal control template and 20µl of Proteinase K is added Mixed well by inverting several time → Incubated at 56ºC for 15min 300µl of Ethanol is added and mixed well → entire sample is then transferred into the PureFast® spin column → Centrifuged for 1 min then the flow-through is discarded and the column placed back into the same collection tube → 500µl Wash buffer-1 is added to the PureFast® spin column → Centrifuged for 30-60 seconds and the flow-through discarded and the column placed back into the same collection tube → 500µl Wash buffer-2 added to the PureFast® spin column → Centrifuged for 30-60 secondsthen the flow-through discarded and the column placed back into the same collection tube → flow-through discarded and centrifuged

for an additional 1 min ⟶ PureFast® spin column transferred into a fresh 1.5 ml micro-centrifuge tube ⟶ 100µl of Elution Buffer added to the center of PureFast® spin column membrane ⟶ Incubated for 1 min at room temperature and centrifuged for 2 min ⟶column discarded and the purified DNA stored at -20°C.

PCR Procedure:

Reactions setting up is done as follows:

Components Quantity

HELINI RedDye PCR Master mix -10µl

HELINI Ready to use - Primer Mix - 5µl

Purified Bacterial DNA - 5µl

Total volume -20µl

It is mixed gently and spun down for short time then placed into PCR machine and program is as followed:

Initial Denaturation: 95ºC for 5 min
Denaturation: 94ºC for 30sec
Annealing: 58ºC for 30sec 35 cycles
Extension: 72ºC for 30sec
Final extension: 72º C for 5 min
Loading:

2% agarose gel is prepared [2gm of agarose in 100ml of 1X TAE buffer]electrophoresis is run at 50V till the dye reaches three fourth distances andbands in UV Transilluminator observed.

Agarose gel electrophoresis:

2% agarose. (2gm agarose in 100ml of 1X TAE buffer and melted using micro

oven) is prepared ⟶ When the agarose gel temperature was around 60ºC,5µl of Ethidium bromide was added ⟶ warm agarose solution is then slowly poured into the gel platform ⟶ the gel is set undisturbed till the agarose solidifies ⟶ 1XTAE buffer is then poured into submarine gel tank ⟶ Carefully the gel platform is placed into tank ; maintaining the tank buffer level 0.5cm above the gel ⟶ PCR samples are then loaded after mixing them with gel loading dye along with 10µl HELINI 100bp DNA Ladder [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1000bp and 1500bp] ⟶ Electrophoresis is then run at 50V till the dye reaches three fourth distance of the gel ⟶ Gel is then viewed in UV Transilluminator and the bands pattern observed.

RESULTS

4.RESULTS

Demographic Data

From January 2016 to August 2016 , 50 patients suffering from diabetic foot ulcers were included in the study.The age group of patients involved in the study were from 40 – 80 years. The mean age of patients included in this study was 57.06 years with (SD 10.08).Males 32 (64%) were affected more than females 18(32%).The number of patients seen in IP were 31 (62%) and OP 19 (38%).39(78%) did not know about their family history while 9 (18%) had family history and 2 (4%) were sure of no family history.

The duration of diabetes as observed was from 1 year to 25 years with mean of 6.94 years.No mortality was noted.

Patients who were on oral hypoglycemic drugs were more 32(64%) that on insulin(18(32%). Irregularity in treatment was found in 44(88%) of the patients.

Doppler study was normal in 47(94%) and abnormal in 3(6%) of patients.

Table 1 and 2 depicts the above data.

Wagners Grading: Maximum patient fell in Grade 2- 24 (48%) followed by Grade 3 -21 (42%) and Grade 4 - 5 (10%).No cases were seen in Grade 1 and Grade 5.

Contributory factors, Comorbid conditions and Complications:

Smoking and alcoholism seen among male patients was 68% (34) and 60% (30) respectively.Hypertension was associated with 20 (40%) and obesity seen in 8 (6%). Complications noted were Neuropathy-40%(20),Nephropathy-10%(5), Retinopathy -14%(7) and Coronary Artery Disease -4%(2).

Treatment

Patients who were treated with surgical debridement and kept on antibiotics for long term were 36(72%).Those who underwent toe disarticulation were 9 (18%) and Below knee amputation was done in 5 (10%).Table 2 enlists the above data.

Aerobic bacteria Isolation and Identification

In this study from 50 patients; 45 pathogenic aerobic bacteria were isolated.In 10(20%) patients there was no aerobic bacterial growth .Gram positives isolated were 10(22.2%) among which Enterococcus was the only one isolated 10(100%).Among the Gram negatives 35 (77.7%) ;Klebsiella spps. was the most common isolate -14 (31.1%) followed by Proteus spps- 13(28.8%) , Pseudomonas aeruginosa - 5(11.1%),Escherichia coli -2 (4.44%) and Citrobacter freundii -1(2.22%). Greater than 1 organism was isolated from 5 (11.1%) patients. Table3 depicts the data on this.

Isolation of aerobic bacteria according to Wagner's Grading

Grade 2– Among 24 patients in this grade ;7 patients (29.1%) did not show any growth. Out of 17 (70.8 %), 18 (40%) pathogens were isolated.Proteus spps.-7(38.8%)

was maximum followed by Enterococcus-5 (27.7%) ,Klebsiella spps,-4 (22.2%),Pseudomonas and Escherichia coli each- 1(5.55%).

Grade 3 – Among 21 patients in this grade ;2 patients (9.52%) did not show any growth.Out of 19 (90.4%) ,22(48.8%) pathogens were isolated.In this group Klebsiella spps showed highest growth 8 (36.3%) followed by Enterococcus - 5(22.7%),Proteus- 5(22.7%),Pseudomonas spps.-3(13.6%) and Escherichia coli- 1(4.54%).

Grade 4 - In this grade out of 5 (1%) patients ,1 (20%) did not show any growth.

Rest 4(80%) patients exhibited 5 (11.1%) pathogens of which Klebsiella was maximum with 40% (2) and rest 20%(1) each of Pseudomonas ,Citrobacter and Proteus. Table 4 further illustrates the above data.

Antibiotic sensitivity and resistance pattern

8 aerobic isolates were recovered in culture and their resistance pattern is as follows:

Enterococcus (10) : These were highly resistant to Penicillin-70% (7) followed by Ampicillin 50% (5) and Ciprofloxacin 40% (4) .No resistance was found with High Level Gentamicin and Vancomycin.

Proteus vulgaris (11) : Gentamicin and Ciprofloxacin showed highest resistance -91%(10) each .Ceftazidime showed 81.9% (9) followed by TMP-SX 72.8% (8),Amikacin 54.5% (6) and Pip/Tz -27.3% (3) . No resistance was found with

Imipenem and Meropenem. 81.8% (9) were MDR. No production of MBL and Amp C was detected .

Klebsiella pneumoniae (10) : Here highest resistance was seen with Ciprofloxacin -90%(9) and TMP-SX 90%(9) followed by Cefotaxime 80%(8) ,Doxycycline 60%(6),Gentamicin 30%(3),Amikacin-20% (2) and Pip/Tz 10% (1).No resistance was seen with Tigecycline ,Polymixin B and Colistin.MDR was found to be 90%(9) and ESBL-80% (8). No MBL and Amp C production was seen.

Pseudomonas aeruginosa (5) :Maximum resistance was seen with Ceftazidime 100%(5) followed by Ciprofloxacin 60% (3),Pip/Tz 60% (3) ,Gentamicin 20% (2) and Amikacin 20% (2).No resistance was seen with Imipenem ,Meropenem and Polymixin B. MDR noted was 60% (3) and Amp C production was seen in (20%).

Klebsiella oxytoca (4):No sensitivity was found with Ciprofloxacin and TMP-SX.Resistance seen with Gentamicin,Amikacin and Cefotaxime was 75%(3) each followed by Doxycycline and Pip/Tz – 50% (2) each.No resistance was seen withTigecycline,Polymixin B and Colistin. MDR was seen with all these isolates 100%(4).ESBL -75% (4) ,MBL – 25%(1) and Amp C -1(25%).

Proteus mirabilis (2): Gentamicin ,Amikacin,Ciprofloxacin,TMP-SX and Ceftazidime showed highest resistance with both isolates being 100%(2) resistant.1 (50%) was resistant to Imipenem ,Meropenem and Pip/Tz each.MDR was found in 2(100%),ESBL -2 (100%) ,MBL- 1(50%) and Amp C production in 1(50%).

Escherichia coli (2):Ciprofloxacin ,TMP-SX and Cefotaxime were 100 % (2).50% (1) resistance was seen with Gentamicin,Amikacin and Doxycycline.No

resistance was seen with Pip/Tz,Imipenem,Meropenem, Polymixin B ,Colistin and Tigecycline.MDR was seen in 50%(1). ESBL production was found in 2(100%).No MBL and Amp C was detected in any of these two.

Citrobacter(1):This isolate was 100% resistant to Amikacin, Gentamicim, Ciprofloxacin ,Ceftazidime and TMP-SX and 100% sensitive to Imipenem, Meropenem, Pip/Tz,Tigecycline,Colistin and Polymixin B.

Table 5 , 6, 7 , 8 and 9 illustrates the above data.

TABLE 1

Patient Characteristics	Numbers	Percentage
Demographic details		
Sex : Male	32	64
Female	18	32
Age(Years) Mean ± SD	57.06 ± 10.08	
< 55	16	32%
55- 65	23	46%
> 65	09	18%
Duration of diabetes (Years) Mean±SD	6.94 ± 5.4	
Duration of ulcer(Mean ± SD)	2.6 ± 2.1	
Duration of Hospital stay(Days)	20 ± 4.8	
Mortality	NIL	
Medications before treatment		
OHA	32	64
Insulin	18	36
Patient on regular treatment	06	12
Patient on irregular treatment	44	88
Associated co-morbidities		
Hypertension	20	40
Obesity	08	16
Smoking habit	34	68
Alcoholism	30	60
Wagners Grading		
Grade 2	24	48
Grade 3	21	42
Grade 4	05	10

TABLE 2

Patients Charaterisctics	Number	Percentage
Doppler Study		
Normal	47	94
Abnormal	03	06
Diabetic Complications		
Neuropathy	20	40
Nephropathy	05	10
Retinopathy	07	14
Coronary Artery Disease	02	04
Treatment		
Debridement	29	58
Toe Disarticulation	09	18
Below knee amputation	05	10

TABLE 3

Pathogens (45)	Numbers	Percentage
Gram Positives	**10**	**22.2%**
1.Enterococcus	10	22.2%
Gram Negatives	**35**	**77.7%**
1.Klebsiella spps. Klebsiella pneumonia (10) Klebsiella oxytoca (04)	14	31.1%
2.Proteus spps Proteus vulgaris (11) Proteus mirabilis (02)	13	28.8%
3.Pseudomonas aeruginosa	05	11.1%
4.Escherichia coli	02	4.44%
5.Citrobacter freundii	01	2.22%

TABLE 4

Aerobic bacterial growth according to Wagners Grading

Characteristics	Grade 2		Grade 3		Grade 4	
	No.	%	No.	%	No.	%
Patients	**24**	48	21	42	05	10
No Growths	**7**	29	2	9.52	4	20
Pathogens Isolated	18	40	**22**	48.8	5	11.1
Proteus spps.	**7**	38.8	5	22.7	1	20
Enterococcus	5	27.7	5	22.7	_	_
Klebsiella	4	22.2	**8**	36.6	2	40
Pseudomonas	1	5.55	**3**	13.6	1	20
Escherichia coli	1	5.55	1	4.54	_	_
Citrobacter	_	_	_	_	1	20

TABLE-5

Antibiotic Sensitivity and Resistance Pattern of AerobicGram positive

Antibiotics	Enterococcus (10)	
	S %	R%
Penicillin	30	70
Ampicillin	50	50
Ciprofloxacin	60	40
High Level Gentamicin	100	0
Vancomycin	100	0

TABLE -6

Antibiotic susceptibility and resistance pattern of Aerobic Gram negative bacterial isolates from diabetic ulcer

Antibiotics	Klebsiella (14)				Proteus (13)			
	Klebsiella Pneumonia(10)		Klebsiella Oxytoca(4)		Proteus vulgaris(11)		Proteus mirabilis(2)	
	S	R	S	R	S	R	S	R
Gentamicin	70	30	25	75	9	91	0	100
Amikacin	80	20	25	75	45.4	54.5	0	100
Ciprofloxacin	10	90	0	100	9	91	0	100
TMP-SX	10	90	0	100	27.2	72.8	0	100
Cefotaxime	20	80	25	75	_	_	_	_
Ceftazidime	_	_	_	_	18.1	81.9	0	100
Imipenem	100	0	75	25	100	0	50	50
Meropenem	100	0	75	25	100	0	50	50
Doxycycline	40	60	50	50	_	_	_	_
Pip/Tz	90	10	50	50	72.7	27.3	50	50
Tigecycline	100	0	100	0	_	_	_	_
Polymixin B	100	0	100	0	_	_	_	_
Colistin	100	0	100	0	_	_	_	_

TABLE 7

Antibiotic susceptibility and resistance pattern of Aerobic Gram negative bacterial isolates from diabetic ulcer

Antibiotics	Pseudomonas Aeruginosa(5)		Escherichia Coli (2)		Citrobacter (1)	
	S%	R%	S%	R%	S%	R%
Gentamicin	60	40	50	50	0	100
Amikacin	60	40	50	50	0	100
Ciprofloxacin	40	60	0	100	0	100
TMP-SX	_	_	0	100	0	100
Cefotaxime	_	_	0	100	_	_
Ceftazidime	20	80	_	_	0	100
Imipenem	100	0	100	0	100	0
Meropenem	100	0	100	0	100	0
Doxycycline	_	_	50	50	100	0
Pip/Tz	40	60	100	0	100	0
Tigecycline	_	_	100	0	100	0
Polymixin B	100	0	100	0	100	0
Colistin	100	0	100	0	100	0

TABLE 8

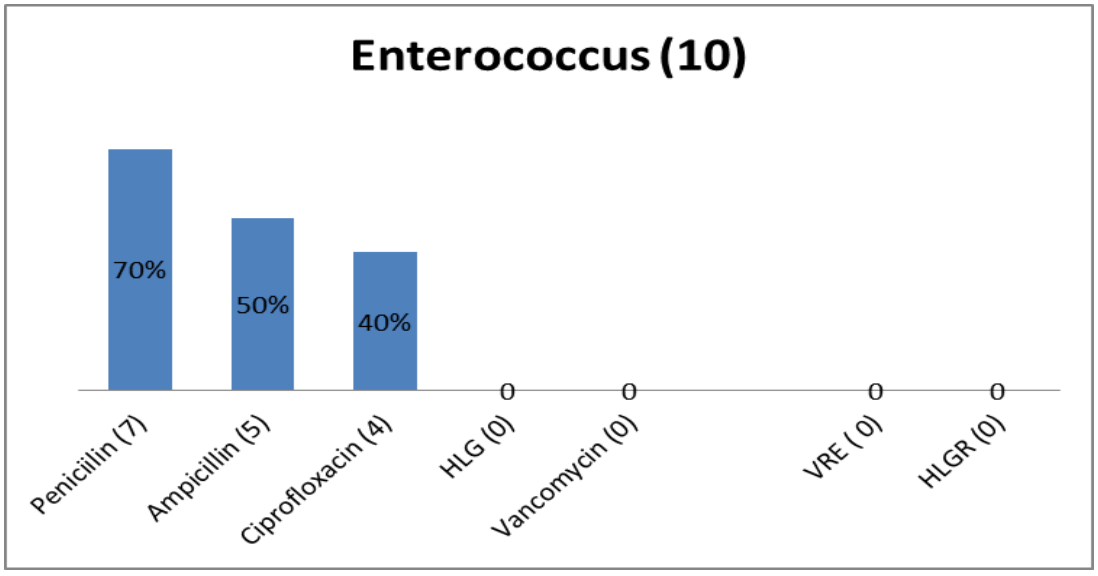
Prevalence of MDR and ESBL in the Aerobic Isolates

Pathogens	MDR		ESBL	
	Nos. out of 45	%	Nos.out of 18	%
Proteus Vulgaris(11)	9	20	-	
Klebsiella Pneumoniae(10)	9	20	8	22.8
Pseudomonas aeruginosa(5)	3	6.66	-	
Klebsiella oxytoca (4)	4	8.88	3	8.57
Proteus mirabilis(2)	2	4.44	2	5.71
Eschericia coli(2)	2	4.44	2	5.71
Citrobacter freundii (1)	1	2.22	_	_
Total	28	62.2	16	88.88

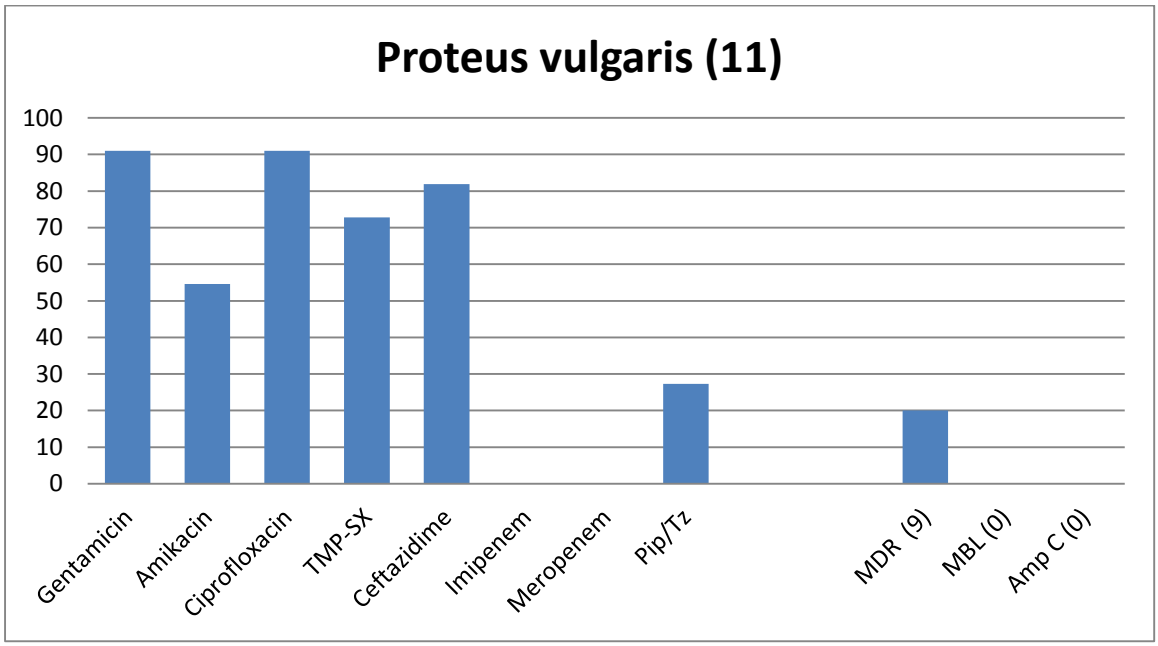
TABLE 9

Prevalence of MBL and Amp C in the Aerobic Isolates

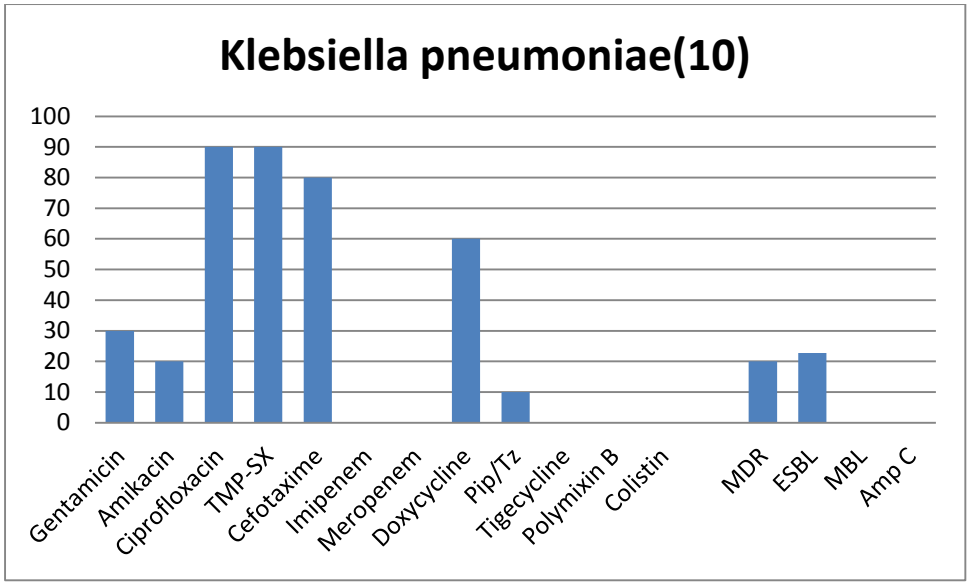
Pathogens(35)	MBL		Amp C	
	No.	%	No.	%
Proteus Vulgaris(11)	0	_	0	_
Klebsiella Pneumoniae(10)	0	_	0	_
Pseudomonas aeruginosa(5)	0	_	1	2.8
Klebsiella oxytoca (4)	1	2.8	1	2.8
Proteus mirabilis(2)	1	2.8	1	2.8
Eschericia coli(2)	0	_	0	_
Citrobacter freundii (1)	0	_	0	_
Total	2	5.71	3	8.5



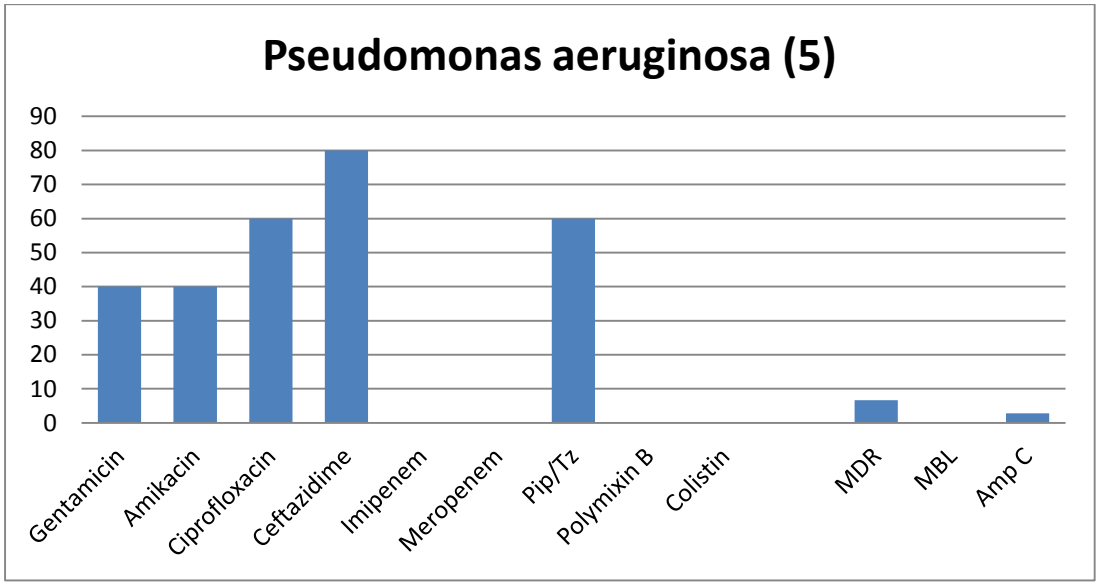
Resistant pattern of Enterococcus with VRE and HLGR status



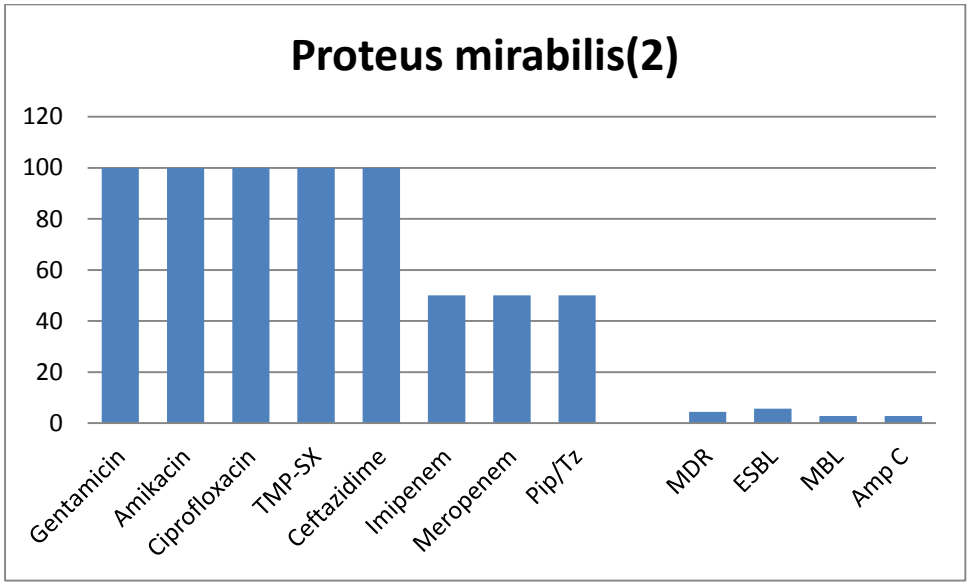
Resistant pattern of Proteus vulgaris



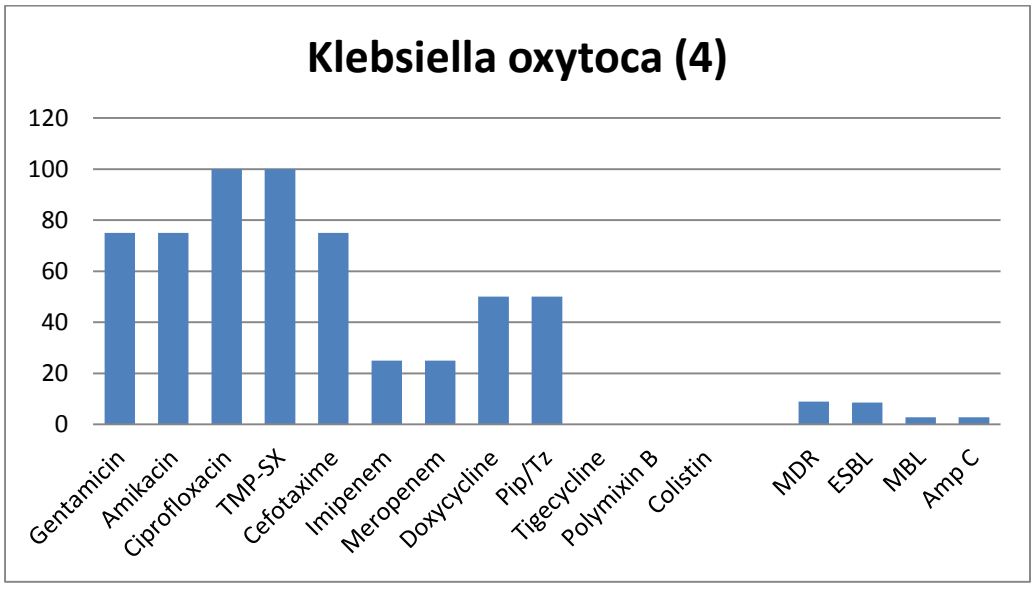
Resistant pattern of Klebsiella pneumonia



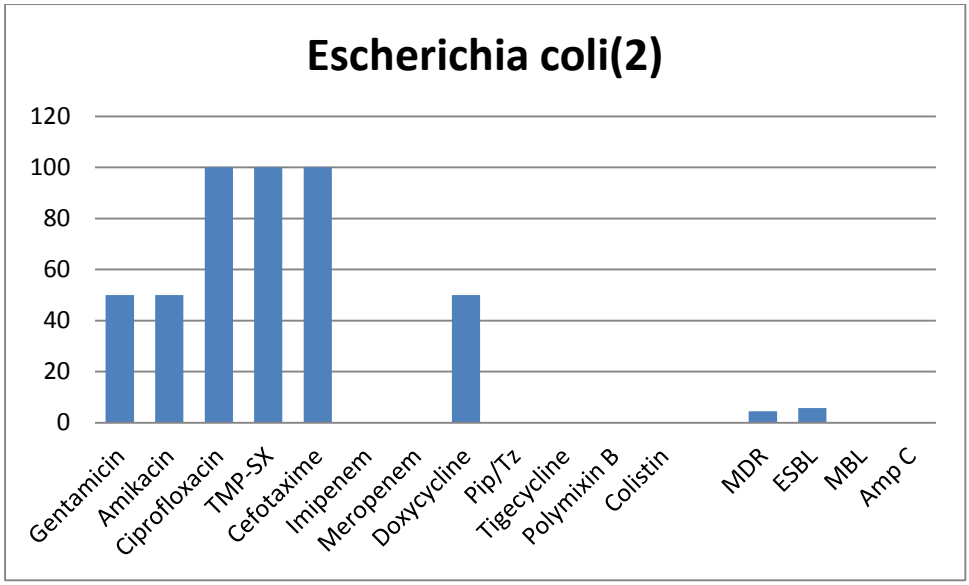
Resistant pattern of Pseudomonas aeruginosa



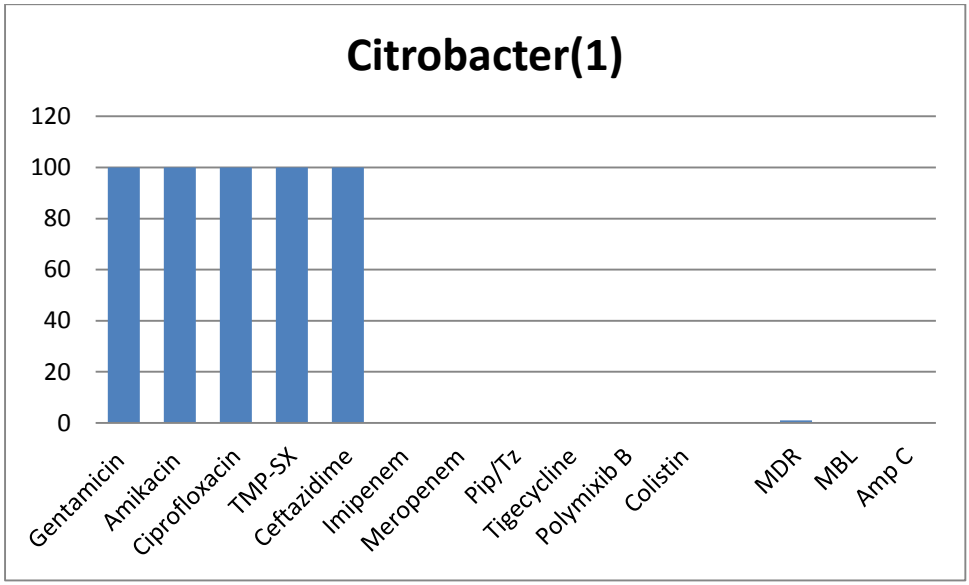
Resistant pattern of Proteus mirabilis



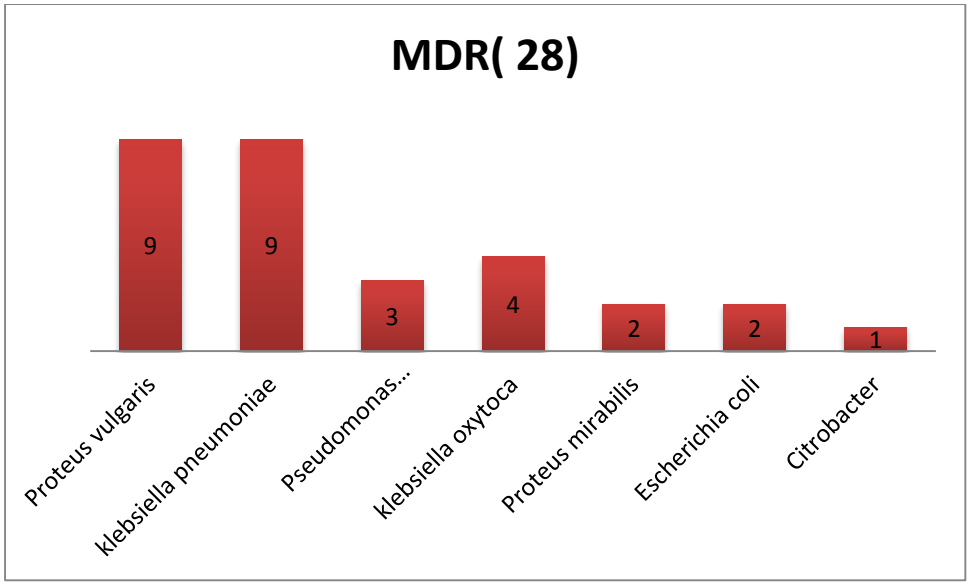
Resistant pattern of Klebsiella oxytoca



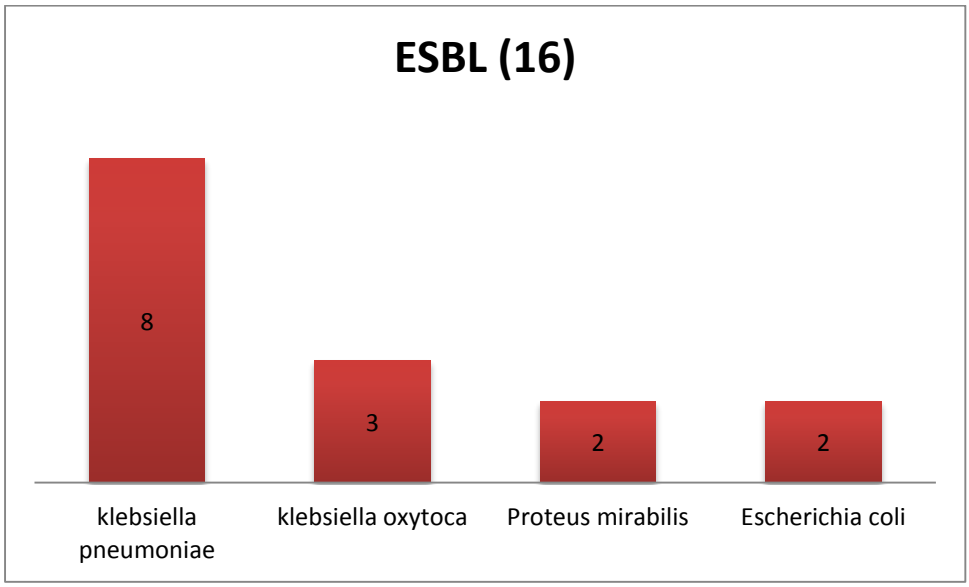
Resistant pattern of Escherichia coli



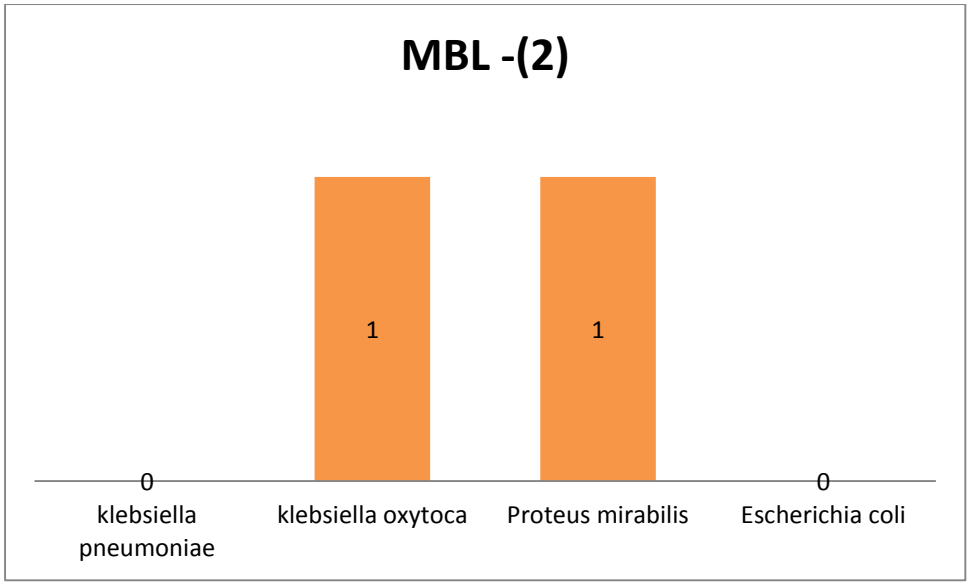
Resistant pattern of Citrobacter freundii



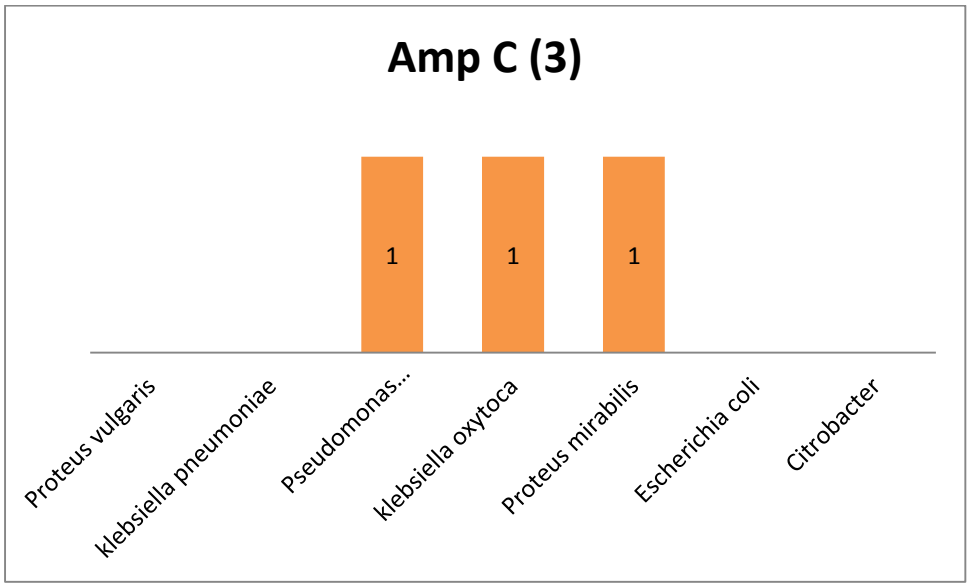
Multi –Drug Resistance in pathogens isolated



Extended spectrum β-lactamases producers



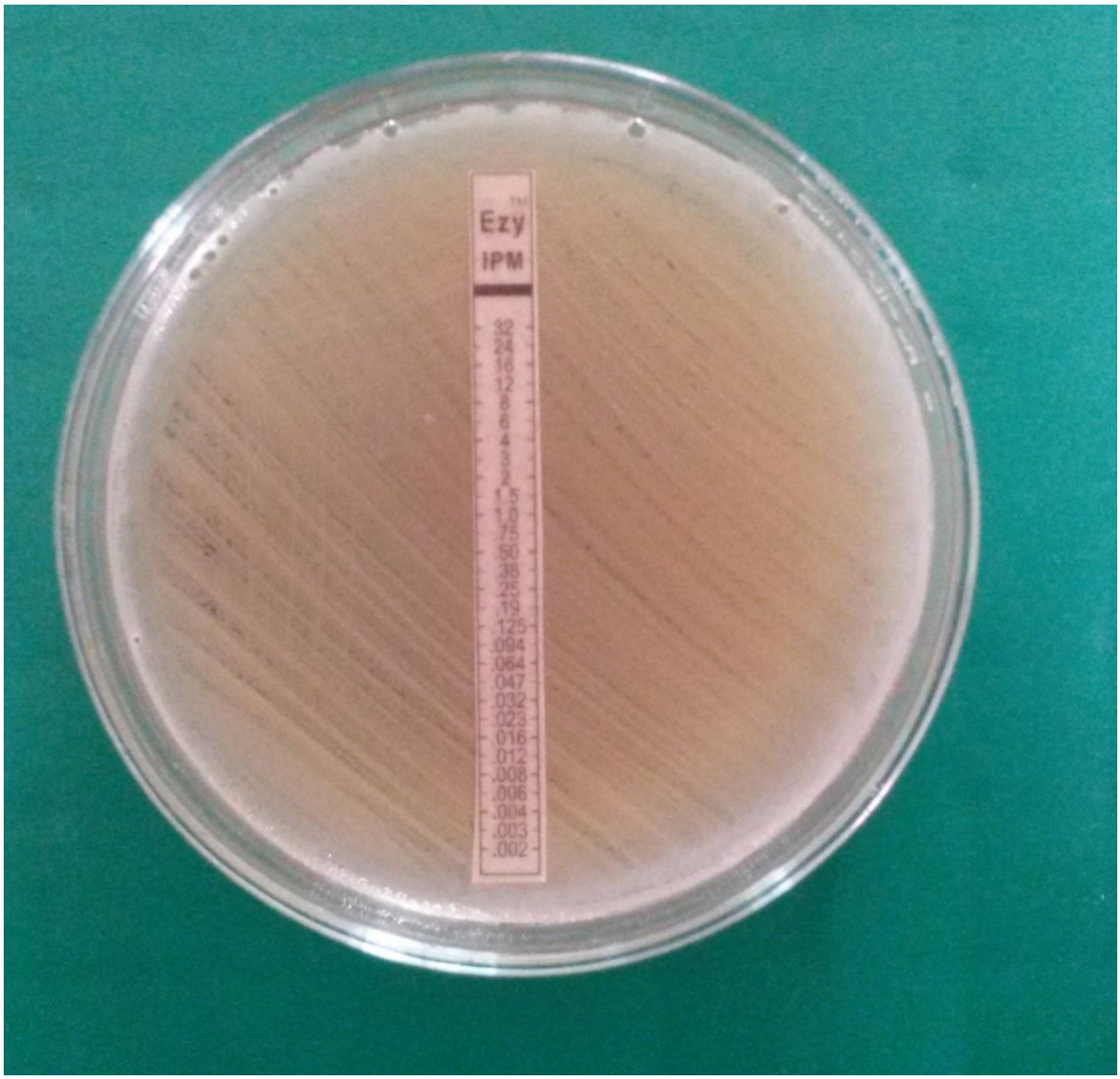
Metallo-β-lactamase producers



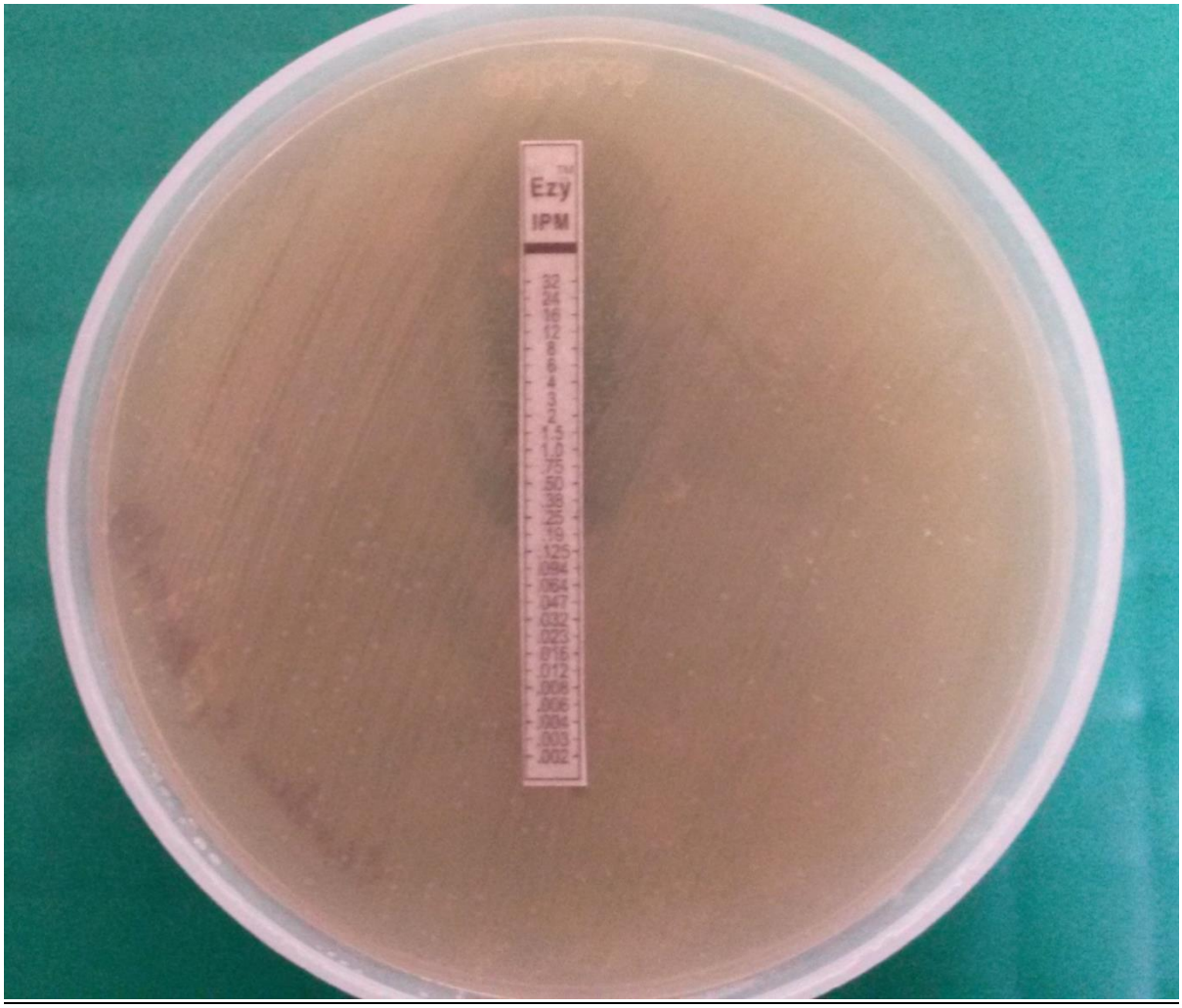
Amp C producers

E –test strip test was done as the steps mentioned in Methodology: Two pathogens were detected resistant to Imipenem and Carbapenem by Kirby Bauer Disk Diffusion method and subjected to this test .Both were found resistant .

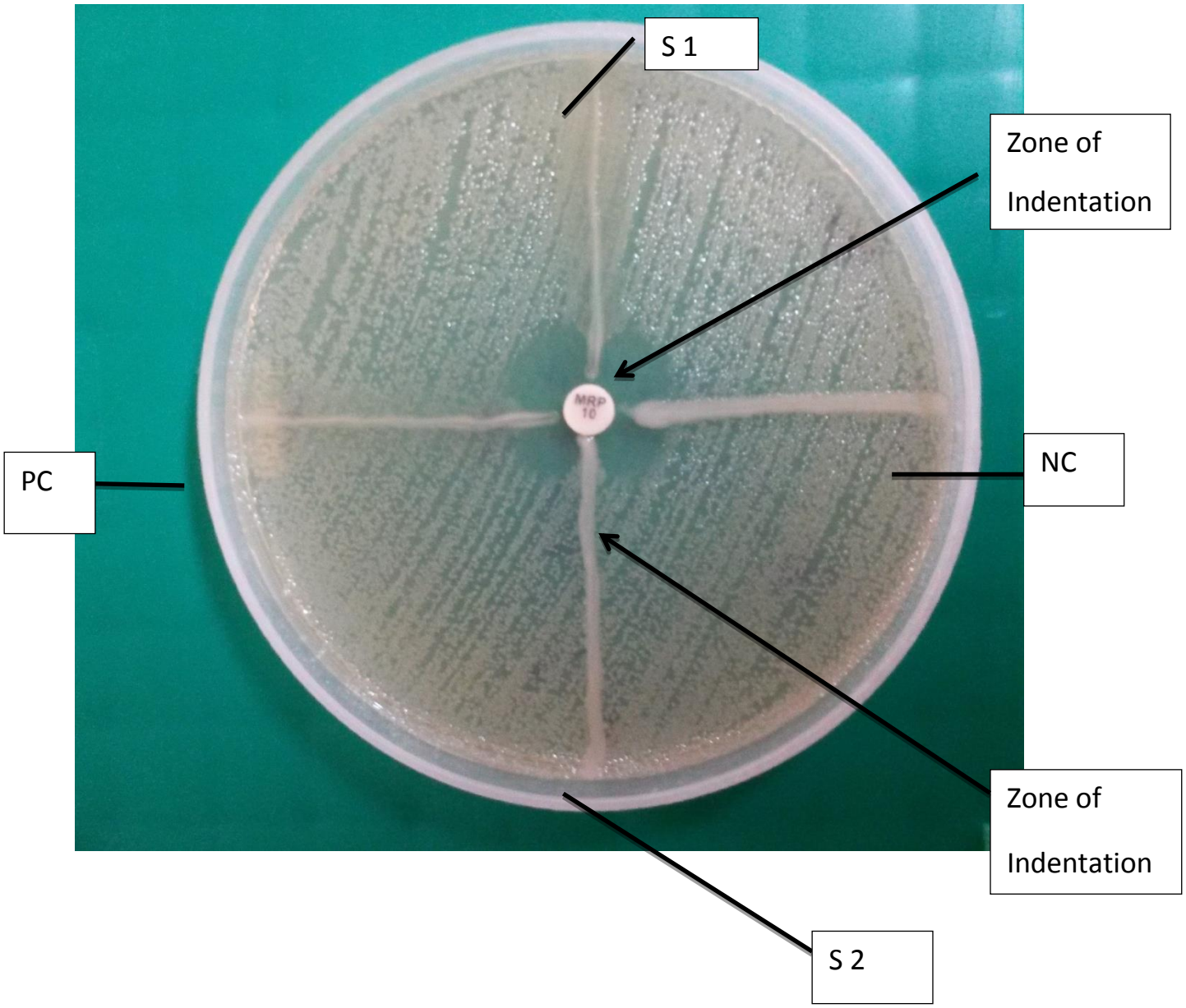
Klebsiella oxytoca- No zone of clearance of the organism around the strip



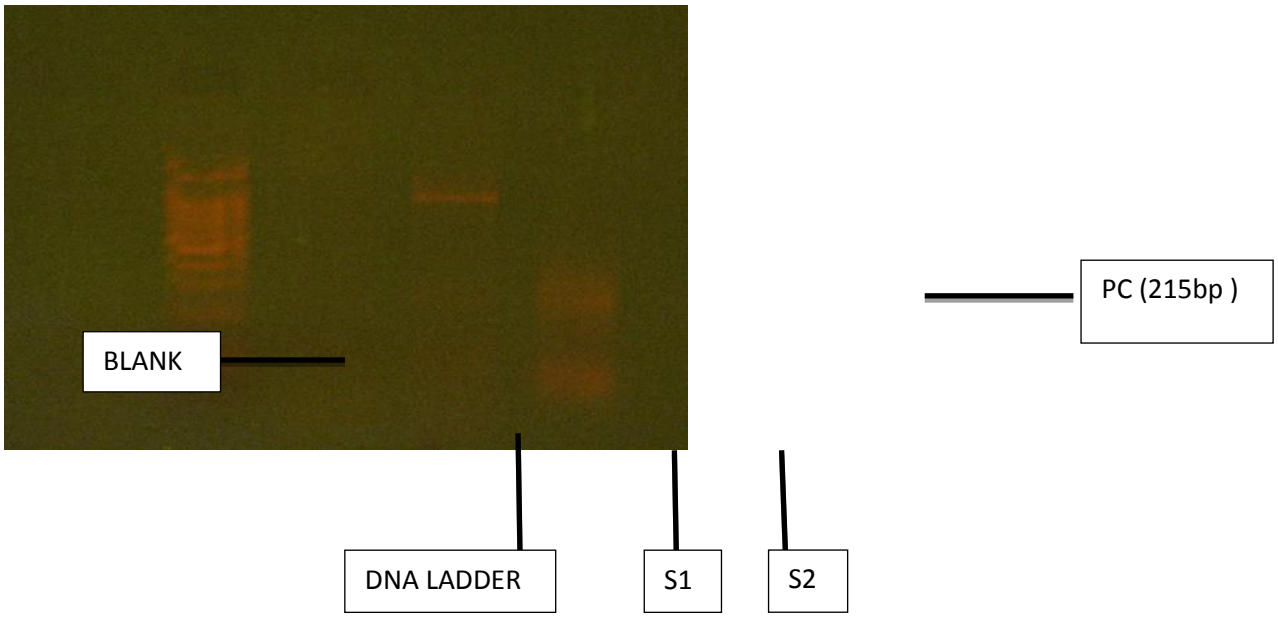
Proteus mirabilis - MIC – 3.0 (CLSI: S- ≥1, I- 2, R -≤ 4



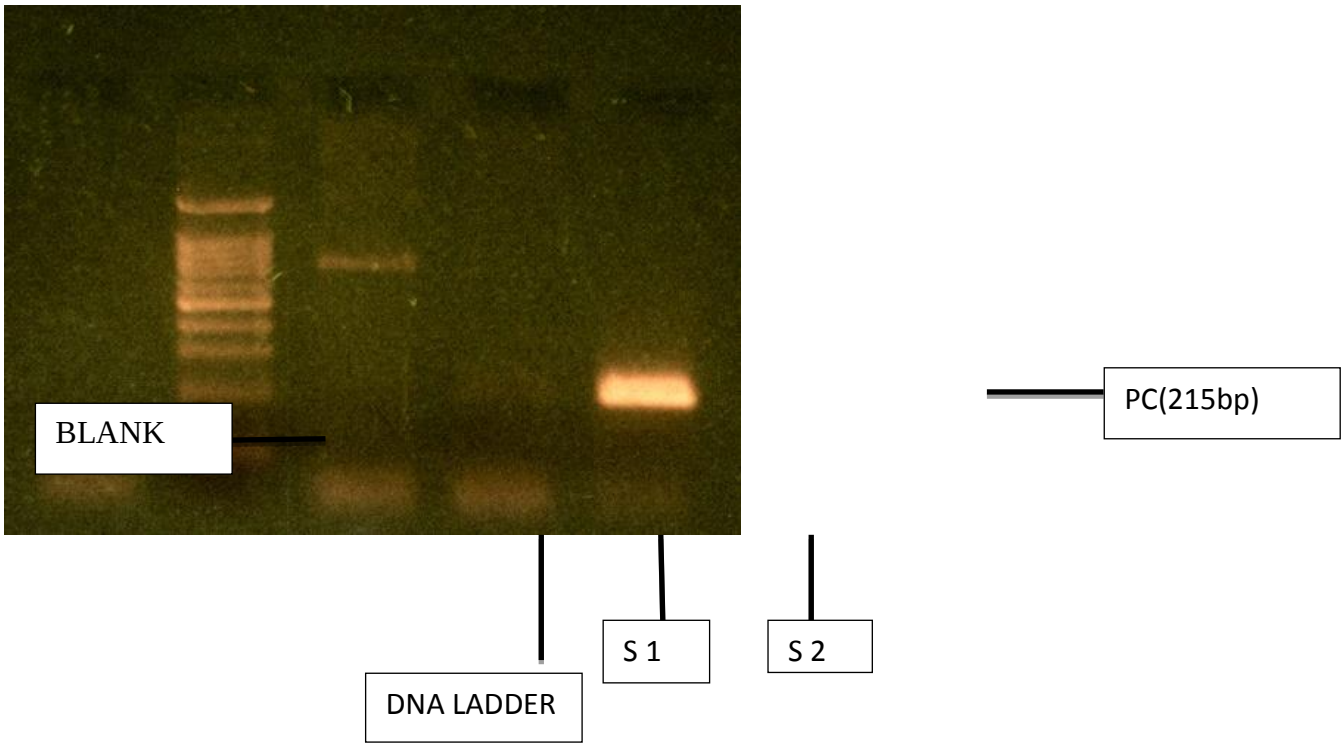
Further Modified Hodge test was done .It's one of the phenotypic methods of detection of Carbapenemase production . Both the samples showed indention and a clover leaf appearance was noted with the positive control and test samples.



PCR- According to procedure illustrated in methodology the carbapenem resistant isolates were processed for gene detection- *bla*$_{KPC}$ and *bla*$_{NDM..}$Both the isolates were negative for these genes tested.



KPC gel Ladders ,showing negative result for both the sample



NMD gel Ladders showing negative results for both the sample

DISCUSSION

6.DISCUSSION

Diabetes mellitus is a serious public health concern worldwide and the prevalence is increasing in India faster than in any other part of this world .[79]Diabetes affects various other parts of the body but this study is focussed on diabetic foot ulcers and its severity .

Its because of the serious or recurrent infections and impaired healing processes that the initially trivial lesion may progress to chronic non healing wounds ,gangrene or untreatable infections that can lead to limb amputations.[80] Most of the diabetic foot ulcers are neglected because they produce few symptoms and their importance is not appreciated by the patients.[81]

Patients who develop foot ulcers have significantly less knowledge of diabetes including footcare.[82]Assal et al : had clearly shown that education on diabetic foot care reduces the number of major amputations in diabetic clinic population. It is therefore of utmost importance to educate the patients to recognize the infection in diabetic foot early and treat it vigorously.[83] Lasarus et al had noted that 'diabetic gangrene is not heaven sent but earth born' so foot problems can be prevented or the associated with it can be diminished. [84]Successful treatment depends on correct evaluation of patient, isolation of pathogens with their antibiotic susceptibility pattern.

Physicians should be adept in managing the cases and should equip themselves with knowledge of the major risk factors of amputation and their prevention with special reference to drug resistance in bacteria.[85,86]

In present study males 32 (64%) were affected more than females 18 (36%).Our result are in concurrent with other investigators .[87,88,89,90]

Diabetes can affects all age group of people with manifold complications .The mean age of the patients was 59.5 years in Kahn et al,[91]study 80.3 years in Delbridge et al,study [92] 58 years in Ramani et al[93] ,study 75 years in Parthare et al[94] ,study ,58 years in Dipali et al,study[95] 53.9 ± 12.1 years in Gadepalli et al, study, [96]

60.8 ± 10.4 years in Shao-Hua-et al ;[97] and Anandi et al, [98]observed the mean age to be 43 years.The mean age of the patients in our study is 57.06 .The patient in our study were of the age 40 to 80 years . The maximum number of patients were in the age group of 51 to 60 years-21(42%). Priyadarshini et al [99]studied showed maximum number of patients (20%) in the age group of 60 – 65 years.

Uncontrolled diabetes lead to various complications and if it is of long duration foot problems become inevitable.Oakely et al ,[100] observed that 19.8 % of patients had diabetes for 5- 10 years and 7.1% of patients had the maximum duration of diabetes for 10 -15 years.Louie et al[101] showed that 12.7% of the patients had 10 -15 years of duration of diabetes.Faiz ur Rehman et al [102] found that most patients had diabetes of more than a decade. Gadepelle et al [96] found that the mean duration of the diabetes mellitus was 11.8 ± 5.6 years. According to study carried out by Atif Sitwat et al ,[103]the mean duration of diabetes mellitus was 15.6± 4.5 years.The mean duration of diabetes in patients in this study is 6.94±5.4 years .The maximum number of cases-29(58%) had diabetes of duration range 1 to 5 years.

Blood sugar level has a direct role on the microbial isolation rate. In the present study ,we observed that 31.1 % (14) of bacterial isolate were obtained from patients having Blood sugar level ≤200 mg/dl whereas 68.8 %(31) was obtained from patients having Blood sugar level ≥ 200 mg/dl .

The good control of blood sugar in diabetic patient is a desirsble goal in the prevention of certain infections and to ensure maintenance of normal host defence mechanism determining resistance and response to infection.There is a significant diminution in intracellular bactericidal activity of leucocytes in patients with poorly controlled diabetes.In the present study 64% of patients who were previously diagnosed and maintained on oral hypoglycemic agents also developed ulcers because of their non compliance. 36 % of patients were on insulin injection .Ozer et al, [104] observed that 38.5% were on OHA and 39.7% on insulin,similarly Mohan Sundaram[105] observed that 47% of the study subjects were on OHA and 36.7% on insulin,Atif Sitwat et al,[103] observed that 64.7% were on OHA and 15.3% on insulin.Excessive hyperglycemia leads to decrease in tissue oxygenation and further hyperglycemia induced pseudohypoxia causing reduced blood supply and formation of gangrene.[106] Neuropathy increases the sufferings of diabetics from making them unaware of injuries due to loss of sensation.In our study 40 % patients had peripheral neuropathy.

Smoking has always been considered a factor in the etiology of foot lesions not only because of increased incidence of atheroma but also because of added shift to the left of oxyhaemoglobin dissociation curve due to glycosylation..In the present study 68% are smokers .Bamberger et al .,[107] observed that 64.7% diabetic patients were

smokers, 47% by Atif sitwat.,[103] 35% by Mohammed Zubair et al.[108] Delbridge et al [92] found that.,71 % of his study patients with foot lesions were smoking or had smoked in the past. In our study smoking habit was found in 68%.

Diabetes and high blood pressure go hand in hand.They occur together so frequently that they are officially considered to be co-morbidities because they share certain physiological traits.In the present study 40% had associated hypertension while 60 % were non hypertensives .Bamberger et al[107] observed that 77% had previous coronary artery or cerebrovascular disease or hypertension.According to Ozer et al ,[104] 44.9% had hypertension, 64% Banashankari et al,[109] 39.7% by Mohan Sundaram,[105]. Our study matches with Mohan Sundaram data.

Diabetic Neuropathy refers to a group of nerve disorders caused by diabetes .Diabetic neuropathy can affect nerves throughout the body,although it most commonly affects the nerves in the feet and legs.The loss of sensation as a part of peripheral neuropathy exacerbates the development of ulcerations.As trauma occurs at the affected site patients are often unable to detect the insult to lower extremities.As a result many wounds go unnoticed and progressively worsen as the affected area is continuously subjected to repetitive pressure and sheer forces from ambulation and weight bearing.Louie et al,[101] observed neuropathy in 100% of cases,Mohan Sundaram et al[105] observed neuropathy in 63.7% of cases,Gadepalli et al,[96] observed neuropathy in 85%. Banashanakari et al,[109] observed neuropathy in 76% of cases and Faiz ur Rehman et al,[285] in 67%-73%.Sapico et al, [110] observed vasculopathy in 88% and Faiz ur Rehman et al,[102] in 26% -33% of the cases .

The Wagner Classification grades diabetic foot ulcers on the basis of depth of tissue penetration and necrosis.Low grades have been seen to be infected with gram positive organisms and higher grades with polymicrobial flora.In the present study the maximum number of bacteria was isolated from Grade 3 ulcers -22 (48.8 %). Study conducted by Pathare et al, [94] showed that maximum number of bacteria was isolated from Grade 3 ulcers (23 %) and maximum numbers of anaerobes per case from Grade 5 ulcers.Study conducted by Banashankari et al, [109] showed that maximum number of bacteria was isolated from Grade 2(46%) ,Study by Mohammad Zubair et al, [108] showed the isolation rate was highest in Grade 1 (33%) followed by Grade 2(35%).Study by Atif Sitwat et al , [103] observed that maximum number of bacteria was isolated in Wagner Grade 4(49.4%) followed by Grade 3 (27%).Although the number of organisms increased progressively in the last four Grades ,the number of organism isolated were for or less same indicative of probably well established poly microbial wound infection.It can be interpreted that to start with ,there is a poor response to injury to the patient with diabetes.This may be because of several factors like vascular insufficiency,neuropathy and poor control of basic diabetic process.The poor defense leads to rapid increase in the number of microbes both aerobes and anaerobes with progressive deterioration of diabetic foot wound.Once the wound has reached Grade 3 or more ,the foot wound is more or less well established with not much further increase in the number of organisms.Anaerobic organisms flourish in deep seated infections .This indicates that with increasing Grade of ulcer,the anaerobic conditions are produced as a result of increase the depth of the wound and decrease in the peripheral blood flow,leading to higher rate of infections by anaerobes. In our study

maximum pathogen isloted were from Grade 3, which was similar to Pathare et al [94] study.

Previously many studies reported the Gram- positive aerobes to be prominent in diabetic foot infections,we found Gram negative aerobic- 35 (77.7%) bacteria the most frequently isolated pathogen. As this is a tertiary centre,we receive patients previously treated in primary and secondary centres ;we isolated more of Gram negatives compared to Gram positives. A study conducted by Sivaraman Umadevi et al,[111] isolated 70.8% of Gram negative bacilli and 29.2% Gram positive cocci with Proteus mirabilis (62.5%),Klebsiella pnemoniae(60%),E.coli (56%) as predominant Gram negative bacilli and Staphylococcus aureus (15%) as predominant Gram positive bacteria. Study by Banashankari et al, [109] isolated Gram negative bacilli in 66% and Gram positive cocci in 32% with Proteus mirabilis 18% and Staphylococcus aureus 24% as predominant bacteria. Mohammad Zubair et al,[108] isolated Gram negative aerobes as the predominant organisms (56%) followed by Gram positive aerobes (44%) and E.coli was isolated as the predominant organisms as (26.6%) followed by Staphylococcus aureus (28%) ,Shalbha Tiwari et al,[112] in the study isolated 68% of Gram negative bacilli and 32% Gram positive cocci and E.coli(28%).In the study by Shao-Hua et al,[97] isolated gram positive cocci in 50.6% followed by gram negative bacilli in 44.4% with Staphylococcus aureus 25.6% being the predominant bacteria followed by Proteus mirabilis (20%).In our study Enterococcus (22.2%) was the only Gram positive bacteria found and among the Gram negative isolates-35(77.7%) ;Klebsialla spps .-14(31.1%) was highest followed by Proteus spps.- 13(28.8%) , Pseudomonas aureginosa – 5(11.1%), Escherichia coli -

2(4.44%) and Citrobacter -1(2.22%)were found .In Priyadarshini Shanmugam et al[99] study done in the vicinity ;Gram negative were more found more (65.1%) than cocci (39.8%).In her commonest was Pseudomonas spps. (16%) then Escherichia coli(14.6%) and Staphylococcus aureus(13.3%).

The incidence of Enterococci in diabetic foot infections has been raising.Gadepalli et al, [96] isolated 11.5%,Mohammad Zubair et al,[108] 5.6% ,Atif Sitwat et al, [103] 2.3%, Sivaraman Umadevi et al , [111] 5.3%,Banashankari et al, [109] 9% and Shao-Hua, [97] 10.6% of enterococcus species.In the present study 22.2% of enterococcus species were the only Gram positive isolated isolated -5 each from Grade II and Grade III ulcers.

Anaerobic bacteria are almost always isolated with aerobes from diabetic foot infections.Gadepalli et al,[96] has isolated 15.3% of anaerobic bacteria with Bacteriodes fragilis (1.6%) being the predominant bacteria.mAnaerobic bacteria were isolated from aspirated pus. Naden et al, has isolated 2.5% of anaerobic bacteria with Bacteriodes fragilis again as the dominant bacteria.Anaerobic bacteria were isolated from grade 2 and grade 5 ulcers.In this study anaerobic bacteria were not cultured due to lack of facility.

The combination of increasing numbers of the population who are at risk of developing chronic wounds, together with the increasing prevalence of antibiotic resistance makes this a highly important issue.The poly microbial nature of chronic wounds is likely to provide an appropriate environment for genetic exchange between

bacteria.Cases of Vancomycin resistance in Staphylococcus aureus was first reported in two patients suffering from chronic wound infection in United States.[97,113]

In this study the antibiotic sensitivity pattern showed Enterococcus to be resistant to Penicillin-70% (7) followed by Ampicillin 50% (5) and Ciprofloxacin 40% (4) .No resistance was found with High Level Gentamicin and Vancomycin. Sivaraman et al[111] found high level aminoglycoside resistance in 33% isolates of Enterococcus. Gordon et al also found high incidence of HLGR and HLSR.[114]

In our study with respect to Pseudomonas ;maximum resistance was seen with Ceftazidime 100% followed by Ciprofloxacin 60% ,Pip/Tz 60% ,Gentamicin 20% and Amikacin 20% .No resistance was seen with Imipenem ,Meropenem and Polymixin B. and Amp C production was seen in 1 (20%).Study done by Priyadarshini Shanmugam et al[99] study done in the vicinity showed more or less same resistant pattern with 61% resistance to cephalosporins and 50% resistance to Gentamicin and Ciprofloxacin and Carbapenem resistance was also found.Shankar et al[89] study showed MDR of 44% in Pseudomonas while in our study MDR noted was 62.2 % with highest percent in Proteus and Klebsiella(20% each).

In case of Enterobacteriaceae; Klebsiella pneumoniae (10) highest resistance was seen with Ciprofloxacin -90%(9) and TMP-SX 90%(9) followed by Cefotaxime 80%(8) ,Doxycycline 60%(6),Gentamicin 30%(3),Amikacin-20% (2) and Pip/Tz 10% (1).No resistance was seen with Tigecycline ,Polymixin B and Colistin.MDR was found to be 90%(9) and ESBL-80% (8). No MBL and Amp C production was seen and in case of Klebsiella oxytoca (4);No sensitivity was found with

Ciprofloxacin and TMP-SX. Resistance seen with Gentamicin, Amikacin and Cefotaxime was 75%(3) each followed by Doxycycline and Pip/Tz – 50% (2) each.No resistance was seen withTigecycline,Polymixin B and Colistin. MDR was seen with all these isolates 100%(4).ESBL -75% (4) ,MBL – 25%(1) and Amp C - 1(25%). Priyadarshini Shanmugam et al[99] study done in the vicinity showed 100% resistance to Gentamicin and Imipenem resistance seen in 20% Studies done by Khoharo et al [115] and Raja et al[116]showed similar results except all were Imipenem sensitive.ESBL production in Klebsiella was 40% in Priyadarshini Shanmugam et al [99] study with MBL production of 20%.In our study ESBL production was high in Klebsiella species 11 (78.5%) and MBL 1(7.1%) was also isolated and Amp C production seen in 1(7.1%).

In Proteus spps. , Proteus vulgaris (11) : Gentamicin and Ciprofloxacin showed highest resistance -91%(10) each .Ceftazidime showed 81.9% (9) followed by TMP-SX 72.8% (8),Amikacin 54.5% (6) and Pip/Tz -27.3% (3) . No resistance was found with Imipenem and Meropenem. 81.8% (9) were MDR. No production of MBL and Amp C was detected . In case of Proteus mirabilis (2): Gentamicin,Amikacin,Ciprofloxacin,TMP-SX and Ceftazidime showed highest resistance with both isolates being 100%(2) resistant.1 (50%) was resistant to Imipenem ,Meropenem and Pip/Tz each. MDR was found in 2(100%),ESBL -2 (100%) ,MBL- 1(50%) and Amp C production in 1(50%). Priyadarshini Shanmugam et al study done in the vicinity showed Proteus mirabilis to be 100% resistant to Cotrimoxazole and Gentamicin; which matched with our study.One isolate(2.8%) was found MBL producer in our study and in their study it as 40%.ESBL producrion

also matched as it was 80% in their study and 84.6% in ours. A study in Pondicherry reported 62.5% ESBL in Proteus.[99]

In Escherichia coli in our study: Escherichia coli (2):Ciprofloxacin ,TMP-SX and Cefotaxime were 100 % (2).50% (1) resistance was seen with Gentamicin,Amikacin and Doxycycline.No resistance was seen with Pip/Tz,Imipenem,Meropenem, Polymixin B ,Colistin and Tigecycline.MDR was seen in 50%(1). ESBL production was found in 2(100%).No MBL and Amp C was detected in any of these two. Priyadarshini Shanmugam et [99] study showed similar pattern with ESBL production(73%) lesser than ours(100%). Citrobacter was resistant to all the first line but sensitive to second line antibiotics. Priyadarshini Shanmugam et [99] study showed MBL production with 75% to Meropenem and 25% resistance to Imipenem.

In our study ; in Gram negatives resistance was not seen with Colistin , Polymixin B,Tigecycline, (0%)and least resistance was seen with Pip/Tz(2.85%) favouring and reserving their use in highly infected diabetic foot ulcers.

In our study ;Conventional Polymerase chain reaction done to find incidence of KPC gene(rare gene in India) and NDM gene (not so rare gene in India) in the two carbapenem resistant and MHT positive isolates was negative for both. Genetic study on multi drug resistant isolates have been done in diabetic foot ulcers in North India,but not for the above mentioned two genes.[117] A study done by Rachana solanki et al [118] in India has revealed presence of KPC (15)and NDM(49) in growths from various clinical samples.In a study done in southern India;NDM was detected in

4 patients from a tertiary care centre.[119] In a study done by Anjana Shenoy K., Jyothi E.K. & R. Ravikumar in south India, 34 isolates out of 74 MDR GNB were positive for blaNDM-1. [120] In a study done by Mohan et al ;presence of NDM-1 gene but absence of KPC gene was noted from the clinical isolates. In their study ,out of 166 isolates which were phenotypically carbapenemase producers ;66 isolates were negative for all the four genes (*bla*$_{\text{VIM}}$,*bla*$_{IMP}$,*bla*$_{\text{NDM}}$ and*bla*$_{\text{KPC}}$) tested by PCR..[121]

Treatment of diabetic foot is a multidisciplinary approach and requires a good co-ordination between all health care professionals involved.The management of diabetic foot involves debridement of the wound, antibiotic treatment, revascularization, conservative surgery and amputation if needed. Treatment offered to Grade 2 and Grade 3 Diabetic foot ulcers is their thorough debridement followed by disarticulation and amputation if needed. Study by Lea Renina et al.[122] revealed that 4 patients out of 12 (33.3%) who had repeated procedures eventually died.89% underwent surgical intervention with concomitant antibiotics. Surgical debridement was the most common intervention in 52.7% followed by toe disarticulation in 16.6% and Below knee amputation in 13.9%.In the study by Catherine Amalia et al.,[123] 86% of the subjects were discharged, while 5% went home against advice and 8.7% died.Out of 8% discharged ,70% underwent amputations of which Below knee amputation was the most frequently performed sugery.In a study by Gadepalli et al ,[96] 42.5% underwent surgical debridement of which 11.8% patients underwent amputation below the knee.According to Anandi et al., 1.97% of patients underwent transmetatarsal amputations and 11.8% underwent Below knee amputation and 1.97% underwent above the knee amputations. In our study,14 (28%) were managed only on

dressing and antibiotic alone and 29 (58%) patients had surgical debridement along with antibotics.18%(9) of the patients had toe disarticulation and 10% (5) underwent Below knee amputation.

CONCLUSION

7.CONCLUSION

Uncontrolled Diabetes with progressing age along with reduced care in foot ,favouring impending infections leading to high cost of hospitalization and further negligence results in disarticulation of toes and amputations of foot and legs thus increasing morbidity.

As in India knowledge about importance of regularity in treatment of Diabetes is poor further progression to complications is inevitable and patient often tend to land up in severe infected foot ulcers ;harbouring resistant pathogens while hopping from one hospital to another.

This study done in our centre has revealed most of the patients on irregular treatment and lack of foot care as most of them belonged to low socio- economic strata .Being a tertiary care centre we receive patients moving from one hospital to another with chronic infections that has reduced the incidence of Gram positive cocci (especially notorious Staphylococcus) in our study.

This study done on these patients for bacterial profile and their antibiotic profile unearths Gram negatives to be most important etiological agents – Klebsiella spps, followed by Proteus ,Enterococcus(Gram positive),Pseudomonas,Escherichia coli and Citrobacter. MDR rate has increased with most cephalosporins alsobecoming resistant leading to elevation in ESBL rate . Carbapenem resistance was also seen in two isolates whose phenotypic test revealed Carbapenemaseproduction. Apart from other higher antibiotics ;Piperacilin /Tazobactam showed least resistance favouring its use for high grade diabetic ulcers as it covers aerobic, anaerobic and is a good β-

lactamase inhibitor. However it does not rule out the necessity of culture and sensitivity tests with rationale surgical procedures to prevent further spread of infection.

The genotypic test done on those two isolates for blaKPC and blaNDM by conventional PCR proved absence of these genes in the those two resistant isolates. Further other molecular studies need to be done to detect the resistance mechanisms.

This evidence –based study will definitely lead to a well guided approach to the management of foot ulcers in diabetics in our centre.

Using evolving technologies in detection of resistance will also help in infection control in health care .

ANNEXURES

Master chart

SN	AG	SX	IP	OP	DD	FH	SE	ED	CO-MOR	DC	S	A	r/l TT	RBS	Dop	WG	Path	Ge	AK	Cip	TMP-SX	Amp	Ctx
1	40	F	IP		5 years	DK	low	low	N	N	N	N	i	329	N	2	Ef	x	x	R	x	R	x
2	65	F	IP		16 years	DK	low	low	HT	N	N	N	i	311	N	3	Kp	S	S	S	R	x	S
																	Pa	S	S	S	x	x	x
3	80	F	IP		1 year	DK	low	low	HT	NEU	N	N	i	145	N	2	Ng	x	x	x	x	x	x
4	48	F	IP		3 years	DK	low	low	N	N	N	N	r	388	N	3	Ko	R	R	R	R	x	s
																	Ef	x	x	R	x	s	x
5	62	M		OP	4 years	DK	low	low	N	N	Y	Y	i	160	Y	2	Ng	X	X	X	X	X	X
6	60	M	IP	OP	3 Years	DK	low	low	N	NEU	Y	Y	i	200	N	2	Pa	R	R	R	x	x	x
7	56	M	IP		4 Years	DK	no	no	N	RP	Y	Y	i	390	Y	3	Pm	R	R	R	R	r	
8	52	M	IP		2 Years	DK	low	low	HT	N	N	Y	I	371	N	3	Ko	R	R	R	R	X	R
9	55	F	IP		12 Years	DK	low	low	HT	NEU	N	N	i	389	N	3	Pv	R	R	R	R	x	x
10	56	M	IP		10 years	DK	low	low	HT	NEU	Y	Y	i	290	Y	4	Ko	R	R	R	R	x	r
11	48	M	IP		5 years	dk	low	low	N	N	Y	Y	i	260	N	3	Kp	S	S	R	R	x	r
12	68	M		OP	2 years	dk	low	low	HT	N	Y	Y	i	180	N	3	Ng	x	x	x	X	x	x
13	43	M		OP	5 YEARS	YES	low	low	N	NEU	Y	N	I	200	N	2	Ef	X	X	S	X	r	X
14	41	M		OP	4 YEARS	YES	LOW	LOW	NIL	N	Y	Y	I	190	N	2	Ef	X	X	S	X	s	X
15	53	M		OP	3 years	NO	LOW	low	N	N	Y	Y	i	180	N	2	Kp	S	S	R	R	x	r
16	60	F	IP	OP	2 years	dk	low	low	N	N	N	N	i	162	N	3	Kp	S	S	R	R	x	r
17	70	M		OP	10 years	dk	low	low	HT	RP	Y	N	r	185	N	3	Ef	x	x	R	x	r	x
18	56	F	IP		15 Years	dk	low	low	HT	N	N	N	i	498	N	2	Pv	R	R	R	S	x	x
19	65	F	IP		5 years	dk	low	low	HT	N	N	N	i	512	N	3	Kp	S	S	S	S	x	s
20	55	M		OP	15 years	dk	low	low	HT	N	Y	Y	i	180	N	2	Ng	X	X	X	X	X	X
21	45	F		OP	1 year	yes	low	low	N	N	N	N	i	250	N	2	Ng	x	x	x	x	x	x
22	63	M	IP		3 years	dk	low	low	HT	NEP	Y	N	r	250	N	2	Pv	R	S	R	R	x	x
23	45	F	IP		8 YEARS	DK	LOW	LOW	N	NEP	Y	N	i	136	N	4	Ng	X	X	X	X	X	X
24	70	F	IP		15 years	dk	no	low	HTN	CA	N	N	i	150	N	4	Cb	R	R	R	R	x	x

25	57	M	IP		15 years	dk	low	low	HT	N	Y	N	i	256	Y	3	Ef	X	X	R	X	S	X
26	60	M	IP		10 years	dk	low	no	N	NEP	N	N	i	340	N	3	Pv	R	R	R	R	x	x
27	52	M	IP		1 year	dk	low	low	N	N	N	N	i	150	N	2	Ng	x	x	x	x	x	x
28	60	M	IP		12 years	dk	low	low	HT	NEP	Y	N	i	300	N	3	Pv	R	R	R	S	x	x
29	58	M	ip		14 years	dk	low	low	HT	NEP	Y	N	i	246	N	3	Pv	S	S	R	R	x	x
30	52	M	IP		5 years	dk	low	low	N	N	Y	Y	i	240	N	3	Ec	R	R	R	R	R	R
31	46	M	IP		2 years	yes	LOW	low	N	N	Y	Y	i	190	N	2	Pv	R	R	S	R	X	X
32	44	F	IP		8 years	dk	LOW	low	N	NEP	N	N	i	434	N	2	Pv	R	S	R	R	x	x
33	80	M	IP		3 years	dk	low	LOW	HTN	N	Y	Y	i	229	N	2	Ko	S	S	R	R	x	R
34	78	F	IP		10 years	yes	low	low	HTN	RP	N	N	I	250	N	3	Pa	S	S	S	x	x	x
35	66	F	IP		6 Years	dk	low	low	N	N	N	N	i	360	N	3	Kp	R	S	R	R	x	r
																	Ef	x	x	S	x	s	x
36	68	M		op	2 Years	dk	low	low	HTN	NEU	Y	Y	i	180	N	3	Ng	x	x	x	x	x	x
37	43	M		op	5 YEARS	YES	low	low	N	N	N	N	I	200	N	2	Ef	X	X	S	X	r	X
38	41	M		op	4 YEARS	YES	low	low	N	N	Y	Y	I	190	N	2	Ef	X	X	S	X	s	X
39	53	M		op	3 years	no.	low	low	N	N	Y	Y	i	180	N	2	Kp	S	S	R	R	x	r
40	60	F	IP		2 years	dk	low	low	N	N	N	N	i	162	N	3	Kp	S	S	R	R	x	r
41	70	M		op	10 years	dk	low	LOW	N	NEU	N	N	r	180	N	3	Ef	x	x	R	x	r	x
42	56	F	IP		15 Years	dk	low	low	HTN	RP	N	N	i	498	N	2	Pv	R	R	R	S	x	x
43	65	F	IP		8 years	dk	LOW	low	HTN	RP	N	N	i	434	N	2	Pv	R	S	R	R	x	x
44	55	M		OP	15 years	dk	LOW	low	N	N	Y	Y	i	180	N	2	Ng	X	X	X	X	X	x
45	45	F		OP	1 year	yes	low	low	N	N	N	N	i	250	N	2	Ng	x	x	x	x	x	x
46	63	M	IP		3 years	dk	low	low	HTN	NEP	N	N	r	250	N	2	Pv	R	S	R	R	x	x
47	50	M	IP		Unknown	dk	low	LOW	N	N	Y	Y	X	180	N	2	Ec	S	S	R	R	r	r
																	Kp	R	R	R	R	X	r
48	65	M	IP		3 YEARS	DK	low	low	NO		NO	NO	i	180	N	3	Pa	S	S	R	x	x	x
49	55	M	IP		25 years	yes	low	low	nil	RP	Y	yes	i	370	N	4	Pa	R	R	R	x	x	x
																	Kp	R	R	R	R	x	r
50	55	M	IP		5 years	dk	low	low	NO		Y	yes	i	340	N	4	Pm	R	R	R	R	x	x

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CLINICAL PROFORMA:DIABETIC FOOT ULCER

NAME	DOA:
AGE / SEX	IP NO.:
OCCUPATION/INCOME	
EDUCATION	
ADDRESS	
CHIEF COMPLAINTS	
HISTORY OF PRESENTING ILLNESS	
H/o trauma - Thorn prick,Nail prick,Shoe bite,Other	
H/o local symptoms-- Swelling, Pain, Wound,Discolouration	
Discharge - foul smelling/ non foul smelling	
H/o neuropathic symptoms- Paraesthesia , Burning sensation, Tingling sensation Others	
Any other relevant history	
PAST HISTORY	
H/o Diabetes Mellitus	Known/Unknown
	Duration
H/o Treatment for Diabetes Mellitus	Insulin/Oral Hypoglycemics
	Regular/Irregular
H/o Hypertension /IHD /Bronchial asthma / Tuberculosis	

FAMILY HISTORY	
H/o Diabetes Mellitus in the family	Yes/No
PERSONAL HISTORY	
Diet	Appetite
Sleep	Bowel/Bladder
H/o Smoking No of Beedies/Cigarettes	
Duration	
H/o Alcohol consumption	
GENERAL PHYSICAL EXAMINATION	

Pulse				BP	
Temperature				Respiratory Rate	
Palor	Icterus	Cyanosis	Clubbing	Lymphadenopathy	Oedema
LOCAL EXAMINATION					
Predisposing Factors:			Callosities		
			Cracks/Fissures		
			Blisters		

Signs Of Ischaemia	Skin Colour	
	Condition Of Hair	
	Nails	
	Wasting Of Muscles	
	Loss Of Sweating	
Type And Site Of Lesion	1. Cellulitis	Foot/Leg
	2. Abscess Heel	
Interdigital		
Dorsum Of Foot		
3 . Wound a) Site	Base Of Metatarsal	
Interdigits		
Toes		
Sole		
b) Size		
c) Shape		
d) Floor		
e) Base		
f) Slough		
Oedema		

g) Discharge:	Blood
Pus	
Serous	

h) Exposure Of Bone & Tendon	
i) Surrounding tissues	
4. Gangrene	Toes
	Fore Foot
	Foot
	Foot & Leg Extension
5. Foot Deformity	
6. Peripheral Pulsations	Dorsalis Pedis Artery
	Posterior Tibial Artery
	Anterior Tibial Artery
	Popliteal Artery
7. Sensations	Temperature
	Vibration
	Touch
	Tendon Reflexes
	Ankle Jerk Present/Absent
	Knee Jerk Present/Absent
	Superficial Plantar Reflex
8. Other Foot	

CLASSIFICATION OF DIABETIC FOOT (Wagner-Meggit)

GRADES	LESION	ASSESSMENT
GRADE 0	FOOT SYMPTOMS LIKE PAIN,ONLY	NIL
GRADE 1	SUPERFICIAL ULCERS	
GRADE 2	DEEP ULCERS	
GRADE 3	ULCER WITH BONE INVOLVEMENT	
GRADE 4	FOREFOOT GANGRENE	
GRADE 5	WHOLE FOOT GANGRENE	

SYSTEMIC EXAMINATION

a) Cardiovascular System

b) Respiratory System

c) Per Abdomen

INVESTIGATIONS

Hb%	
TC	
DC	
Blood Urea	
Sr. Creatinine	
Blood Sugar:	Random Blood Sugar
	Fasting Blood sugar
	Post Prandial Blood Sugar
Urine Sugar	
Urine For Ketone Bodies	
X-ray Foot:	
ECG	

Doppler Ultrasound

DIAGNOSIS:

MANAGEMENT	
1.Control Of Diabetes	Diabetic Diet

	Insulin/Oral Hypoglycemics/Both
2. Treatment Of Diabetic Foot :	Conservative
	Antibiotics :
	Daily Dressings
	Wound Debridement
	Skin Grafting
	Toes/Foot Disarticulation
Major Amputations :	Below Knee/Above Knee

PROGNOSIS :

KEY TO MASTER CHART

DD= Duration of Diabetes

FH= Family history

IP= In- Patient

OP =Out-Patient

Y=Yes

N=No

SE=Socio economic

ED=Education

CO MOR=Co Morbidities

DC=Diabetes complication

SM=Smoking

AL=Alcoholism

r/t TT= regular/irregular treatment

RBS=Random blood sugar

HT=Hypertension

WG=Wagner Grading

PATH=Pathogens

Ef=Enterococcus faecalis

Kp=Klebsiella pneumoniae

Ko=Klebsiella oxytoca

Pv=Proteus vulgaris

Pm=Proteus mirabilis

Pa=Pseudomonas aeruginosa

Cb=Citrobacter

Ec=Escherichia coli

Ng=No Growth

RP=Retinopathy

NEP=Nephropathy

NEU=Neuropathy

CA=Coronary Artery Disease

OH=Oral Hypoglycemic Drugs

In=Insulin

TD=Toe disarticulation

BKA=Below knee Amputation