THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

M.D. BRANCH IV

MICROBIOLOGY

Dissertation on

A MICROBIAL STUDY OF DIABETIC FOOT ULCER INFECTIONS

INSTITUTE OF MICROBIOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003

SEPTEMBER 2006

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "***A MICROBIAL STUDY OF DIABETIC FOOT ULCER INFECTIONS"*** submitted by **Dr.C.Y.RANJINI,** is a work done by her during the period of study in this department from July 2003 - September 2006.

.....

Dr.A.LALITHA M.D., D.C.P.
Director and Professor
Institute of Microbiology
Madras Medical College
Chennai - 600 003.

.....

Dr.KALAVATHY PONNIRAIVAN,
B.Sc., M.D.
DEAN
Madras Medical College
Chennai - 600 003.

CONTENTS

INTRODUCTION

"I was angered, for I had no shoes. Then I met a man who had no feet. "

- Chinese Proverb

Poets have waxed eloquent of their grace, romantics have offered their hearts under their beloved's feet, yet the feet remain the most neglected part in our body. As the Chinese proverb has succinctly pointed out the pathos of a life without feet, the prevention of amputation becomes poignant in today's world where chronic diseases like diabetes have increased the rates of foot amputation.

Diabetes mellitus with its multisystem affliction has emerged as the scourge of the 21st century, reaching pandemic proportion and causing devastating consequences. Foot ulcers are a significant complication of diabetes mellitus and often lead to lower-extremity amputation. Approximately 85 percent of all diabetes-related lower-extremity amputations are preceded by foot ulcers[84].They are the most common cause of disability and hospitalization in patients with diabetes and are the disease's most expensive complication. The WHO defines diabetic foot as "Infection, ulceration and/or destruction of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular disease in the lower limb" (WHO, 1985)[12].

The underlying etiologies are neuropathy, trauma, deformity, high plantar pressures, infection, impaired wound healing, limited joint mobility and peripheral arterial disease. These factors lead to foot ulceration, gangrene and, finally, amputation if appropriate intervention is not applied.

Diabetic ulcers are at high risk of infection secondary to impaired leukocyte chemotaxis and phagocytosis. High glucose levels and poor tissue perfusion may compound this condition. Decreased ability to fight off infection combined with tissue hypoxia creates an ideal environment for a necrotizing infection. Limb-threatening diabetic infections are usually polymicrobial involving multiple aerobic and anaerobic infections. *Staphylococcus aureus, Streptococcus spp., Enterobacteriaceae*,

Bacteroides fragilis, Peptococcus spp. and Peptostreptococcus spp. may be cultured from diabetic ulcers.

Proper choice of antimicrobials in the treatment of a limb-threatening diabetic foot ulcer infection is imperative as it should include those with activity against Gram-positive and Gram-negative organisms and provide aerobic and anaerobic coverage. Prompt initiation of appropriate antibiotic therapy, as well as surgical debridement of necrotic or devascularized soft tissue and bone, are essential for controlling the infection and preventing additional morbidity.

Management of the diabetic foot requires a thorough knowledge of the major risk factors for amputation, frequent routine evaluation and meticulous preventive maintenance. A careful physical examination, buttressed by monofilament testing for neuropathy and noninvasive testing for arterial insufficiency, can identify patients at risk for foot ulcers and appropriately classify patients who already have ulcers or other diabetic foot complications.

Patient education regarding foot hygiene, nail care and proper footwear is crucial to reducing the risk of an injury that can lead to ulcer formation. The aim of therapy should be early intervention to allow prompt healing of the lesion and prevent recurrence once it is healed. Multidisciplinary management programs that focus on prevention, education, regular foot examinations, aggressive intervention, and optimal use of therapeutic footwear will lead to significant reductions in the incidence of lower-extremity amputations.

AIM OF THE STUDY

1. To evaluate the precipitating cause, severity and complications of foot ulcers of patients with diabetic foot treated in our hospital.

2. To identify the aerobic, anaerobic and fungal pathogens involved in the different grades of diabetic foot ulcers.

3. To establish an effective antimicrobial regimen for empirical treatment of diabetic foot infections.

REVIEW OF LITERATURE

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. It is one of the oldest metabolic diseases known to mankind. The earliest known record of diabetes written in Egyptian papyrus of 1500 BC mentions polyuria (frequent urination) as a symptom. In 500 BC, two Indian Physicians, Susruta and Charaka were the first to recognize the sweetness of diabetic urine .The term ‘diabetes’ was first described in 2nd Century A.D by Arateus of Cappadocia from the Greek word for a siphon. He gave a graphic account of the disease as 'the melting down of flesh and limbs into urine’. Avicenna (960-1037) defined the clinical features of diabetes and described gangrene and impotence as its effects. Up to the 11th Century, diabetes was commonly diagnosed by 'water tasters,' who drank the urine of those suspected of having diabetes as the urine of people with diabetes was thought to be sweet-tasting. The Latin word for honey (referring to its sweetness), 'mellitus', was added to the term diabetes as a result. In 1869 Paul Langerhans, a German medical student discovered that the pancreas contains two systems of cells. One set secretes the normal pancreatic juice while the function of the other was unknown. Several years later, these cells were identified as the 'islets of Langerhans. In 1893 Gustav Laguesse suggested that these islets were responsible for regulating glucose metabolism. In 1909, Jean de Meyer gave the name ‘*insuline*’ to this hypothetical product of the islets. On clinical grounds, Diabetes was subdivided initially into *diabete maigre* (lean) *and diabete gras* (obese) and later into insulin dependent (type 1) and insulin non-dependent (type 2) subtypes. **(33)**

Neuropathic symptoms in diabetic patients had been mentioned by Rollo at the end of the 18th century and Marchal de Calvi concluded in 1864 that nerve damage was a specific complication of diabetes. In 1885, the Guy’s Hospital physician, Frederick Pavy gave a description of neuropathic symptoms as “The usual account given by these patients of their condition is that they cannot feel properly in their legs, that their feet are numb, and that their legs seem too heavy” **(85)**

Insulin was discovered at the University of Toronto in 1921 by Frederick Banting and Charles

Best. The term ‘insulin’ was coined by Macleod. The primary sequence of insulin was reported in 1955 by Frederick Sanger. Milestones in insulin pharmacology include the preparation of delayed action insulin by Hagedorn in 1930s; the production of synthetic human-sequence insulin in 1979 and recently of novel insulin analogues by recombinant DNA technology. The first oral hypoglycemic agents available were the sulphonylureas in the early 1940s.The biguanides phenformin and metformin became available in 1959 and 1960 respectively[33].

EPIDEMIOLOGY OF THE DIABETIC FOOT PROBLEM

One third of all diabetic patients have significant peripheral neuropathy and/or peripheral vascular disease, the main risk factors for foot ulceration. 3-10% of those with diabetes have a foot ulcer and the life time risk for the diabetic patient to develop one is around 15%. The average age of the patient suffering from diabetic foot problem is over 60 years[84].

Amputations: People with diabetes are 10-15 times more likely to have a lower limb amputation than non-diabetic individuals[5]. 50% of all Lower Extremity Amputations (LEA) are diabetes related and >70% of LEA’s are preceded by a foot ulcer. Infection was the second most frequent indication, next to gangrene for diabetic lower extremity amputation [22].Up to 20% of patients with diabetic foot undergo an ipsilateral amputation within 12 months and nearly 50% undergo a contra lateral amputation within 1-3 years. Life expectancy is also reduced as a result. The 3 year mortality after amputation is 20-50%[5, 12]. The recurrence rates of a healed neuropathic ulcer may be as high as 70% over the next 5 years. Hospitalized diabetics are 2.8 times more likely to die in the hospital and their length of stay is 59% more than that of non diabetics[29].

ETIOPATHOGENESIS OF FOOT ULCER

Foot ulceration occurs as a result of trauma in the presence of neuropathy and/or peripheral vascular disease with infection occurring as a secondary phenomenon. Interplay of all four factors is usually involved. Diabetic foot ulcers can be broadly classified into three types:

1. Neuropathic
2. Ischemic
3. Neuroischemic.

TRAUMA:

Trauma is usually trivial and often goes unnoticed due to the loss of pain sensation. The various mechanisms of injury that destroy the foot include:

1) Direct mechanical disruption of tissue

2) Small amount of force that is sustained over time that leads to ischemia and

3) Moderate amount of force that is repeated over and over that leads to inflammation and enzymatic autolysis of tissue.

NEUROPATHY

Neuropathy accounts for over one half of ulcers. Motor, Sensory and Autonomic neuropathy occurs .Sensory neuropathy occurs as symmetrical glove and stocking distribution and causes loss of protective pain sensation [15].The intrinsic muscles of the foot are weakened due to the motor component of neuropathy and leads to the characteristic 'neuropathic posture' of raised arch, clawed toes and high pressure concentrated on the metatarsal heads and heel. Shear forces generated during walking stimulate skin thickening (callus) on these pressure points and areas of hemorrhage or necrosis that commonly develop within the callus can breakthrough to the surface to form an ulcer. Callus formation is therefore an important prediction for ulceration.

Autonomic neuropathy leads to reduced sweating which causes the skin to dry out and crack easily, allowing the entry and spread of infection[16]. Severe autonomic dysfunction leads to Charcot arthropathy, a rare but important cause of foot deformity that leads to pressure ulceration.

ISCHEMIA

Ischemia is primarily due to peripheral arterial disease. It compromises the supply of oxygen and nutrients to the tissues and delays the healing of foot ulcers. In diabetics, there is a predilection for the smaller arteries arising at the trifurcation of the popliteal artery to get affected. Pure ischemia accounts for less than 10% of cases while one third of foot ulcers are neuroischemic.

INFECTION

Infection may begin superficially in an ulcer or crack in the skin, but may spread to involve the deep tissues including tendons and bones. It is usually caused by organisms from the surrounding tissues. The potential for a wound to become infected is determined by two main factors: the micro-organic contamination of the wound; and the person's resistance to that contamination[44]. Infections related to superficial ulceration are most commonly caused by aerobic gram-positive cocci. Cellulitis of non- ulcerated skin is nearly always caused by *Streptococci sp. or S aureus. S. aureus* is the most commonly isolated pathogen and accounts for most infections in which only a single pathogen is recovered. *Coagulase-negative staphylococci, enterococci, and group B streptococci* are also frequently isolated. *Streptococci* secrete hyaluronidase which facilitates spread of the necrotizing toxins of *Staphylococci*. Enzymes from these bacteria are also angiotoxic and cause in situ thrombosis of the vessels. If both vessels are thrombosed in the toe it leads to necrosis and gangrene.

In deep seated infections, aerobic gram negative bacteria and anaerobic bacteria flourish. *Proteus species, Escherichia coli*, and members of the *Klebsiella sp* and *Enterobacter* species have been isolated most often. Anaerobic isolates are often found when appropriate collection techniques are used. *Bacteroides species, Clostridium species, and anaerobic streptococci, such as Peptococcus spp and Peptostreptococcus species*, are the most common anaerobic pathogens involved in diabetic foot infections.

Necrotizing infections involving gas forming organisms can cause gas gangrene and septicemia

might occur. Both aerobic and anaerobic bacteria can spread to the blood stream and cause life threatening septicemia. Severe sepsis is often associated with gas in the soft tissues. Although *Clostridial* organisms have been previously held responsible for this presentation, non Clostridial organisms are more frequently the offending pathogens. These include *Bacteroides, Escherichia and anaerobic Streptococci*.

Fungal infections also occur but usually do not cause systemic upset. However, infections of toe nails and interdigital spaces by *Trichophyton* and *Candida albicans* can serve as portal of entry for bacteria[21].

RISK FACTORS FOR FOOT ULCERATION

Early identification of risk factors for diabetic foot ulcer and initiation of proper treatment reduce the occurrence of complications, including the need for amputation. The various factors are neuropathy, structural deformity or limited joint mobility, previous history of ulceration or amputation, and poor glucose control[37]. Effects of these risk factors are cumulative. The demographic risk factors include older age, male gender, ethnicity and even social situations like living alone.

Diabetes Related Risk Factors

Lavery et al[47] in their study showed that an elevated plantar pressure (>65 N/cm^2), history of amputation, lengthy duration of diabetes (>10 years), foot deformities (hallux rigidus or hammer toes), male sex, poor diabetes control (glycosylated hemoglobin >9%), 1 or more subjective symptoms of neuropathy, and an elevated vibration perception threshold (>25 V) were significantly associated with foot ulceration.

Peripheral vascular disease though 4 times more common in those with diabetes were more associated with a risk for amputation than the development of foot ulcer per se.

Increased plantar pressures: Veves[79, 38] and colleagues demonstrated that plantar foot pressures in diabetic patients are strongly predictive of subsequent plantar ulceration, especially in the presence of neuropathy.

Advanced Glycosylated End products cause glycation of collagen and restrict movement of key joints and limits joint mobility.

Diabetes mellitus being an immune suppressed state, impaired neutrophil chemotaxis and phagocytosis makes the foot more prone for infections [61]

Past history of amputation and ulcer were independently related to risk of ulcer. About 60% of diabetic patients with a history of foot ulcer will develop another ulcer within a year following healing. This is due to three distinct factors. First, the risk factors responsible for the previous ulceration are still present. Second, the skin over the previous ulcer site maybe weakened and finally, the deformity and biomechanical imbalances may create areas of high pressure leading to recurrence. After a great toe amputation, pressure distribution of the foot is significantly altered. Because pre-amputation risk factors such as peripheral neuropathy, foot deformity, and limited joint mobility for many of these patients remain unchanged, an increase in foot pressures contributes to an increased risk of reulceration and reamputation in these patients[45].

Behavioral risk factors: Self management skills are highly correlated to the presence of diabetic foot complications.

Other risk factors include body weight which is in higher prevalence in type 2 diabetics. One potential mechanism for the higher risk associated with greater weight is increased plantar pressure in heavier subjects[23].

Insulin use and history of poor vision are independently related to higher ulcer risk. It is possible that both of these factors reflect diabetes severity. Also, poor vision may interfere with a patient's ability to detect early foot lesions that without attention might progress to non-healing ulcer.

Interaction of risk factors

Boyko et al in the Seattle Diabetic Foot Study had concluded that multiple mechanisms contribute to the development of diabetic foot ulcer. Certain foot deformities, reduced skin oxygenation and foot perfusion ,poor vision, greater body mass, and both sensory and autonomic neuropathy independently influence foot ulcer risk, thereby providing support for a multifactorial etiology for diabetic foot ulceration.[23] .In a prospective study Adler et al identified peripheral sensory neuropathy, PVD, foot ulcers (particularly if they appear on the same side as the eventual LEA), former amputation, and treatment with insulin as independent risk factors for LEA in patients with diabetes[2].

CLINICAL PRESENTATION OF FOOT ULCER

The most frequent presentation of the diabetic foot is the neuropathic ulcer. These ulcers appear as punched out and clean unless infected. Its classic position is under the metatarsal heads, heel and the tip of the toes [21].Ischemic ulcers arise at the margins of feet and have a ragged appearance; the foot is usually cold, with absent pulses. Infected ulcers show inflammatory signs of warmth, erythema and tenderness. Constant foul smelling discharge and crepitus is indicative of gas gangrene. Systemic signs of fever, leukocytosis and raised levels of acute phase proteins occur when the patient becomes septicemic but maybe absent when infection remains confined to the deep tissues [84].

CLINICAL EXAMINATION AND SCREENING OF FEET AT RISK OF ULCERATION

Routine examination of the diabetic feet should include screening for risk factors, clinical inspection, neurological examination and vascular assessment. Clinical evaluation begins with a history of previous ulceration or amputation. Clinical examination includes inspection for foot deformities, limitation of joint mobility, osseous prominence secondary to Charcot arthropathy, areas of erythema and callus formation.

Neurological testing is done for identification of loss of protective sensation. The perception of pain, touch and vibration is done using pin prick, cotton wool and tuning fork. Other screening tests that predict ulceration include a vibration perception threshold (VPT) >25v (measured using a

biothesiometer) and the inability of the patient to feel the 5.07 Semmes-Weinstein monofilament at the great toe or metatarsal head [3].

Vascular assessment includes eliciting history for claudication and the palpation of lower extremity pulses. The absence of two or more foot pulses on palpation is widely used as a diagnostic criterion for peripheral vascular disease. The use of Doppler ultrasound is a useful adjunct for measurement of ankle systolic pulses. An ankle –brachial index of <0.8[13] is taken as abnormal vascularity and <0.6 indicates significant arterial stenosis.

CLASSIFICATION OF FOOT ULCERS

Numerous classification schemes have been proposed for describing diabetic foot ulcers. The most commonly used and most often referred to is the **Meggitt-Wagner system** which categorizes diabetic foot ulcers into five grades on the basis of anatomical location, depth and presence of ischemia [74,81,84].

MEGGITT-WAGNER CLASSIFICATION OF DIABETIC FOOT ULCERS

GRADE	CONDITION
0	High risk foot, no ulcer
1	Superficial skin ulcer
2	Deep ulcer extending through dermis.Tendons,ligaments,joint capsule or bone may be exposed
3	Deep ulcer with abscess, osteomyelitis and joint sepsis
4	Localized gangrene of forefoot or heel
5	Gangrene of foot

Wagner Grade 0 describes a pre ulcerative or post ulcerative lesion. There is no break in the skin, but risk factors such as calluses or foot deformities are present. Grade 1 ulcers are superficial, full thickness ulcers with penetration past the dermis. They are indicative of the presence of peripheral sensory neuropathy along with at least one other risk factor for ulceration. Continued weight bearing on them will cause deepening of the foot ulcers past the dermis with involvement of deeper structures such as tendons, ligaments, joint capsules and neurovascular structures. These are the grade 2 ulcers and they do not probe to the bone. Wagner grade 3 ulcers are characterized by the presence of deep infection and bone involvement. Abscess formation or osteomyelitis is usually present. Grade 4 ulcers present with partial gangrene of the foot demonstrating extensive vascular insufficiency, sepsis and tissue necrosis which necessitates aggressive management for limb salvage. Grade 5 ulcers are characterized by extensive necrosis and gangrene which is best addressed by limb amputation. Though this system is easy to use it has its limitations in that it is not descriptive enough to allow for cross comparison of ulcers between different centers and misclassifies infective digital gangrene (grade 4, localized) which has little or no macrovascular disease[84]. The criticisms include its failure to gauge the degree of ischemia in grade 1 to 3 and its inability to address the combination of infection and ischemia. Additionally, it does not provide predictions on healing and outcomes.

The other classification systems include the Nottingham system (UK); the University of Texas' St.Antonio wound classification system [84,53,46] ,the International working group classification [20] and one by Brodsky and Lavery. A study by Samson Oyibo et al compared the Wagner's system and the

University of Texas (UT)system and found the UT system (Appendix II) a better predictor of the healing time and outcome [64].A new scoring system called The DEPA scoring system which includes the depth of the ulcer (D), the extent of bacterial colonization (E), the phase of ulcer healing (P) and the associated underlying etiology(A) has been studied .An increasing DEPA score was associated with increased risk of amputation and poor healing[60] .

While these classification systems are more inclusive, the comprehensiveness makes them difficult to remember and use in the clinical setting. Hence the American Diabetic Association has formed a working committee to propose a new system[74].

MANAGEMENT

Treatment of diabetic foot varies greatly depending on the severity of the ulceration as well as the presence of ischemia. The cornerstone of treatment for full thickness ulcer consists of adequate debridement, off loading of pressure, treatment of infection and local wound care.

REMOVAL OF CALLUS : The callus that surrounds the ulcer must be removed by podiatry. Excess keratin must be pared away to expose the floor of the ulcer and allow efficient drainage and proper re-epithelisation.A simple non adhesive dressing should be applied after cleaning the ulcer.

DEBRIDEMENT : The treatment of choice for the complete removal of all necrotic, dysvascular and non viable tissue is sharp, surgical debridement .Other debridement techniques are also available.Autolytic debridement refers to the body's own mechanism of removing devitalized tissue done primarily by the macrophages. Enzymatic debridement involves the use of topical agents with ability to degrade necrotic tissue via proteolytic enzymes. Mechanical debridement gently loosens the slough and removes it from the wound bed. The simplest form of this technique is the commonly applied wet to dry saline gauze. While this is an inexpensive and relatively easy method, it removes both viable and non viable tissue and causes pain in the sensate foot.

PRESSURE OFF LOADING: Reduction of pressure is essential in the healing of plantar foot

ulcers. The most popular methods include total contact casting, half shoes, and short leg walkers and felt foam dressings.

ERADICATION OF INFECTION: A proper understanding of the pathogens involved in the foot infection is required before instituting antimicrobial chemotherapy. The ulcer culture and sensitivity report is therefore imperative. The wound swab should be taken from the floor of the ulcer. Swab cultures usually grow out numerous surface contaminants and may not provide information on the pathogen(s) causing the deep tissue infection.[30] .Two swabs should be collected, one for gram stain and the other for culture .A superficial ulcer may be treated on an outpatient basis and oral antibiotics prescribed, according to the organism isolated. Cultures are most reliable when a deep tissue specimen is obtained. All organisms recovered from deep tissue cultures should be treated as pathogens unless there is evidence to support that the culture was contaminated from another source.

Culture of material carefully collected from abscess cavities or by surgical biopsy of deep soft tissue or bone provides the most useful guide to treatment and minimizes the potential for contamination. Culture of material from sinus tracts is unreliable.

Before an infected wound is cultured, care should be taken to remove any overlying necrotic debris from the site. Vigorously scrubbing the wound with saline-moistened sterile gauze[43] often can accomplish this. Culture of the wound base, preferably from expressed pus, can then be attempted. Specimens obtained from curettage of the base of the ulcer correlate best with results from deep-tissue culture.

Gram's stain is often helpful for interpreting culture results A surface culture may grow several organisms, while Gram's stain may reveal only a single bacterial morphology.

The polymicrobial nature of most infections of the diabetic foot is well known, with an average of five or six organisms involved [43]. A mixture of aerobic and anaerobic organisms is common. In one study, anaerobic organisms were recovered from 90% of cultures. Foul-smelling drainage and the

presence of gas in the tissues, detected by clinical or radiographic evaluation, often predict a mixed polymicrobial infection. Adequate coverage for these organisms is important. Some anaerobes will be killed by contact with molecular oxygen for only a few seconds. Specimens are to be collected from a prepared site using sterile technique. Overlying and adjacent areas must be carefully disinfected to eliminate contamination with indigenous flora. Ideally, pus or other fluid obtained by needle aspiration through intact skin or mucosal surface which has been cleaned with antiseptic should be collected. Sampling of open lesions is enhanced by deep aspiration using a sterile plastic catheter. If irrigation is necessary, nonbacteriostatic sterile normal saline may be used. Anaerobic transports must be used for swabs and for aspirates. Specimens for anaerobic culture should be maintained at room temperature. Under these conditions, aerobes and anaerobes will survive 24-72 hours when properly collected in the anaerobic transport tube [72].

OTHER LABORATORY MEASUREMENTS

Total count, differential count, ESR, plasma glucose levels, HbA1C, renal parameters and radiography provide information regarding the infection status and extent of involvement. Bone detected by probing a pedal ulcer has a sensitivity of 66 percent for osteomyelitis and a specificity of 85 percent [43]. While standard radiographs may be of assistance in diagnosing osteomyelitis, MRI is considered the more sensitive and specific pathologic confirmation of osteomyelitis. Serial radiographs compared with baseline radiographs may be of significant value in determining progressive bone changes resulting from osteomyelitis.

ANTIBIOTIC THERAPY: Antimicrobial selection should take into account the causative organisms while bearing in mind the potential toxicity of the agents. In the diabetic foot, the bacteria mostly responsible for non limb threatening infection are staphylococci and streptococci, while limb threatening infections are the consequence of polymicrobial infection [50,59].

Empirical antibiotic selection should be based on the suspected pathogen along with the modifications to address anticipated resistant pathogens that may have been selected during prior hospitalization. Broad spectrum antibiotics should be begun empirically with reassessment following the result of culture and sensitivities. The duration of antibiotic therapy should be based on the clinical response and wound care. Two weeks of therapy are the usual guideline; however, recalcitrant infections may require longer courses. For treatment purposes, the ulcers are classified as non – limb threatening, limb threatening and life threatening.

CATEGORY	ANTIMICROBIAL REGIMEN
NON-LIMB THREATENING	Cephalexin 500mg p.o.q6h Clindamycin 300 mg p.o.8h Amoxicillin Clavulanate (875/125 mg)1q12h Dicloxacillin 500mg q6h Levofloxacin 500-750mg qd
LIMB THREATENING	Ceftriaxone 1g IV daily plus Clindamycin 450-600 mg ivq8h Ciprofloxacin 400mg iv q 12h plus Clindamycin 450-600 mg ivq8h Ampicillin/sulbactam3g iv 6h Ticarcillin/clavulanate3g iv q4-6h Piperacillin/tazobactam 3.375 g q4h or 4.5g iv q6h Flouroquinolone iv plus metronidazole 500mg iv q8h
LIFE THREATENING	Imipenem cilastatin 500mg iv q6h Piperacillin/tazobactam 4.5g iv q6h plus Gentamicin 1.5mg/kg iv8h Vancomycin 1g ivq12h plus Gentamicin plus metronidazole

EMERGENCE OF ANTIMICROBIAL RESISTANCE

This is of prime concern today as widespread usage of antibiotics has led to emergence of novel resistance mechanisms among the microbes. Among the Gram positive cocci Methicillin Resistant Staph Aureus (MRSA) and Vancomycin Resistant Enterococci (VRE) are highly dangerous.

The most serious resistance patterns now emerging among Gram-negative organisms include resistance to extended-spectrum cephalosporins and penicillins. This resistance is commonly mediated by ESBLs in *Escherichia coli* and *Klebsiella* species, or by the hyper production of chromosomally mediated cephalosporinases (Bush group I amp C enzymes) in *Citrobacter*, *Serratia* and *Citrobacter*

species. The ESBL genes generally result from point mutations in the genes of broad-spectrum β-lactamase Ambler class A enzymes, such as TEM-1, TEM-2 or SHV-1. They are usually located in conjugative mega plasmids, which often carry genes responsible for resistance to other antibacterial drugs, making it extremely difficult to treat infections caused by bacteria that produce these enzymes.

Along with ESBLs, plasmid-mediated Ambler class C cephalosporinases (or Bush group 1 cephalosporinases) have been found in clinical isolates of the *Enterobacteriaceae*. These enzymes can produce resistance to cephamycins, extended spectrum cephalosporins and aztreonam, and unlike class A ESBLs, β-lactamase inhibitors do not inhibit these bacteria.

GLYCEMIC CONTROL

The concept that improved glycemic control could delay or prevent the appearance of micro vascular complications was studied by Jean Pirart and conclusively proven by the Diabetes Control and Complications trial[75] and the UK Prospective Diabetes Study [76], respectively in 1993 and 1998.

WOUND CARE

The effective use of dressing is essential to ensure the optimal management of diabetic foot ulcers. A clean, moist wound healing environment prevents tissue dehydration and cell death, accelerates angiogenesis and facilitates the interaction of growth factors with the target cells. Standard dressing care for the treatment of diabetic foot ulcers in the US is still the use of wet-to-dry or wet-to-moist saline gauze dressings. Hutchinson, et al., studied the incidence of infection under occlusion and found it to be four times more likely to occur under dry gauze than under occlusion. High levels of exudates warrant the choice of a moisture-absorbing material, which may include alginates, foams, collagen-alginate combinations, carboxymethylcellulose materials, or gauze. Low exudates and desiccated wounds respond well to hydro gels. Occlusive hydrocolloids are not recommended over highly exuding wounds in weight-bearing areas.

ADVANCED WOUND CARE PRODUCTS

New biologic products and drugs directly interact with the wound environment to manipulate and direct activity at a cellular level. These include biological skin substitutes and recombinant PDGF.

Skin Replacements

Cultured human keratinocytes have been developed and used in the past. They are of limited benefit on full-thickness defects and have not been shown to be of benefit in the treatment of diabetic foot ulcers.

Derma graft® is a semi synthetic material composed of human neonatal dermal fibroblasts cultured onto a bioabsorbable mesh. The metabolically active cells are responsible for the secretion of human dermal collagen, growth factors, and other proteins, which may contribute to wound closure. Dermagraft is FDA approved for use in the treatment of diabetic foot ulcers.

Apligraf® is a bilayered, allogeneic skin equivalent with a fully differentiated epidermis and a dermis. The dermis consists of bovine collagen containing human fibroblasts derived from human foreskins, while the epidermis is derived from keratinocytes also attained from infant foreskins. Apligraf is currently indicated for the treatment of venous ulcers and diabetic foot ulcers.

Growth Factors

Becaplermin is recombinant human platelet-derived growth factors and is the only currently approved growth factor indicated for the use of diabetic neuropathic plantar foot ulcers. Becaplermin is a recombinant, dosed, and regulated product. While currently indicated and FDA approved for use on diabetic ulcers, becaplermin's mechanism of action is not specific to diabetic wounds.

Prior to the introduction of becaplermin, the only available growth factor was an autologous, unregulated product called Procuren® Autologous materials, to date, are neither produced in regulated doses nor FDA controlled.

Granulocyte colony–stimulating factor (G-CSF) is an endogenous hematopoietic growth factor

that induces terminal differentiation and release of neutrophils from the bone marrow. G-CSF stimulates the growth and improves the function of both normal and defective neutrophils, including in patients with diabetes and has immunomodulatory and antibiotic-enhancing functions. In its purified, cloned recombinant form, commercially approved G-CSF has been used to treat various difficult infectious problems. Because G-CSF specifically enhances neutrophil functions, several investigators have explored using it as an adjunct to treating diabetic foot infections. In a metanalysis study conducted by Lipsky et al adjunctive G-CSF treatment does not appear to hasten the clinical resolution of diabetic foot infection or ulceration but is associated with a reduced rate of amputation and other surgical procedures. Treatment with G-CSF was also associated with a tendency toward a shorter duration of parenteral antibiotic therapy [55].

PREVENTION AND FOOT CARE

Many diabetic foot ulcers and their complications can be avoided by simple preventive measures. It has been estimated that upto 80% of diabetic foot ulcers are preventable. High-risk patients without ulceration should be examined every three months, while those with open wounds not requiring hospitalization should be seen at least once a week. Even people with diabetes at low risk for ulceration and amputation should have their feet examined on an annual basis, while moderate risk patients may be seen twice a year. The latter examination schedule would be considered appropriate care.

FOOTWEAR: Footwear is one of the most critically important components in preventing foot ulcer. Use of footwear with a thick insole , avoiding barefoot walking and use of open toed shoes or sandals, avoiding reuse of sweaty socks and keeping the dry foot moist are some of the guidelines given by the American Diabetic Association [83].

MATERIALS AND METHODS

This is a cross-sectional study conducted at the Institute of Microbiology at Madras Medical College, Chennai for a period of one year from December 2004 to December 2005.Samples of wound isolates taken from 104 patients with diabetic foot ulcer attending as outpatients in the Diabetology OP and those admitted in the surgical wards of the Diabetology Department were collected and processed. 30 outpatients and 74 inpatients were included in the study.

DISEASE DEFINITION

Diabetic foot infection was defined as the presence of a non-healing wound with evidences of inflammation, with or without systemic toxicity, and with a definite growth on culture that correlated with the Gram's stain result.

SELECTION CRITERIA

Inclusion criteria

Male and female Diabetic patients in the age range of 20 to 90 having type 1 or type 2 diabetes and presenting with grade 1 to grade 5 foot ulcers were selected in the study. Each patient was included only once in the study.

Exclusion criteria

Patients presenting with just callus and no foot ulcer (grade 0) were excluded from the study.

CLINICAL ASSESSMENT

The patients were clinically assessed and history regarding the duration of diabetes, smoking, alcohol intake, trauma preceding the ulcer, previous history of ulcer or amputation ,duration of stay in the hospital, associated medical illnesses like ischemic heart disease or renal disease, glycemic control status, the use of OHA / insulin and antibiotics used were appraised. Physical examination included inspection of the foot ulcer and musculoskeletal examination for any foot deformity. The location, size, depth, margin, colour, grade of the ulcer, presence of granulation tissue, necrotic tissue, edema, erythema, foul odour, and purulent discharge was noted.

NEUROLOGICAL EXAMINATION

Neurological assessment for peripheral neuropathy was done using the 5.07 Semmes-Weinstein monofilament (SWM). The ability of the patient to feel the filament at the great toe and the metatarsal heads was recorded. Scoring was done as mild, moderate, or severe peripheral neuropathy.

VASCULAR ASSESSMENT

Vascular assessment was done by palpation of all lower extremity pulses and DOPPLER Examination (for inpatients) of the brachial, posterior tibial, popliteal, dorsalis pedis and anterior tibial arteries .Estimation of the ankle –brachial index was done. An ankle brachial index of <0.9 was taken as impaired vascularity.

ULCER GRADING

Ulcer grading was done using the **Megitt Wagner Classification** of diabetic foot ulcers. (Appendix III).

SAMPLE COLLECTION

The ulcer site and size were examined with the patient laying supine on an examination table. Superficial dead tissue was removed with sterile scissors and a scalpel blade. After local debridement of devitalized tissue, the ulcer wound was scrubbed thoroughly with normal saline to remove surface colonizers. Sample collection was then done by

a) Using sterile cotton swabs for all cases

b) Scrapings of the ulcer base collected in a sterile manner where necrotic tissue was present

c) Pus aspirates where appropriate (presence of any deep abscess).

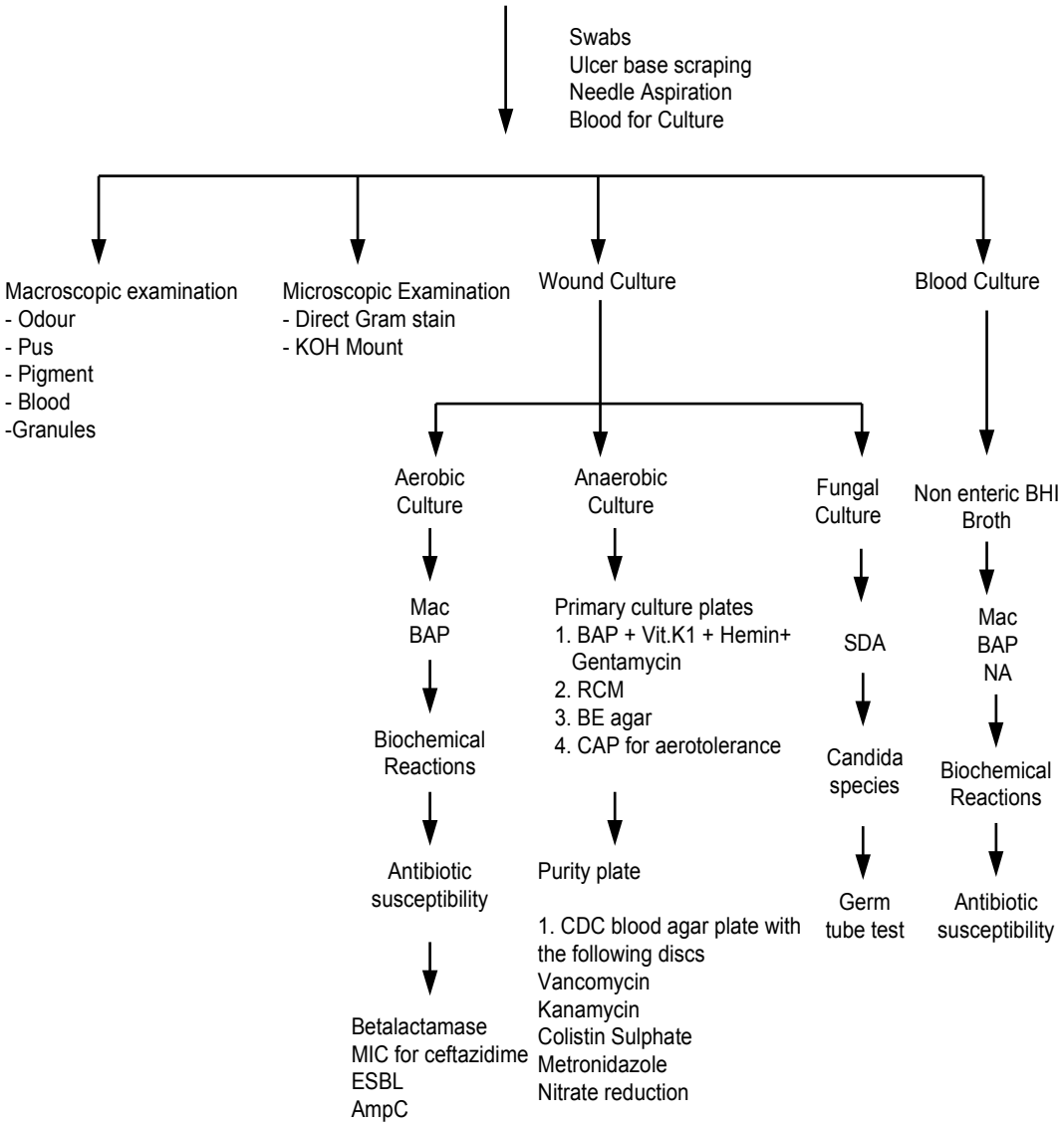
Wound swabs were taken from the base of the grade 1 ulcer. Two swabs were collected, one for Gram stain and the other for aerobic culture. For Grade 2 to Grade 5 ulcers, swabs and scrapings of the ulcer base were taken. Anaerobic isolation was done when clinically suspected and for this the overlying and adjacent areas were carefully disinfected with 70% ethanol to eliminate contamination with indigenous flora. When swabs and tissue scrapings were collected they were immediately inoculated into the transport media. In deep abscesses pus was obtained by needle aspiration, the tip of which was immediately plunged into a sterile rubber cork to prevent air exposure.

TRANSPORTATION OF SAMPLES

Samples were taken immediately to the laboratory .Specimens meant for anaerobic processing were transported in brain heart infusion broth in 0.1% agar base.

SAMPLE PROCESSSING CHART

Sample Collection



MACROSCOPIC EXAMINATION

The presence of blood, pus, foul odour, granules, or pigment was noted.

MICROSCOPIC EXAMINATION

The type and relative number of microorganisms and host cells was identified by a direct Gram stain smear of all the samples. (Appendix IV) The direct smears were fixed in methanol for 30 seconds instead of heat fixation so as to preserve the tissue cell and bacterial cell morphology.

DIRECT EXAMINATION FOR FUNGAL ELEMENTS

Samples collected using sterile swabs were examined with 10% KOH mount. Gram staining was performed to observe the presence of yeast and pseudohyphae.

ISOLATION OF ORGANISMS

AEROBIC CULTURE

The specimens were cultured on Blood agar and MacConkey agar plates for aerobic culture .Incubation was done at 37^0 C for 24-48 hours. The bacterial isolates were then identified and antimicrobial sensitivity performed by the standard microbiological techniques as per the NCCLS guidelines [54].

ANAEROBIC CULTURE:

Anaerobic culture was done when clinically indicated by inoculation of specimens immediately on sampling into Robertson's cooked meat broth and brain heart infusion broth in 0.1% agar topped with paraffin wax (Appendix VII). The tubes were immediately overlaid with sterile liquid paraffin and transported to the lab without delay. Level I identification included information from the primary plates in conjunction with Gram stain and colony morphology. Level II identification was based on colony and cell morphology, gram stain, susceptibility to antibiotic identification discs and nitrate

reduction disc test. The sample was inoculated onto the following freshly poured *primary culture plates* [6].

A) 5%sheep blood agar plates supplemented with vitamin k1 (10µg/ml) and Haemin (5µg/ml) and Gentamicin 20 µg/ml. (Appendix VIII)

B) Robertson's cooked meat media.

C) Bacteroides Bile Esculin Agar as the selective medium for identification of Bacteroides fragilis. (Appendix IX)

D) CAP incubated aerobically in 5-10% CO_2 atmosphere in candle jar for aerotolerance check.

Incubation was done in anaerobic Macintosh and Fildes jar (the inlet and outlet closed) at 35-37ºC for 48 hours. Gas pak was used as the reducing agent with palladianised asbestos as the catalyst. Reduced methylene blue was used as the indicator.[24]

EXAMINATION OF PRIMARY PLATES AND SUBCULTURE

Following incubation, the primary plates were examined for colony morphology, hemolysis and pigmentation. The individual colonies were identified by Gram stain and sub cultured to the following purity plate:

CDC Blood agar plates: (Appendix VIII) The following antibiotic discs were placed on the first quadrant of the purity plate.Vancomycin 5µg, Kanamycin 1000µg and Colistin sulphate10µg .Metronidazole 5µg discs and nitrate discs were placed in the second quadrant. The plates were then incubated anaerobically as mentioned above for 48 hours at 35ºC.

INTERPRETATION OF THE ANTIBIOTIC DISC IDENTIFICATION ON THE PURITY PLATES

The colony characteristics were noted. A Zone of 10 mm or less around the antibiotic discs indicated resistance and greater than 10 mm indicated susceptibility. A brown colour change of the nitrate on adding the nitrate test reagent indicated test positive for nitrate reduction.

FUNGAL CULTURE

Fungal isolates were identified by inoculation into Sabouraud's dextrose agar[35] and incubated at 25^0C and 37^0C for 3-4 weeks before discarding .The slants were examined every day for the first week and then thrice a week subsequently. Fungal isolates grown were identified by gram stain. Candida species were tested for germ tube production (Appendix V)

BLOOD CULTURE

In patients with clinical signs of sepsis blood culture was performed .5-10 ml of blood was collected after sterile precautions and inoculated into brain heart infusion broth bottles. After 6-18 hours bacterial growth that appeared was sub cultured into NAP, 5 % sheep BAP and Mac Conkey Agar plates. The colonies grown after 24 hrs were identified by the standard microbiological techniques. If no visible growth was seen in the broth, blind subculture was done at 24 hrs, 48 hrs and 1 week. Anti microbial drug susceptibility was performed in Mueller Hinton agar by Kirby Bauer disc diffusion method.

I. LEVEL II IDENTIFICATION OF ANAEROBES

	Cell Shape	Spores	Box Car Shaped Cells	Double zone βhemolysis	Kanamycin 1 mg	Vanco mycin 5µg	Colistin 10 µg	Nitrate Reduction	Pits the Agar	Growth in 20% bile	Pigment	Esculin Hydrolysis
Gram positive Cocci	C,CB	-	-	-	V	S	R	-	-	-	-	-
Peptostreptococcus anaerobius	C,CB	-	-	-	R	S	R	-	-	-	-	-
Peptostreptococcus asaccharolyticus	C	-	-	-	S	S	R	-	-	-	-	-
Clostridium Sp.	B	+	-	-	V	S	R	+	-	-	-	-
C.perfringens	B	-	+	+	S	S	R	+	-	-	-	-
C.baratii	B	+	-	-	S	S	R	V	-	-	-	-
C.sordeilli	B	+	-	-	S	S	R	-	-	-	-	-
C.bifermentans	B	+	-	-	S	S	R	-	-	-	-	-
Propionibacterium acnes	B,CB	-	-	-	V	S	R	+	-	-	-	-
Eubacterium lentum	B	-	-	-	S	S	R	+	-	-	-	-
Bacteroides fragilis group	B	NA	NA	NA	R	R	R	-	-	+	-	+
Bacteroides urealyticus gp	B	NA	NA	NA	S	R	S	+	+	-		
Fusobacterium sp	B	NA	NA	NA	S	R	S	-	v	v	-	v
Veillonella sp	C	NA	NA	NA	S	R	S	+	-	-	-	-

V- variable **S- Sensitive** **R - Resistant**
B - Bacilli **C - Coccobacilli** **NA - Not applicable**

ANTIMICROBIAL SUSCEPTILITY TESTS

Antibiotic sensitivity was performed on Mueller Hinton agar plates by the Kirby Bauer disc diffusion method using antibiotic discs obtained from HI MEDIA, Mumbai. Standard 0.5 McFarland saline suspensions of bacteria were used to inoculate the Mueller Hinton agar media confluently with a cotton swab. The resultant zones of inhibition were measured with a scale using transmitted light. (Appendix X)

GRAM POSITIVE COCCI

The antibiotics used for Gram positive organisms were

Penicillin G(10 U) ,Oxacillin (1µg),Erythromycin (25µg) , Ampicillin(10µg) , Ampicillin-Sulbactam (10/10µg), Ceftazidime (30µg), Cefaperazone (75µg), Ofloxacin (5µg) and Vancomycin(30µg).

GRAM NEGATIVE BACTERIA

The antibiotics employed for Gram negative bacteria were

Amikacin(30µg), Gentamicin(10µg), Ofloxacin(5µg), Ceftazidime (30µg),Cefaperazone (30µg) , Cefaperazone – Sulbactam, (75/30µg) Pipericillin (100 µg), Cotrimoxazole (25µg)and Imipenem (10µg)

BETA LACTAMASE DETECTION

The isolates were tested for β lactamase production by the iodometric method[82]. (Appendix XI)

MINIMUM INHIBITORY CONCENTRATION

The MIC is the lowest concentration of antibiotic which inhibits the growth of the bacterium. MIC_{50} of all isolates for Ceftazidime was put up according to the agar dilution method. (Appendix XII)

DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCTION (ESBL) AND AmpC

Two methods were employed to detect ESBL production. Forty seven isolates of Gram-negative bacteria exhibited resistance to Ceftazidime (30µg disc) under the disc diffusion method. A zone size <18 mm was interpreted as resistance or decreased susceptibility (intermediate by NCCLS criteria).As per the NCCLS guidelines any isolate showing a zone of inhibition <22 mm or an MIC <=2µg/ml for Ceftazidime should be tested for ESBL.Thus fifty four isolates which met this criteria were tested for ESBL.

Screening for ESBL and AmpC beta lactamases

We simultaneously tested for ESBL and AmpC beta lactamases by a modified double disc synergy test (DDST) [71].

Modified Double Disk Synergy Test (DDST)

A lawn culture of test strain on Mueller Hinton agar was exposed to disc of Ceftazidime (30 µg) and a disc of amoxiclav (augmentin) (20 µg amoxicillin/10 µg clavulanic acid) arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone produced by Ceftazidime tested on its own. A cefoxitin (30µg) disc was also placed at a distance of 20mm from the Ceftazidime disc. The test isolate was considered to produce ESBL, if the zone size around the antibiotic disc increased towards the Augmentin disc. Isolates showing reduced susceptibility to Ceftazidime and cefoxitin or blunting of

the Ceftazidime adjacent to the cefoxitin disc were considered as screen positive for AmpC.

Combined Disc Method

In the combined disc method (CDM), we used a modified procedure of NCCLS procedure, in that a Cefaperazone disc (75 µg) and a Cefaperazone–Sulbactam disc (75–30µg) were used. A ≥5 mm increase in zone diameter for the Cefaperazone–Sulbactam disc versus the Cefaperazone disc was interpreted as production of ESBL.

Controls: Known ESBL positive and negative strains were used as the controls. The result of the DDST method was taken for interpretation.

CONFIRMATORY METHOD FOR AmpC BETA LACTAMASES

AmpC DISC TEST: All the fifty four isolates were checked for AmpC production by the AmpC disc test. A lawn culture of ATCC E.coli 25922 was prepared on MHA plate. Sterile disc (6mm) were moistened with sterile saline (20µl) and inoculated with several colonies of the test organism. The inoculated disk was then placed beside a cefoxitin disc (30µg) almost touching it. The plates were incubated at 35^{o}C overnight.

INTERPRETATION: A flattening or indentation of the cefoxitin inhibition zone for ATCC E.coli in the vicinity of the test disc was interpreted as a positive test. An undistorted zone was taken as negative test.

STATISTICAL ANALYSIS

Chi-square test was used for the statistical analysis of the result.

RESULTS

TABLE 1

SEX DISTRIBUTION

MALES	FEMALES
63 (60.57%)	41 (39.42%)

N = 104

Males were more commonly affected. Male: female ratio =1.53:1

TABLE 2
AGE DISTRIBUTION

AGE RANGE	NO.OF PATIENTS =104	% of patients
20-30	3	2.88
31-40	14	13.46
41-50	17	16.34
51-60	46	44.23
61-70	17	16.34
71-80	6	5.77
81-90	1	0.96

N= 104

The age ranges of the patients varied from 25 to 82 years.

Majority (44.23%) of the patients were in the 51-60 age group.

Mean age of patients = 54.93. (Standard deviation = 10.926)

TABLE 3

FOOT INVOLVEMENT

FOOT INVOLVED	N=104	%

Right	65	62.5
Left	37	35.57
Bilateral	2	1.92

Right foot (62.5%) was more involved than the left.

TABLE 4
GRADE OF ULCERS

GRADE	N=104	%
1	13	12.5
2	16	15.38
3	30	28.84
4	42	40.38
5	3	2.88

Majority of the patients had Grade 4 ulcers. (40.38%)

Since ours is a tertiary care hospital catering to the poor socioeconomic group, patients presented with the advanced stage of the disease more frequently.

TABLE 5
TYPE OF DIABETES

TYPE OF DIABETES	N=104	%
TYPE 1	7	6.73
TYPE 2	97	93.26

Patients were predominantly suffering from type 2 diabetes (93.26%).

TABLE 6

THE DURATION OF DIABETES MELLITUS

DURATION OF ILLNESS(YEARS)	N	%
<1	3	2.88
1-5	36	34.61
6-10	44	42.30
11-15	5	4.80
16-20	3	2.88
Not Known(Diag At Time Ofadmission)	13	12.5

Duration of diabetes mellitus was another important risk factor and it varied from as less as 4 months to 17 years.

Thirteen of them were diagnosed as diabetic only on admission.

Most (42.30%) of the patients had DM for 6-10 years.

TABLE 7

ASSESSMENT OF RISK FACTORS FOR FOOT ULCER

RISK FACTORS	N=104	%
Smoker	32	30.76

Alcoholic	24	23.07
Trauma	35	33.65
Previous ulcer	16	15.38
Prior amputation	6	5.76
Previous ulcer leading to amputation	9	8.65

Trauma (33.65%) and smoking (30.76%) were the most common risk factors, followed by alcohol intake and previous ulcer.

None of the risk factors assessed were significant (p value >0.05) for the development of foot ulcer.

TABLE 8
DISTRIBUTION OF PATIENTS WITH NEUROPATHY

NEUROPATHY	N=104	%
Mild	39	37.5
Moderate	29	27.88
Severe	2	1.92
No neuropathy	34	32.69

N=104.

Neuropathy was seen in 67.30% of patients. Most of them had Mild neuropathy (37.5%).Neuropathy was significant for the development of foot ulcer. (p <0.05)

TABLE 9
VASCULAR ASSESSMENT

VASCULAR ASSESSMENT	N=74	%
Ischemic	9	12.16
Neuroischemic	18	24.32
Normal vascularity	41	55.40

N=74.Vascular assessment using Doppler was done for the in-patients.

Impaired vascularity was seen in 27 patients. (25.96%)

Nine patients (12.16%) had purely ischemic while 18 (24.32%) had neuro-ischemic ulcers.

TABLE 10
ASSOCIATED MEDICAL DISEASES

ASSOCIATED MEDICAL DISEASE	N	% of total patients
Coronary heart disease	8	7.69
Hypertension	8	7.69
Renal dysfunction	5	4.80

The morbidity of the disease is increased by other associated medical illnesses.

Coronary heart disease and hypertension were present in eight patients each, while renal dysfunction was present in 5 patients.

TABLE 11

DAYS OF STAY IN HOSPITAL

DURATION OF STAY IN HOSPITAL(DAYS)	N=74	%
1-5	10	13.51
6-10	38	51.35
11-15	16	21.62
16-20	5	6.75
21-25	3	4.05
26-30	2	2.70

N= 74 (No. of inpatients)

More than half of the hospitalized patients (51.35%) required a stay of

6-10 days.

TABLE 12

NO.AND (%) OF PATIENTS WHO WENT FOR AMPUTATION

AMPUTATION	N	%
AK	3	2.88
BK	10	9.61
Toe disarticulation / Ray amputation	20	19.23

33 patients (31.73%) underwent amputation.

Nearly 20% of the subjects had minor (toe disarticulation /Ray amputation) amputation done.

TABLE 13

THE CLINICALPROFILE OF PERSONS WITH AND WITHOUT AMPUTATION DUE TO DIABETIC FOOT ULCERS

Clinical characteristics	Total		Patients who underwent amputation(33)		Patients without amputation (71)		P value
	N	S.D.	N	S.D.	N	S.D.	
Mean duration of diabetes	7.14	3.71	8.33	3.75	6.37	3.43	N.D 57;p<0.05
Mean RBS	190.88	46.1	192.69	58.20	189.07	34	S.E of mean =0.332 p>.05
Medications	N	%	N	%	N	%	
OHA	42	40.38	18	42.85	24	57.14	p>.05
Insulin	26	25	16	61.53	10	38.46	p>.05
Fever	7	6.73	5	71.42	2	28.57	
Leukocytosis	3	2.88	3	100%	0	0	
Blood culture	3	2.88	3	100%	0	0	
Wagner's grade							

1	13	12.5	0	0	13	12.5	
2	16	15.38	0	0	16	15.38	
3	30	28.84	5	4.80	25	24.03	
4	42	40.38	25	24.03	17	16.34	P<0.05
5	3	2.88	3	2.88	0	0	P<0.05

SD = Standard Deviation;

SE = Standard Error of Mean

ND = Normal Deviate

Mean duration of diabetes was slightly more among amputees (8.33) than non amputees (6.37).

Only 68(65.38%) patients were on medications-OHA (40.38%) and insulin (25%).

The average blood glucose level was 190.88 indicating poor glycemic control.

7 patients had fever of whom 3 had leukocytosis; all 3 were blood culture positive and underwent amputation.

Amputation rate increased with grade of foot ulcer; 59.52% of grade 4 ulcer and 100% of pts with grade 5 ulcer underwent amputation.

TABLE 14
AEROBIC ORGANISMS ISOLATED
IN WOUND CULTURE

Aerobic Isolates	N	% of aerobic isolates (n=138)	% of total isolates (n=169)
GPC			
Staphylococcus aureus	21	15.21	12.43
Enterococcus faecalis	8	5.79	4.73
TOTAL	**29**	**21.01**	**17.16**
GNB			
Escherichia coli	25	18.11	14.79
Klebsiella pneumonia	13	9.42	7.69
Klebsiella oxytoca	1	0.72	0.43
Citrobacter koseri	1	0.72	0.43

Proteus vulgaris	5	3.62	2.96
Proteus mirabilis	36	26.08	21.30
Proteus penneri	1	0.72	0.43
Morganella morganii	9	6.52	5.33
Ac. baumanii	1	0.72	0.43
Pseudomonas aeruginosa	17	12.32	10.06
TOTAL	**109**	**78.98**	**81.66**

GNBs (78.98%) were isolated more than the GPCs (21.01%).

Proteus mirabilis was the commonest isolate (26.08%).

Staphylococcus aureus was the commonest GPC isolate (15.21%).

The ratio of GNB: GPC=3.75:1.

TABLE 15

ANAEROBIC ORGANISMS ISOLATED IN WOUND CULTURE

Anaerobic Isolates	N	% of anaerobic isolates	% of total isolates
Bacteroides fragilis	8	32	4.73
Peptococcus sp.	8	32	4.73
Peptostreptococcus sp.	5	20	2.95
Clostridium welchii	3	12	1.77
Clostridium tetani	1	4	0.59
Total	25	100	14.79

Anaerobes constituted 14.79% of the total isolates.

Bacteroides fragilis and Peptococcus sp. were the common anaerobes obtained.

Cl.tetani was isolated from a patient with grade 3 ulcer; no toxic symptoms were observed.

TABLE 16

FUNGAL ORGANISMS GROWN IN WOUND CULTURE

Fungal Isolates	N	% of fungal isolates	% of total isolates
Candida albicans	5	83.33	2.96
Non – albicans Candida sp.	1	16.67	0.59
Total	6	100	3.55

Fungal organisms comprised only 3.55%of the total isolates.

Among the fungi Candida albicans was the commonest fungal pathogen (83.33%).

TABLE 17

CORRELATION OF BLOOD CULTURE RESULTS AND WOUND CULTURE RESULTS

Organisms isolated in blood culture	Organisms isolated in wound culture	Grade of ulcer	Amputation
E.coli	E.coli,P.mirabilis, B.fragilis	5	AK
E.coli	E.coli,P.mirabilis,	4	Forefoot amputation
S.aureus	S.aureus,E.coli, Peptostrep.	5	AK

Blood Culture was done in 7 patients who presented with fever.

Among them 3 were culture positive.3 isolates were obtained- E.coli=2 and S.aureus =1.

The isolates were the same as the organisms obtained in wound swab culture. Two of them had grade 5 ulcer and underwent AK amputation while one patient had grade 4 ulcer and had forefoot amputation done.

TABLE 18

COMPARISON OF MONOMICROBIAL AND POLYMICROBIAL ISOLATES IN PATIENTS WITH AND WITHOUT AMPUTATION

Microbial Profile	With amputation n=33	Without amputation n=71	Total n=104
Monomicrobial	**9 (27.27%)**	**31(43.66%)**	**40(38.46%)**
GPC	3	9	12(11.53%)
GNB	6	22	28(26.92%)
Polymicrobial	**22(66.66%)**	**37(52.11%)**	**59(56.73%)**
GPC + GNB	2	4	6(5.76%)
>=2 GNBs	12	7	19(18.26%)
>=2 GPC	1	2	3(2.88%)
GPC/GNB+Anaerobe	6	19	25(24.03%)
Gpc/gnb+fungi	1	5	6(5.76%)

No growth	**2(6.06%)**	**3(4.22%)**	**5(4.80%)**
Anaerobes	**6**	**19**	**25(24.03%)**
Fungi	**1**	**5**	**6(5.76%)**

More than half of the cases (56.73%) had polymicrobial infection.

The combination of aerobe (GPC/GNB) +Anaerobe constituted nearly half of the polymicrobial group. (24.03%)

The ratio of Polymicrobial: Monomicrobial infection was 3:2.In amputees it was 2.4:1 while in the non amputees group it was 1.2:1

Anaerobic and fungal organisms always occurred in mixed infection.

No growth was seen in 5 cases.

TABLE 19

COMPARISON OF MICROBIAL PROFILE BETWEEN IN-PATIENTS AND OUT-PATIENTS

Microbial Profile	Outpatients(30)	In patients (74)	% (N=104)
Monomicrobial	**23(76.66%)**	**17(22.97%)**	**40(38.46%)**
GPC	**10**	**2**	12(11.53%)
GNB	**12**	**16**	28(26.92%)
Polymicrobial	**4(13.33%)**	**55(74.32%)**	**59(56.73%)**
GPC + GNB	**1**	**5**	6(5.76%)
>=2 GNBs	**2**	**17**	19(18.26%)
>=2 GPC	**0**	**3**	3(2.88%)
GPC/GNB+Anaerobe	**1**	**24**	25(24.03%)
Gpc/gnb+fungi	**0**	**6**	6(5.76%)
No growth	**3(10%)**	**2(2.7%)**	**5(4.80%)**

Monomicrobial infection was seen more among out-patients (76.66%).

In hospitalized patients, infections were often polymicrobial (74.32%).

Majority of the anaerobic (24/25) and all fungal infections were seen among hospitalized patients.

TABLE 20
ISOLATION OF AEROBIC AND ANAEROBIC BACTERIAL
ORGANISMS FROM DIFFERENT GRADES OF FOOT ULCERS

	Gram positive cocci n=29		Gram negative bacilli n=109		Anaerobes n=25		Total	n=163
	NO	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**
Grade 1	**7**	*24.13*	**15**	*13.76*	**0**	*0*	**22**	*13.49*
Grade 2	**3**	*10.34*	**16**	*14.67*	**0**	*0*	**19**	*11.65*
Grade 3	**7**	*24.13*	**24**	*22.01*	**5**	*20*	**36**	*22.08*
Grade 4	**11**	*37.93*	**50**	*45.87*	**14**	*56*	**75**	*46.01*
Grade 5	**1**	*3.44*	**4**	*3.66*	**6**	*24*	**11**	*6.74*
N=163	**29**	*17.79*	**109**	*66.87*	**25**	*15.33*	**163**	*100*

Gram negative bacilli were predominant in all the grade of foot ulcers.

Maximum (46.01%) isolates were obtained from grade 4 ulcers.

Isolation of Anaerobic organisms increased with the grade of foot ulcers.

GPC were found in higher % in grade 1 ulcer. They formed 34% of the total isolates in Grade 1 ulcer and were found in < 20% of the total isolate in the other grades of foot ulcer.

TABLE 21.ANTIMICROBIAL SENSITIVITY PATTERN FOR GRAM POSITIVE COCCI.

All the gram positive isolates were 100% sensitive to Vancomycin and 100% resistant to penicillin ; they were less sensitive to Ampicillin and Erythromycin. In addition poor sensitivity to Gentamicin was seen among Enterococcus faecalis.

Ampicillin - sulbactam was effective in all Methicillin Sensitive Staph aureus (MSSA) and Enterococci sp.

Staph aureus exhibited moderate sensitivity to Ceftazidime, Cefaperazone and Ampicillin - sulbactam (76.19%) while enterococcus faecalis was 75% sensitive to both Ceftazidime and Amikacin.

Table 22

BETA LACTAMASE PRODUCTION

Gram Positive Cocci	Beta Lactamase producers	%
Staph.aureus (21)	12	57.14
Enterococcus faecalis (8)	2	25
Total (29)	14	48.27

Beta lactamases were detected among the GPCs by the iodometric method and constituted 48.27%.

Staph.aureus had a moderate degree of beta lactamase production (57.14%).

TABLE 23

ANTIBIOTIC SENSITIVITY PATTERN OF

GRAM NEGATIVE BACILLI

GRAM NEGATIVE BACILLI	Ak		G		Of		Ca		Cs		Cfs		Pc		Co		I	
	No.	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
E. coli (n= 25)	21	84	10	40	6	24	15	60	7	24	22	88	10	40	2	8	25	100
K.pneumoniae (n=13)	11	84.61	9	69.23	11	84.61	10	76.92	8	61.53	12	92.30	9	69.23	1	7.69	13	100
K. oxytoca (n= 1)	1	100	0	0	1	100	0	0	0	0	1	100	1	100	0	0	1	100
C.koseri (n= 1)	1	100	1	100	1	100	0	0	0	0	1	100	1	100	0	0	1	100
Pr.mirabilis(n=36)	17	47.22	7	19.44	14	38.88	12	33.33	6	16.66	32	88.88	7	19.44	6	16.66	36	100
Pr.vulgaris (n= 5)	3	60	1	20	3	60	3	60	2	40	5	100	1	20	0	0	5	100
Pr.penneri (n= 1)	1	100	0	0	0	0	0	0	1	100	1	100	0	0	0	0	1	100
M.morganii (n= 9)	5	55.55	2	22.22	6	66.66	6	66.66	4	44.44	8	98.69	2	22.22	2	22.22	9	100
Ps.aeruginosa (n= 17)	9	52.94	5	35.29	10	58.82	9	52.94	9	52.94	15	88.23	15	88.23	1	5.88	17	100
Ac.baumannii	0	0	0	0	0	0	0	0	0	0	1	100	0	0	0	0	1	100

(n= 1)																	

All the Gram negative bacilli were 100% sensitive to Imipenem and around 90% sensitive to Cefaperazone - Sulbactam

E.coli was moderately sensitive to Amikacin (84%).

Klebsiella spp. were moderately sensitive to Amikacin and Ofloxacin (84.61% each).

Only one isolate of Citrobacter spp was isolated and it was sensitive to Amikacin, Ofloxacin and Gentamicin.

Proteus species exhibited moderate to poor sensitivity to all the drugs.

Pseudomonas spp was highly sensitive to Pipericillin but poorly sensitive to all the other antimicrobials.

Acinetobacter sp. was resistant to all drugs except Imipenem and Cefaperazone - Sulbactam.

All the isolates were poorly sensitive to Cotrimoxazole.

Multidrug resistance (>3 drugs) was observed in 28.26% of isolates. E.coli-9, Klebsiella spp-7, Proteus species -15, Pseudomonas spp-4, Morganella sp-3 and Acinetobacter sp.-1.

50

52

TABLE 24

RESISTANCE OF GNBS TO THIRD GENERATION CEPHALOSPORINS

Organisms	Ceftazidime Resistant Isolates (zone size<18mm)	No. of isolates with MIC >=2µg/ml
E.coli (25)	7 (28%)	10 (40%)
Kleb.pneumoniae (13)	5 (38.46%)	6 (46.15%)
Kleb.oxytoca (1)	1 (100%)	1 (100%)
Citrobacter koseri (1)	1 (100%)	1 (100%)
Proteus mirabilis (36)	22 (61.11%)	24 (66.66%)
Proteus vulgaris (5)	2 (40%)	2(40%)
Proteus penneri (1)	1 (100%)	1(100%)
M. morganii (9)	2 (22.22%)	3 (33.33%)
Ps.aeruginosa (17)	5 (29.41%)	5(29.41%)
Ac.baumanii (1)	1 (100%)	1(100%)
Total 109	47 (43.11%)	54(49.54%)

Forty seven isolates exhibited resistance to Ceftazidime phenotypically (zone size <18mm) and Fifty four isolates had MIC greater than 2µg/ml.

TABLE 25

MINIMUM INHIBITORY CONCENTRATION OF CEFTAZIDIME

Organisms	64µg/ml	128µg/ml	256µg/ml	Total	MIC_{50}	MIC_{90}
E.coli	2	7	1	10	128µg/ml	128µg/ml
Kleb.pneumoniae	1	4	1	6	128µg/ml	128µg/ml
Kleb.oxytoca	0	1	0	1	128µg/ml	128µg/ml
Citrobacter koseri	0	1	0	1	128µg/ml	128µg/ml
Proteus mirabilis	5	15	4	24	128µg/ml	256µg/ml
Proteus vulgaris	1	1	0	2	64 µg/ml	128µg/ml
Proteus penneri	0	1	0	1	128µg/ml	128µg/ml
M. morganii	0	1	2	3	256µg/ml	256µg/ml
Ps.aeruginosa	0	1	4	5	256µg/ml	256µg/ml
Ac.baumanii	0	0	1	1	256µg/ml	256µg/ml
TOTAL	**9**	**32**	**13**	**54**		

All the members of Enterobacteriaceae had an MIC_{50} of 128 µg/ml except for Proteus vulgaris (64µg/ml).

Morganella sp. and the non-fermenter GNBs, Pseudomonas sp. and Acinetobacter sp.had a higher MIC_{50} of 256µg/ml.

TABLE 26

NO. AND % OF ESBL PRODUCERS

Organisms	No of Ceftazidime resistant isolates			% of ESBL producers among CAZ. resistance isolates	% of ESBL producers among total isolates
	Total	No. of isolates with ESBL phenotype			
		DDST	CDM		
E.coli (25)	10	6	6	60%	24%
K.spp.(14)	7	5	5	71.42%	30.76%
C. koseri(1)	1	0	0	0%	0%
P. mirabilis(36)	24	14	17	58.33%	38.88%
P. vulgaris(5)	2	1	1	50%	20%
P. penneri(1)	1	0	1	0%	0%
M. morganii(9)	3	2	2	66.66%	22.22%
Ps.aerug (17)	5	2	3	40%	11.76%
Ac.baumanii(1)	1	0	1	0%	0%
Total 109	54	30	36	55.55%	27.52%

The results of the Modified Double Disc Synergy Test was taken for interpretation.

In addition to E.coli and Klebsiella pneumoniae, ESBL detection was performed for other enterobacteriaceae also.

55.55% of the Ceftazidime resistance was due to ESBL production.

The % of ESBL among the total isolates was 27.52%.

Proteus mirabilis (38.88%) was the commonest organism exhibiting ESBL followed by Klebsiella sp (30.76%).24% of E.coli strains were ESBL producers.

The combined disc method using Cefaperazone-Sulbactam gave a better identification of ESBLs.

TABLE 27

NO.AND % OF AmpC PRODUCERS

Organisms	No of CAZ resistant isolates			% of AmpC producers among CAZ. resistance isolates	% of AmpC producers among total isolates
	Total	AmpC Phenotype screening test positive	AmpC confirmatory test positive		
E.coli (25)	10	4	4	40	2.89
K.spp.(14)	7	2	1	14.28	7.14
C. koseri(1)	1	1	1	100	100
P. mirabilis(36)	24	14	8	33.33	22.22
P. vulgaris(5)	2	1	1	50	20
P. penneri(1)	1	1	1	100	100
M. morganii(9)	3	3	1	33.33	11.11
Ps.aerug (17)	5	2	1	20	7.24
Ac.baumanii(1)	1	1	1	100	100
Total 109	54	29	19	35.18	17.43

Screening for AmpC was positive in 53.70 of Ceftazidime resistant isolates. (29/54)

AmpC production was seen in 19 isolates (35.18%) that exhibited Ceftazidime resistance. They constituted 17.43% of the total isolates.

Only one isolate each of Acinetobacter, Citrobacter and Proteus penneri were obtained and all were AmpC producers.E.coli showed significant (40%) AmpC production.

No mechanism of cephalosporin resistance was explainable for 5 isolates.

DISCUSSION

Foot ulcer is one of the most feared complications in persons with diabetes. The problem gets compounded with infection causing considerable distress to the patient. Their quality of life is severely deteriorated. It is one of the most common precursors to lower extremity amputation, thereby affecting the mobility of the patients and increasing their morbidity. This study was done to analyse the risk factors for foot ulceration and amputation, to understand the microbial agents involved in the infection and their antimicrobial resistance pattern so that an effective antibiotic regimen can be employed to curtail the infection at the earliest.

The demographic profile of our patients showed that males (60.57%) were more commonly affected than females (39.42%) and the male - female ratio was 1.53:1 (Table 1). This is probably attributable to the greater out door activity of males and thereby increased predisposition to trauma. In a study conducted in Delhi by Dhanwal [18] and colleagues the male: female ratio was as high as 9:1.

Majority of our patients were in the fifty to sixty age groups (44.23%) (Table 2). The mean age was 54.93. 93.26% of our patients had type 2 diabetes (Table 5). A multicenter prospective trial done by Morbach and Vishwanathan et al[57] to assess the regional differences in risk factors at Germany, Tanzania and Chennai had also shown that diabetic foot ulcer patients were predominately male and had Type 2 diabetes and the corresponding patient ages were 71, 56 and 51 years respectively.

The right foot (62.5%) was more affected than the left (35.57%) in our population with a ratio of 1.75:1 (Table 3). Faiz ur Rehman et al [26] in their study also had found that diabetic foot affected the right foot (64.7%) more commonly than the left foot (35.3%), in

a ratio of 1.8:1.They had reasoned out that the right foot was the dominant foot and hence more prone for trauma.

The characteristic of the wound in the foot ulcer is another poignant feature that influences prognosis. Majority of our patients had grade 4 ulcers. (40.38%) (Table 4). Margolis et al [14] in their study found that the risk factors that most dramatically were associated with a wound failing to heal were increasing wound size, increasing wound duration, and the grade of the wound.

Duration of diabetes mellitus of our study group varied from as less as 4 months to 17 years (Table 6). Thirteen of the patients were diagnosed only on admission while most of them were diabetic for 6-10 years. In our study the mean duration of diabetes was 7.18 years while it was 6.6 years in a study by Raymundo et al.[55] Morbach and Vishwanathan et al[57] showed that the average diabetes duration until the onset of the initial foot lesion was 14 years in Germany and 12 years in India, but only 5 years in Tanzania.

Numerous studies have been done to find out the risk factors associated with diabetic foot. In the analysis of risk factors and precipitating causes for foot ulceration in diabetes, the present study showed the following findings. Trauma (33.65%) and smoking (30.76%) were the most common risk factors though their significance was not established (p value>0.05) (Table 7). Past history of foot ulcer (15.38%), previous amputation (5.76%) and alcohol intake (23.07%) were also not significant.

Neuropathic sign was present in 67.30% of the study population and was a significant risk factor (Table 8). Mild neuropathic affliction was predominant (37.5%). Reiber[29] and colleagues have indicated that Neuropathy was the most common component cause leading to ulceration. His study showed neuropathic changes in 78% followed by a minor traumatic event (77%) and ischemia in 35% that contributed to the development of

foot ulcers. In our study 25.96% of the subjects had impaired vascularity (Table 9). Nine patients (12.16%) had purely ischemic while 18 (24.32%) had neuro-ischemic ulcers. A study by Samson Oyibo et al[64] also showed that only 1.0% was ischemic while 67.0% of the ulcers were neuropathic, 26.3% were neuroischemic.

The morbidity of the disease is increased by other associated medical illnesses. Eight patients each had coronary heart disease and hypertension. Renal dysfunction was present in 5 patients (Table 10). More than half of the hospitalized patients (51.35%) required a stay of 6-10 days (Table 11).

Following foot infection, amputation is the most dreaded complication. Thirty three (31.73%) patients underwent amputation of whom three had Above Knee amputation done (Table 12). Below knee amputation was done in 10 patients-(grade 3=2, grade 4=7, grade5=1) of whom two had bilateral foot ulcers. Great toe disarticulation/transmetatarsal amputation was performed in twenty patients. (grade3=4, grade4=16). Among the amputees, nine (27.27%) gave history of previous ulcer and six of them had undergone prior amputation. Adler et al[2] had reported in a prospective study that former amputation and treatment with insulin are independent risk factors for amputation apart from peripheral sensory neuropathy and peripheral vascular disease.

In our study mean duration of diabetes was slightly more among amputees (8.33) than non amputees (6.37) (Table 13). Only 68(65.38%) patients were on medications-OHA (40.38%) and insulin (25%).The study showed that increasing grade of ulcer had an adverse effect on the wound resolution. Amputation rate increased with the grade of ulcer. All the patients who went in for amputation had ≥ grade 3 ulcers. 59.52% of patients with grade 4 ulcers and all patients with grade 5 ulcer underwent amputation. Raymundo et al's[55] study revealed that the chief risk factors leading to limb amputation among patients

admitted for infected diabetic foot were duration of diabetes of more than 10 years and, advanced Wagner's grade of foot ulceration on admission.

Poor glycemic control was another common feature in our patients who had an average plasma glucose level of 190.88 (Table 13). A study by Reiber et al [28] showed that the outcome of diabetic foot ulcer is related to poor glycemic control and longer diabetes duration. This point is further stressed in a study by Lavery et al[47] that revealed an elevated glucose level (Glycated haemoglobin HbA1C >9 %) as one of the risk factors for ulceration.26 patients (25%) were insulin dependent and 42 patients (40.38%) were on oral hypoglycemic agents.13 patients were diagnosed only on admission. The rest (23 patients 22.01%) were not on any medications.

Many of the studies on the risk factors have conflicting results. In the Seattle Diabetic Foot Study by Boyko et al, diabetes duration and type, smoking status, and also other factors like race, education, joint mobility, hallux blood pressure, and other foot deformities were found to be unrelated to foot ulcer risk in multivariate models [23].

Various methodologies have been tried by different workers for optimal sampling of infected diabetic ulcers. Most of the studies show deep tissue sampling by curettage of the ulcer base /needle aspiration and collection of the tissue or bone at the time of debridement to be superior to routine surface sampling. The yield of anaerobes has substantially increased by these methods and risk of surface contamination is also reduced. In a study by Uday Kelkar and Anju et al[77] the yield of aerobic and anaerobic organisms from diabetic foot ulcers, significantly more organisms were isolated from deep tissue samples (average 3.7 organisms per sample) than from properly collected swabs (150; average 3.0 organisms per sample) indicating that deep tissue sampling is a more sensitive method. Kessler et al had recommended needle aspiration for deep direct sampling in diabetic patients with osteomyelitis related to foot ulcer when surgical debridement is

contraindicated or delayed [41]. In the present study we used swabs, deep tissue scrapings and needle aspiration of abscesses.

The microbiology of diabetic lower extremity infection varies depending on the patient population studied. In this study the isolation rate of gram negative bacteria was higher compared to the gram positive cocci depicting a ratio of 3.75:1 .An average of 1.629 organisms were isolated per specimen. Sharp et al[69] had in their study obtained an average of 2.3 organisms per specimen among patients. Sapico[67] et al had a high isolation rate with a mean of 4.7 bacterial species per specimen. In our study, aerobes constituted 81.6% while anaerobes comprised of 14.79%of the total. In Unachukwu's study [78] aerobes and anaerobes constituted 95.4% and 4.6% of the total bacterial isolates respectively. In a study by Sharp et al[69] the anaerobic isolation rate was 27%. Bartlett et al [52] in their study showed a high isolation rate of anaerobes. There were a total of 116 isolates with an average of 5.8 species per specimen (3.2 aerobes and 2.6 anaerobes).

In our study GPC were 21.01% and GNBs were 78.98% of the total aerobic isolates(Table 14). The ratio of GNB: GPC was 3.75:1. *Staphylococcus aureus* has been the most common isolate in numerous studies[1, 26, 31, 40, 78].Though not the commonest organism, a significant number of *Staph.aureus* (12.43%) have been isolated in our study. Several reasons could be given, 56% of the subjects had received antibiotics prior to admission primarily oral cloxacillin or amoxicillin, which could have eradicated the gram-positive organisms in the initially mixed infected lesions.

The predominant organisms isolated in our study were Proteus mirabilis, Escherichia coli, Staph.aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Morganella morganii and Enterococci in that order. Proteus mirabilis was the commonest isolate (26.08%) and Staphylococcus aureus was the commonest GPC isolate (15.21%). Similar bacterial isolates were found by Sharp et al whose study had Proteus sp.,

Enterococcus spp, Staphylococcus aureus, and other Enterobacteriaceae in that order. (63) In Carvallho's study[9] also the most frequently occurring pathogens were Enterobacteriaceae (83.7%), Staphylococcus aureus (43.3%) and anaerobic bacteria (17%).

We isolated 25 anaerobes in culture of which Bacteroides fragilis and Peptococcus spp were the common organisms (Table 15). Other isolates include Peptostreptcoccus sp. (5), Clostridium welchii (3) and Clostridium tetani (1). The principal anaerobes isolated by Bartlett and colleagues also were Bacteroides species , Peptococcus sp. and Clostridia sp. Wheat et al reported that most infections were heavily mixed; Common anaerobic isolates in his study included Peptostreptococcus magnus, Peptostreptococcus prevotii, and Bacteroides species. GPAC, cultured from 36% of specimens, were the major anaerobic group in their study[82]. All the anaerobes were 100% sensitive to metronidazole in our study. We found that BHI with 0.1% agar gave a better isolation rate of the anaerobes compared to RCM. The competency to isolate all the species of anaerobic organisms was not yet maximized as of present time; therefore the low isolation rate of anaerobes. Fungal organisms comprised only 3.55% of the total isolates. Candida albicans was the commonest isolate (83.33%) (Table 16) In a study by Chincholikar et al, Candida albicans was the commonest fungal pathogen.

Seven patients presented with systemic signs of fever of whom three were blood culture positive.All 3 of them had higher grade of ulcer and underwent amputation (Table 17). This showed that systemic toxicity occured in the advanced stage of the infection and was associated with an increased rate of amputation.

Among the amputees the isolation rate was higher. More than half of the cases (56.73%) had polymicrobial infection.The combination of aerobe (GPC/GNB) +Anaerobe constituted nearly half of the polymicrobial group. (24.03%) The ratio of Polymicrobial:

Monomicrobial infection was 3:2.In amputees it was 2.4:1 while in the non amputees group it was 1.2:1. Scher and Steele[68] in their study on amputation showed that infections among amputees were polymicrobial, with 5.8 bacterial isolates and 2.3 anaerobes recovered per patient (Table 18).

Monomicrobial infection was seen more among out-patients (76.66%).Among hospitalized patients, infections were often polymicrobial (74.32%) comprising of aerobic gram-positive and gram negative organisms, as well as obligate anaerobes (Table 19). Anaerobic (24/25 isolates) and fungal infection was also seen predominantly among hospitalized patients only. Most of these patients had limb threatening infection. Boyko and Lipsky[22] have in their work noted that among diabetic outpatients with lower extremity infection aerobic gram-positive cocci were isolated as the sole pathogen in 42% of cases, while anaerobes and aerobic gram-negative bacilli were infrequently recovered. Thus, infection severity appears related to number and type of infecting organism.

While studying the variation in the type of organisms in different grade of foot ulcers our work showed that Gram negative bacilli were predominant in all the grade of foot ulcers. Maximum (45.87%) isolation was seen in grade 4 ulcers. Anaerobic isolation rate also increased with grade of the ulcer (Table 20).

Regarding the antimicrobial sensitivity pattern, we found that Staph. aureus was relatively better sensitive to Ceftazidime, Cefaperazone and Oxacillin to which it was moderately sensitive (76.19% each) (Table 21). 57.14% were beta lactamase producers and 5 of the 21 isolates were MRSA (23.80%). In a prospective study done by Goldstein et al [31] to analyze the microbial culture and sensitivity pattern, *Staphylococcus aureus* was the most common isolate (76% of patients), including *methicillin-resistant S. aureus (MRSA)* in 5 of 25 (20%) patient wounds. An investigation by Tentolouris et. Al [73], among outpatients reported that *S. aureus* was the most prevalent pathogen of gram-positive

aerobes isolated from wounds and MRSA organisms comprised 40 percent of *S. aureus* isolates. They reasoned that the high prevalence of *MRSA* in patients with foot ulcers may reflect the increased prevalence of *MRSA* in the community. They also found that *MRSA* infection or colonization was not associated with factors like previous hospitalization, use of antibiotics, etc that are known to predispose to *MRSA* colonization or infection. For patients with a documented MRSA or those who are at high risk for MRSA, linezolid is becoming the drug of choice. Sharpe [69] found that linezolid was not only superior to vancomycin but also noted there were seven amputations in the patients treated with vancomycin and none in the patients treated with linezolid. In our study all the isolates were sensitive to vancomycin. 57.14% of Staph aureus were beta lactamase producers (Table 22).

Enterococcus faecalis was moderately sensitive to CA and Ak (75% each). Both Staph.aureus and Enterococcus faecalis were 100% resistant to penicillin while they were 100% sensitive to Vancomycin. Ampicillin - sulbactam was effective in all MSSA and enterococci.

The sensitivity pattern of the Gram negative isolates revealed 100% sensitivity to Imipenem and around 90% sensitivity to Cefaperazone-Sulbactam (Table 23). Kavitha et al[39] had demonstrated the antimicrobial sensitivity pattern in diabetic foot ulcers. Their study showed sensitivity of the Enterobacteriaceae and non-fermenters to Cefaperazone + Sulbactam (78.4%) and Meropenem (97.8%).In our study the Enterobacteriaceae was better sensitive to Amikacin and Ofloxacin than the third generation cephalosporins. While Citrobacter spp was100% sensitive to Amikacin, E.coli and Klebsiella spp showed a sensitivity of 84% and 84.61% respectively to Amikacin. Klebsiella spp. was equally sensitive to Ofloxacin (84.61%).Proteus species exhibited moderate to poor sensitivity to all the drugs. A study by Anandi et al had also shown that E.coli (97%), and Klebsiella spp

(94%) were sensitive to Ciprofloxacin and Ofloxacin and all aerobes were sensitive to Amikacin.[4] Pseudomonas spp was highly sensitive to Pipericillin and poorly sensitive to all the other antibiotics. Acinetobacter sp. was resistant to all drugs except Imipenem. All the isolates were poorly sensitive to Cotrimoxazole (<25%).

Multi drug resistance was seen in 28.26% of the organisms predominantly occurring in Acinetobacter sp(100%), Klebsiella sp(50%) and Proteus species (41.66%).All the patients had ≥ grade 3 ulcers. In a study conducted by Hartemann et al, it was found that MDROs are often present in severe diabetic foot wounds. They found that about one-third of patients with a history of previous hospitalization for the same wound, and 25% of patients with osteomyelitis had MDRO-positive specimens [32].

Ceftazidime resistance was seen in 47 isolates phenotypically and the MIC of 54 isolates was greater than 2µg/ml (Table 24). All the members of Enterobacteriaceae had an MIC 50 of 128 µg/ml except for Proteus vulgaris (64µg/ml).Morganella sp. and the non-fermenter GNBs, Pseudomonas sp. and Acinetobacter sp. had a higher MIC_{50} of 256µg/ml (Table 25).

Resistance to third generation cephalosporins due to acquisition and expression of Extended Spectrum β-lactamase (ESBL) enzymes among Gram-negative bacteria are on the increase. Presence of ESBL producing organisms has been reported to significantly affect the course and outcome of an infection. In our study we employed two methods, the modified double disc approximation method using Ceftazidime and amoxiclav and combined disc method using Cefaperazone alone and Cefaperazone –Sulbactam disc. (Table 26)

Cefoperazone/sulbactam (1:1) is a unique combination of III generation cephalosporin that is more stable to b-lactamases then penicillins with inhibitor at the

highest available ratio. Among all b-lactam-inhibitor combinations tested Cefoperazone/sulbactam revealed the highest activity against ESBL-producing organisms. Its superior activity is probably attributed to the improved stability of Cefaperazone and to the high concentration of the inhibitor component- Sulbactam. [19].

We found that the combined disc method was more effective in the detection of ESBL producers.ESBL production was observed in 28.84% of the study subjects and 55.55% of the Ceftazidime resistant strains. The % of ESBL among the total isolates was 27.52%. The pattern observed in our study was E.coli 24%, Klebsiella spp.30.76%, Proteus mirabilis 38.88% and no ESBL production in Citrobacter spp. In a study by Raghavendra Rao et al[63], ESBL production was observed in 10.6% of E.coli,10% of Klebsiella,9.3% of Proteus and 5.1% of Citrobacter spp. In Carvallho's study [9] the ESBL producing strains were detected only in 6% of the patients. Gargritu and Gulati et al[27] had employed Cefaperazone and Cefaperazone-Sulbactam for the detection of ESBL production. Their study showed ESBL production among Pseudomonas strains as high as 61.25%.

The other resistance mechanism that we simultaneously screened for while detecting ESBL production was the presence of AmpC beta lactamases (Table 27). The presumptive AmpC producers were later confirmed by the AmpC disc method .AmpC production was seen in 19 isolates (35.18%) that exhibited Ceftazidime resistance and they constituted 17.43% of the total isolates.E.coli showed significant AmpC production (40%).No mechanism of cephalosporin resistance was explainable for 5 isolates. In a study by Singhal et al the AmpC production was seen in 8% of the gram negative clinical isolates. Kenneth Thomson had in his work reported that high-level expression of AmpC may prevent recognition of an ESBL[40], especially in species or strains that produce a chromosomally encoded inducible AmpC beta-lactamase (e.g., *Enterobacter, Serratia*,

Providencia, *Aeromonas* spp., *M. morganii*, *C. freundii*, *Hafnia alvei*, and *P. aeruginosa*). With these organisms, clavulanate may act as an inducer of high-level AmpC production while Tazobactam and Sulbactam are much less likely to induce AmpC beta-lactamases and are therefore preferable inhibitors for ESBL detection tests with these organisms.

In many hospitals, beta-lactam/beta-lactamase inhibitor compounds, such as piperacillin/tazobactam or ampicillin/sulbactam, are considered first line therapy for complicated diabetic foot infections. These drugs have a spectrum of activity that consists of excellent gram positive and anaerobic coverage with variable activity against the gram negatives. We have proposed the following empiric antibiotic regimen according to the Wagner grades of foot ulcers.

- For Grade 1 and Grade 2 ulcers oral Cephalosporins /Cloxacillin can be given for GPCs and oral quinolones like Ciprofloxacin and Ofloxacin can be given for GNBs. Parenteral aminoglycoside + cephalosporins can be used for enterococcal infections.

- In Grade 3 and Grade 4 ulcers, GPCs can be empirically treated with Ampicillin –Sulbactam; Cefaperazone + Sulbactam along with Metronidazole IV will cater for the GNBs and anaerobic organisms.

- For Grade 5 ulcer, Vancomycin /Imipenem +Metronidazole IV can be employed.

SUMMARY

- Males (60.57%) were more affected than females (39.42%).
- Majority of the patients affected were in the 51- 60 years age group. The mean age of the patients was 54.93.
- Significant risk factors for ulceration include neuropathy and longer mean duration of diabetes mellitus .Other factors like smoking, trauma, previous ulceration and alcohol intake were not contributory in the development of foot ulcer.
- Significant risk factor for amputation was polymicrobial infection and increased grade of ulcer.
- Diabetic foot disease causes significant morbidity as the average duration of hospitalization in more than half the patients (51.35%) was 6-10 days.
- Associated co-morbid conditions included coronary heart disease, hypertension and renal dysfunction though the presence of these had no effect on the course of the foot ulcer.
- Amputation was the main complication.31.73% of the patients were amputated; AK amputation in 3, BK amputation in 10 and forefoot amputation in 20 patients.
- Aerobic (81.66%), anaerobic (14.79%) and fungal (3.55%) isolates were obtained on culture.
- Among the aerobes GNB (78.98%) were isolated more than the GPC (21.01%).
- Proteus mirabilis (26.08%) was the commonest isolate.

- Staph. aureus (15.21%) was the commonest GPC isolate.

- Among the anaerobes Bacteroides fragilis and Peptococcus sp.were the common isolates. (32% each).

- Candida albicans was the commonest fungal isolate. (5 of 6 fungal organisms).

- Blood culture was positive in 3 patients. All three underwent amputation and had higher grade of foot ulcer. (grade 5=2, grade 4=1).This indicated that the risk for sepsis increases with the grade of foot ulcer and in that eventuality chances of amputation also went up.

- Polymicrobial infection was present in 56.73% of the cases.74.32% of the hospitalized patients had polymicrobial infection. Their rate was higher among amputees.

- Anaerobic (24/25 isolates) and all fungal infections were seen among hospitalized patients only.

- Anaerobic isolation rate increased with the grade of foot ulcer.

- All the GPC were sensitive to Vancomycin. Ampicillin-Sulbactam was effective for all MSSA and enterococci sp. All the GNBs exhibited 100% sensitivity to Imipenem and around 90% sensitivity to Cefaperazone-Sulbactam.

- Beta lactamases were detected in 48.27% of the GPCs by the iodometric method. 57.14% of Staph.aureus strains were beta-lactamase producers.

- MRSA constituted 23% of the Staph. aureus recovered.

- The Gram positive cocci were better sensitive to Cephalosporins and Oxacillin.

Enterobacteriaceae were better sensitive to Amikacin and Ofloxacin than the third generation Cephalosporins. Pseudomonas sp was highly sensitive to Pipericillin.

- Multi Drug Resistance was seen in 28.26% of the aerobic isolates.

- Resistance to third generation Cephalosporins was seen in 39.13% of gram negative isolates.

- All the members of Enterobacteriaceae had an MIC_{50} of 128 µg/ml for Ceftazidime except for Proteus vulgaris (64µg/ml). Morganella sp. and the non-fermenter GNBs Pseudomonas sp. and Acinetobacter sp.had a higher MIC_{50} of 256µg/ml.

- ESBL production was observed in 28.84% of the total GNBS and 55.55% of Ceftazidime resistant isolates. Proteus mirabilis (38.88%) was the commonest organism exhibiting ESBL followed by Klebsiella sp (30.76%).24% of E.coli strains were ESBL producers**.** The detection of ESBL was better observed by the combined disc method using Cefaperazone and Cefaperazone-Sulbactam combination.

- AmpC producers comprised of (35.18%) that exhibited Ceftazidime resistance and they constituted 17.43% of the total isolates.

- The mechanism of cephalosporin resistance was not clear in 5 isolates.

CONCLUSION

The study revealed that

- Peripheral neuropathy and longer duration of diabetes are the factors that predispose to foot ulcers while polymicrobial infection and increasing grade of foot ulcer aggravated the chance of amputation.

- The most common microorganisms were gram-negative aerobes, and the isolation pattern, according to the grade of ulcer was primarily *Staphylococcus aureus* in Wagner I diabetic foot. Gram-negative organisms, majority of which were *Proteus mirabilis sp. and Escherichia coli sp.*, were isolated as the foot grade advanced to gangrene. Significant anaerobic growth was observed in Wagner's IV and V lesions.

- Management of early stages include treatment with oral quinolones / cloxacillin / Cephalosporins. Ampicillin- Sulbactam and Cefaperazone-Sulbactam were found to be very effective drugs. Imipenem monotherapy or third-generation Cephalosporins with beta lactamase inhibitors plus an anti-anaerobe drug are attractive regimens for the advanced stage of the disease.

RECOMMENDATIONS:

- Patient education on prevention of foot ulceration and increasing their awareness on early management of a non healing wound is pivotal in reducing the incidence of the foot disease.

- Once admitted, patients with diabetic foot infections should be managed aggressively with regular wound care and if necessary, repeated debridement to prevent the progression of infection to deeper tissues and subsequent development of gangrene.

- Culture and sensitivity results should be followed up as early as possible and antimicrobial coverage should be adjusted accordingly. A polymicrobial growth and presence of anaerobes should be aggressively managed.

- Appropriate and timely surgical procedure with the administration of the correct antimicrobials based on the culture and sensitivity results is still the dictum in the successful management of diabetic foot infection.

ACKNOWLEDGEMENTS

First and foremost I thank the **Lord Almighty,** who has been the guiding light and the source of inspiration throughout this study.

I extend my gratitude to the Dean of Madras Medical College, **Dr.Kalavathy Ponnirivan, B.Sc., M.D.,** for having allowed me to do this study in this hospital.

I acknowledge my sincere regards to the Director and Professor of Institute of Microbiology, **Dr.A.Lalitha,M.D.D.C.P.,** for having supervised this work and helped in the successful completion of the project.

I am deeply indebted to the former Director and Professor of Institute of Microbiology, **Dr.T.S.Vijayalakshmi, M.D.,** for her invaluable support and suggestions while conducting this study.

I sincerely thank the Vice-Principal of Madras Medical College, **Dr.S.Geetalakshmi, M.D.,** Professor of Microbiology, who gave me useful insights during the course of this study.

I owe my gratitude to my guide, **Dr.Kalavathy Victor, M.D., D.C.P.,** Professor of Microbiology, for her constant encouragement and continuous monitoring of the work.

It's my privilege to thank **Dr.A.S.Shameem Banu, M.D., Dr.G.Sumathi, M.D.,** and **Dr.G.Sasirekha, M.D.D.G.O.,** Professors of Microbiology for their immense support and invaluable guidance.

I would like to thank the Chief of Diabetology Department, Madras Medical College and Govt. Hospital, **Dr.Hariharan, M.D.,** for allowing me to do the study in his department.

My sincere regards are also to the Professor of General Surgery, **Dr.G.Sivakumar, M.S.,** for his kind cooperation in helping me do the study in his wards.

I wish to express my sincere thanks to **Dr.K.Radhika, M.D., D.A.D.N.B.,** who has helped me in the study design and constantly appraised the project work.

My heartfelt regards are to **Dr.Sujatha Varadarajan,M.D., Dr.Sheila Doris, M.D.,** who have closely monitored my work and helped improve the study with their suggestions.

I wish to thank **Dr.T.Girija Devi, M.D.,P.G.D.H.M., Dr.K.Kaveri, M.D.,D.C.H., Dr.R.Selvi,M.D., Dr.M.Indumathi,M.D.,D.G.O., Dr.T.Latha, M.Sc., Ph.D.,** Assistant Professors of Institute of Microbiology, for their support and encouragement.

I wish to thank **Dr.P.Madhumathi,M.D.,** for her help in the anaerobic culture work and **Dr.Ilayakumar,M.S.,** for helping me in the neurovascular assessment.

I also extend my warm regards to the laboratory technical staff for their cooperation and help in the preparation of media and other aspects of the study.

My heart felt reverence and regards to my husband and parents for their relentless support and motivation which helped me in the successful completion of this study.

APPENDIX

I. PROFORMA OF STUDY

NAME :
AGE :
SEX :
OCCUPATION :
ADDRESS :

OP / IP NO :

PRESENTING COMPLAINT :

DURATION OF DIABETES :

ON INSULIN / OHA :

H/O NUMBNESS / TINGLING SENSATION / PAIN / PARAESTHESIA :

SMOKER / ALCOHOLIC / HYPERTENSIVE

IF INPATIENT - NUMBER OF DAYS IN HOSPITAL :

PREVIOUS HISTORY OF FOOT ULCER / ANY CHRONIC NON - HEALING INFECTION :

OTHER ASSOCIATED DIABETIC ILLNESSES :

LOCAL TRAUMA / ANY PRECIPITATING CAUSE FOR FOOT ULCER :

TYPE OF FOOTWEAR :

FAMILY HISTORY OF DIABETES :

GENERAL EXAMINATION

BUILD / NOURISHMENT - WELL NOURISHED / ILL NOURISHED

LOCAL EXAMINATION

FOOT ULCER
SIZE
DEPTH
EDGE
ANY BLEEDING
BONE & JOINT INVOLVEMENT
SENSATION

TYPE OF DRESSING

ANY SURGICAL INTERVENTION :

GRADING OF ULCER :

ASSESSMENT OF OTHER FOOT :

NEUROLOGICAL ASSESSMENT :

ASSESSMENT OF VASCULARITY :

INVESTIGATIONS

TEMPERATURE :
TC :
DC :
ESR :
BLOOD UREA :
BLOOD SUGAR :
[F] :
[PP] :
[R] :
URINE SUGAR :
HBA_1C :
SERUM ELECTROLYTES:
SERUM CREATININE

MICROBIAL STUDY : PUS C/S

BLOOD CULTURE IF PATIENT IS FEBRILE

TREATMENT

ANTIBIOTICS

ANTIDIABETICS

OTHERS

OTHERS SYSTEMS

CVS
RS
ABDOMEN
CNS
ENDOCRINES
RENAL
OPHTHALMIC

ABBREVIATIONS USED IN THE TEXT

ABI	ANKLE BRACHIAL INDEX
AK AMPUTATION	ABOVE KNEE AMPUTATION
BAP	BLOOD AGAR PLATE
BK AMPUTATION	BELOW KNEE AMPUTATION
BL	BETA LACTAMASE
CAP	CHOCOLATE AGAR PLATE
CDC	CENTER FOR DISEASE CONTROL
DDST	DOUBLE DISC SYNERGY TEST
ESBL	EXTENDED SPECTRUM BETA LACTAMASE
G-CSF	GRANULOCYTE COLONY STIMULATING FACTOR
GNB	GRAM NEGATIVE BACILLI
GPAC	GRAM POSITIVE ANAEROBIC COCCI
GPC	GRAM POSITIVE COCCI
KOH	POTASSIUM HYDROXIDE
LCB	LACTOPHENOL COTTON BLUE
LEA	LOWER EXTREMITY AMPUTATION
Mac	MAC CONKEY AGAR PLATE
MDRO	MULTI DRUG RESISTANT ORGANISMS
MIC	MINIMUM INHIBITORY CONCENTRATION
NAP	NUTRIENT AGAR PLATE
NCCLS	NATIONAL CENTRE FOR CLINICAL AND LABORATORY STANDARDS
OHA	ORAL HYPOGLYCEMIC AGENT
PVD	PERIPHERAL VASCULAR DISEASE
RBS	RANDOM BLOOD SUGAR
RCM	ROBERTSON'S COOKED MEAT MEDIUM

II) UNIVERSITY OF TEXAS FOOT ULCERATION CLASSIFICATION

STAGE	GRADE			
	0	1	2	3
A	Pre or post lesion, intact	Superficial ulcer	Penetrating to tendon or joint capsule	Penetrating to bone or joint space
B	+Infection	+Infection	+Infection	+Infection
C	+Ischemia	+Ischemia	+Ischemia	+Ischemia
D	+Infection and Ischemia	+Infection and Ischemia	+Infection and Ischemia	+Infection and Ischemia

III) MEGGIT AND WAGNER GRADING OF FOOT ULCER

GRADE	CONDITION
0	High risk foot, no ulcer
1	Superficial skin ulcer
2	Deep ulcer extending through dermis.Tendons,ligaments,joint capsule or bone may be exposed
3	Deep ulcer with abscess, osteomyelitis and joint sepsis
4	Localized gangrene of forefoot or heel
5	Gangrene of foot

IV) GRAM STAIN

REQUIREMENTS:

1. METHYL VIOLET -0.5-2%
2. GRAM'S IODINE
3. ACETONE DECOLORISER
4. DILUTE CARBOL FUCHSIN

PROCEDURE:

1. For direct gram stain the smear is fixed with methanol for 30 seconds as the cell morphology is better preserved .For culture smears heat fixation can be

done.

2. The slide is then covered with the primary stain, methyl violet for one minute. Wash the slide with water.
3. Cover the whole slide with Gram's iodine and leave for a minute .Wash the slide with water.
4. Decolourise with acetone as follows: Hold the slide with a forceps at a steep slope and pour acetone over the slide allowing it to act for 2-3 seconds. End decolourisation by removing all acetone in the stream of water from the tap.
5. Counterstain the smear with Dilute Carbol Fuchsin and allow it to act for 30 seconds.
6. Wash thoroughly with water from tap and blot dry, placing the smear upwards.
7. Completely dry the slide in warm air by passing high above the Bunsen flame.
8. Observe the smear under oil immersion objective.

V) GERM TUBE TEST

1. Lightly touch a single colony with a loop; remove the excess inoculum and then emulsify the yeast cells in 0.5 ml serum in a simple test tube.
2. Incubate at 37ºC in a water bath for 2-4 hrs. Prolonged incubation is not required.
3. A loopful is then taken and observed under coverslip under low power microscope. Germ tube appears as extension of the yeast cells and gives a drum stick appearance.

VI) HAEMIN AND MENADIONE FOR ANAEROBIC ISOLATION

HAEMIN 500 µg /ml: Dissolve haematin hydrochloride 50 mg in 1ml of 1 mol/litre NaOH solution and make up to 100 ml with distilled water. Filter sterilize.

MENADIONE 100 µg /ml: Dissolve 10mg menadione in 2 ml ethanol and make upto 10ml with distilled water. Filter sterilize .Protect from light.

The final concentration in the medium should be Haemin 5 µg /ml and menadione 1µg /ml. For 100 ml media, 1ml each is added.

VII) BRAIN HEART INFUSION AGAR SEMISOLID MEDIUM

CONTENTS

Calf Brain Infusion	-	200 g/l
Beef heart infusion	-	250 g/l
Proteose peptone	-	10 g
NaCl	-	5 g/l
Disodium phosphate	-	2.5 g
Dextrose	-	2.0 g
Sodium Polyanethol Sulphate	-	0.5 g
Agar	-	1g/l

Dissolve 37.5 g in 1 litre distilled water. Heat well to ensure complete dissolution. Dispense into tubes and sterilize by autoclaving at 121ºC for 15 minutes. .1 g/l agar added to ensure anaerobic growth. Final pH adjusted to 7.4. The tubes are then immediately sealed at their mouth using cotton wool plug soaked in molten sterile paraffin wax.

VIII) CDC BLOOD AGAR

CONTENTS

Trypticase soy agar	-	15 g
Phytone	-	5 g
NaCl	-	5 g
Agar	-	20 g
Yeast Extract	-	5 g
Haemin	-	5 g
Vitamin K	-	400 mg
Deionised water	-	1 litre.
Sheep/ Rabbit blood	-	50 ml.

METHOD

The basic medium except Haemin and Vitamin K is dissolved in 1 liter water and autoclaved at 121ºC for 20 minutes. The medium is then cooled to about 50ºC, when Haemin, Vitamin K and 5% sheep blood is added. The final concentration in the medium should be Haemin 5 µg /ml and menadione 1µg /ml.

IX) BACTEROIDES BILE ESCULIN AGAR

CONTENTS		**g/l**
Peptic digest	-	5
Beef extract	-	3
Oxgall	-	20
Esculin	-	0.5

Ferric citrate - 0.25

Agar - 15

METHOD

Dissolve 64.5g of the medium in 1 litre distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121ºC for 15 minutes.

X) SYMBOL AND STRENGTH OF THE ANTIBIOTICS USED AND THEIR ZONE SIZE INTERPRETATION CHART.

ANTIBIOTIC	SYMBOL	STRENGTH (µg)	RESISTANT (mm)	INTERMEDIATE (mm)	SENSITIVE (mm)
AMIKACIN	Ak	30	14	15-16	17
AMPICILLIN+SULBACTAM	As	10/10	11	12-14	15
AMPICILLIN (Staph.aureus)	A	10	28	-	29
CEFAPERAZONE	Cs	75	15	16-20	21
CEFAPERAZONE+SULBACTAM	Cfs	75/30	15	16-20	21
CEFTAZIDIME	Ca	30	14	15-17	18
CIPROFLOXACIN	Cf	5	15	16-20	21
COTRIMOXAZOLE	Co	25	10	11-15	16
ERYTHROMYCIN	E	15	13	14-22	23
GENTAMICIN	G	10	12	13-14	15
IMIPENEM	I	10	13	14-15	16
OFLOXACIN	Of	5	12	13-15	16
OXACILLIN	OX	1	10	11-12	13
PENICILLIN	P	1O U	28	-	29
PIPERICILLIN	Pc	100	17	-	18
PIPERICILLIN WHEN TESTING ENTERIC GRAM NEGATIVE BACILLI	Pc	100	17	18-20	21
VANCOMYCIN	Va	30	-	-	15

XI) BETA – LACTAMASE DETECTION

Iodometric method:

Requirements:

a) 1%soluble starch solution-prepared by dissolving the starch at 100ºC.

b) Iodine reagent-consisting of 2.03 g iodine and 5.32 g potassium iodide in 100 ml distilled water.

c) Micro titer plate.

Method:

From an overnight culture of the test organism, a heavy suspension was made containing 10 ^{9}cfu/ml. in 100 mM sodium phosphate buffer at pH 7.3 containing

penicillin at 6 g/litre.A negative control was put up without the test organisms. An organism known to produce β lactamase was put up as positive control. The test and the control organisms were then inoculated into the micro titer plate and incubated for one hour at 37ºC .1% soluble starch solution was then added to each well. A drop of the iodine reagent was then added. Loss of blue colour within 10 minutes was inferred as production of β-lactamase.

XII) DETECTION OF MINIMUM INHIBITORY CONCENTRATION

AGAR DILUTION METHOD

REQUIREMENTS:

a) Mueller Hinton Agar broth
b) Petri plates
c) Ceftazidime powder reconstituted with distilled water.
d) Test strains

Procedure:

Medium: Mueller Hinton agar was heated at 100ºC and dispensed as 19 ml each in tubes, autoclaved and allowed to cool in a 50ºC water bath.

Antibiotic: The Ceftazidime powder was dissolved in Saturated NaHCO3 and sterile water. Serial dilutions of the antibiotic from 256mg/l to 2mg/l were then made in the Mueller Hinton Broth at ten times the final concentration of the antibiotic in the plate. One ml of the antibiotic was added to 9ml of the medium. After adding the antibiotic the medium was mixed well and poured into Petri dish. A control plate containing the test medium without the antibiotic was prepared for each series of the dilution.

Test inoculum: 4-5 individual colonies of same morphology was picked up and inoculated into peptone water and incubated at 35ºC for 2 hours. The turbidity of the

inoculum was then adjusted with sterile saline to get s turbidity optically comparable to the 0.5 McFarland standard. Using a micropipette 2µl of the inoculum was delivered on the agar surface which gave an approximate strength of 10^4 cfu/ml. The plates were then incubated at 35ºC overnight.

RESULT: The lowest concentration of the antibiotic which inhibited the growth of the organisms was taken as the MIC.

MIC_{50}: The concentration of the antibiotic at which 50% of the test strains was inhibited was taken as the MIC_{50}.

MIC_{90}: The concentration of the antibiotic at which 90% of the test strains are Inhibited was taken as the MIC_{90}.

BIBLIOGRAPHY

1. Abdulrazak A, Bitar Z.I, Al-Shamali A, Mobasher L.A. Bacteriological study of diabetic foot infections**.** J Diabetes Complications. 2005 May-Jun;19(3):138-41.

2. Amanda I. Adler, Edward J. Boyko, Jessie H. Ahroni, Douglas G. Smith Lower- Extremity Amputation in Diabetes The independent effects of peripheral vascular disease, sensory neuropathy, and foot ulcers Diabetes Care, 1999 ; 22:1029–1035.

3. Ambrosch A, Lehnert H, Lobmann R.Zentrallabor, St. Joseph-Hospital, Bremerhaven. Microbiological aspects and antibiotic therapy of diabetic foot infections. American Family Physicians november1, 2002; 66/no.9:1655-62.

4. Anandi ,Alagaraju D,Natarajan V,Bacteriology of Diabetic foot lesions,Indian Journal of Medical Microbiology,2004;22(3):175-178.

5. Anne L Carrington, Caroline A Abbott, Joan Griffiths, Ross , Andrew J.M.Boulton,A foot Care Program for diabetic unilateral Lower limb amputees.; Diabetes journals.org/24/2/216.

6. Bailey and Scott's Diagnostic Microbiology, 9th edition; Processing clinical specimens for anaerobic bacteria: isolation and identification procedures, Ch 35:474-501.

7. Betty A Forbes, Daniel F Sahm, Alice S. Weissfield, Bailey and Scott's Diagnostic Microbiology, 11th edition, 2002 Pgs 273 - 283.

8. Caroline A. Abbott,Loretta Vileikyte, Sheila Williamson, Anne L. Carrington, Andrew J.M. Boulton, Multicenter study of the incidence of and predictive risk factors for diabetic neuropathic foot ulceration diabetes care, July 1998; volume 21, number 7.

9. Carvalho CB, Neto RM, Aragao LP, Oliveira MM, Nogueira MB, Forti AC. Diabetic foot infection. Bacteriologic analysis of 141 patients Arq Bras Endocrinol Metabol. 2004 Jun; 48(3):398-405. Pub 2004 Aug 26.

10. Chincholikar DA, Pal RB. Study of fungal and bacterial infections of the diabetic foot. Indian Journal of Pathology and Microbiology.2002 Jan; 45(1):15-22.

11. Citron M D, Specimen, Collection and Transport, Anaerobic Culture Techniques and Identification of Anaerobes. Review of Infectious Diseases, Mar-Apr 1984; Vol 6: Supplement 1:S51-S57.

12. Craig Payne, www.latrobe.edu.au .

13. David G. Armstrong, and Lawrence A. Lavery, Diabetic Foot Ulcers: Prevention, Diagnosis and Classification, American Family Physician, March 15, 1998.

14. David J. Margolis, Lynne Allen-Taylor, Ole Hoffstad, Jesse A. Berlin, Diabetic Neuropathic Foot Ulcers: The association of wound size, wound duration, and wound grade on healing Diabetes Care 25:1835-1839, 2002.

15. Davidsons Principles and Practice of Internal Medicine 19th Edition: Diabetes Melitus p644.

16. Davidsons Principles and Practice of Internal Medicine 19th Edition: Diabetes Mellitus p676-678.

17 Department of Microbiology, King Abdulaziz University Hospital, PO Box 6615, Jeddah 21452, Kingdom of Saudi Arabia.

18. Dhanwal D. Kumar P, Nehru R Clinical and investigation profile of patients with diabetic foot in North India.poster display, Diabetic foot; 18th International Diabetic Federation Conference.

19. Edelstein, Stratchounski L, I., A. Narezkina, M. Edelstein, M. PimkinIn vitro activity of cefoperazone/sulbactam vs. amoxicillin/clavulanic acid and piperacillin/tazobactam against extended spectrum b-lactamase (ESBL)-producing strains of Escherichia coli and Klebsiella pneumoniae

20. Edgar J.G. Peters and Lawrence A. Lavery; Effectiveness of the ***Diabetic Foot*** Risk Classification System of the International Working Group on the ***Diabetic Foot*** Diabetes Care, *2001; 24:1442-1447*

21. Edmonds M.E. and P.J.Watkins; Plantar neuropathic ulcer and charcot joints: risk factors, presentation and management; diabetic neuropathy: Dyke & Thomas; 2nd edition. , chapter 29; 398 to 405.

22. Edward J. Boyko and Benjamin A. Lipsky, Infection and Diabetes: Chapter *22;* http://diabetes.niddk.nih.gov/dm/pubs/america/pdf/chapter22.pdf .

23. Edward J. Boyko, Jessie H. Ahroni, Victoria Stenseluby C. Forsberg Denise R. Davignon, Douglas G. Smith, A Prospective Study of Risk Factors for Diabetic Foot Ulcer The Seattle Diabetic Foot Study Diabetes Care, 1999; 22:1036–1042.

24. Elmer W Koneman, Colour Atlas and Textbook of Diagnostic Microbiology, 5th edition, 1997: Lippincott Publications. Anaerobic jar techniques pg.725.

25. Eneroth M, Apelqvist J, Stenstrom A. Clinical characteristics and outcome in 223 diabetic patients with deep foot infections. Foot Ankle International 1997; 18(11):716-22.

26. Faiz-ur-Rehman, Shahnaz Nadir, Seema Noor Department of Medicine, Khyber Teaching Hospital, Peshawar.Diabetic Foot,2004 ;Vol 18 No 3 : Page

463 – 469.

27. Gargritu, Gulati V.L, Arora Usha; Department of Microbiology, Govt.Medical College Amritsar;Extended spectrum beta lactamase production by Pseudomonas aeruginosa isolated from surgical wound infections and its resistance pattern. Poster presentation in IAMM Microcon 2005.

28. Gayle E. Reiber, Loretta Vileikyte, Edward J. Boyko, Michael Del Aguila, Douglas G. Smith, Lawrence A. Lavery, Andrew J.M. Boulton, Causal Pathways for Incident Lower- Extremity Ulcers in Patients With Diabetes From Two Settings Diabetes Care, January 1999 Volume 22, Number 1 .

29. Gayle E. Reiber, MPH, PhD; Edward J. Boyko, and Douglas G. Smith, Lower Extremity Foot Ulcers and Amputations in Diabetes chapter 18 **;**http://diabetes.niddk.nih.gov/dm/pubs/america/pdf/chapter18.pdf

30. Gerit Mulder, David Armstrong, Susie Seaman, Standard, Appropriate, and Advanced Care and Medical-Legal Considerations: Part One -- Diabetic Foot Ulcerations Wounds, 2003; 15(4):92-106.

31. Goldstein EJ, Citron DM and Nesbit CA, Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases of Diabetic foot infections. Diabetes Care, Vol 19, Issue 6 638-641.

32. Hartemann-Heurtier,J. Robert S. Jacqueminet G. Ha Vanj. L. GolmardV. JarlierA. Grimaldi, Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact; Diabetic Medicine, Journal of Diabetes UK Volume 21: Issue 7.

33. History of diabetes, Canadian Diabetic Association, www.diabetes.ca .

34. Hutchinson JJ, McGluckin M. Occlusive dressings: A microbiologic and clinical review. Am J Infect Control 1990; 18:257.

35. Jagdish Chander, Textbook of Medical Microbiology, 2nd edition; pg.222.

36. Jan Cuzzell Wound Assessment and Evaluation: Diabetic Ulcer Protocol Dermatology Nursing 15(2):153, 2003.

37. Jeffrey Muha, Local wound care in diabetic foot complications Aggressive risk management and ulcer treatment to avoid amputation; Postgraduate Medicine, July 1999: Vol 106 / No 1.

38. Jessie H. Ahroni, Edward J. Boyko, Ruby C. Forsberg; Clinical Correlates of Plantar Pressure among Diabetic Veterans; *Diabetes Care,* 1999: 22:965–972.

39. Kavitha.R.Dinesh,Shashikala Shivaprakasha,Rajesh.R,Usha Menon; Department of Microbiology and Endocrinology,Amrita Institute of Medical Sciences,Kochi,Kerala.Empiric regime in Diabetic foot infections.Are we making the right choice? Poster presentation in IAMM Microcon 2005.

40. Kenneth S. Thomson, Controversies about Extended spectrum and AmpC beta lactamases;Centre for Disease Control:Emerging infectious diseases:Mar-Apr 2001,Vol 7, No.2.

41. Kessler L, Piemont Y, Ortega F, Lesens O, Boeri C, Averous C, Meyer R, Hansmann Y, Christmann D, Gaudias J, Pinget M. Comparison of microbiological results of needle puncture vs. superficial swab in infected diabetic foot ulcer with osteomyelitis. Diabet Med. 2006 Jan; 23(1):99-102.

42. Zgonis T, Roukis TS. A systematic approach to diabetic foot infections**.** Adv Ther. 2005 May-Jun; 22(3):244-62.

43. Kevin W. Shea, Antimicrobial therapy for diabetic foot infections a practical approach; Postgraduate Medicine, July 1999: VOL 106: NO 1.

44. Kingsley A proactive approach to wound infection: Nursing Standard 2001:15; 30, 50-58.

45. Lavery LA, DC Lavery and TL Quebedeax-Farnham Increased foot pressures after great toe amputation in diabetes Diabetes Care, Vol 18, Issue 11 1460-1462.

46. Lavery LA,Armstrong DG,Harkles LB.Classification of diabetic foot wounds Foot ANKLE Surgery 1996;35:528-31.

47. Lavery Lawrence. A. David G. Armstrong, Steven A. Vela, Terri L. Quebedeaux, John G. Fleischli, Practical Criteria for Screening Patients at High Risk for Diabetic Foot Ulceration Arch Intern Med 1998;158:157-162.

48. Lipsky BA, Baker PD, Landon GC, et al. Antibiotic therapy for diabetic foot infections: comparison of two parenteral-to-oral regimens. Clin Infect Dis.

49. Lipsky BA, Pecoraro RE, Larson SA, et al. Outpatient management of uncomplicated lower-extremity infections in diabetic patients. Archive of Internal Medicine 1990; 150(4):790-7.

50. Lipsky BA, Pecoraro, Wheat LJ.The diabetic foot: soft tissue and bone infection. Infectious disease Clinics of North America, 1990; 4:409-32.

51. Lipsky, RE Pecoraro, MS Chen and TD Koepsell, Factors affecting staphylococcal colonization among NIDDM outpatients Diabetes Care, Vol 10, Issue 4 483-486.

52. Louie TJ, Bartlett JG, Tally FP, et al. Aerobic and anaerobic bacteria in diabetic foot ulcers. Ann Intern Med 1976; 85(4):461-3.

53. MacFarlane R, Jeffcoate WJ, Classification of diabetic foot ulcers; the SAD system; Diabetic Foot 1999;2:123-6.

54. Mackie and McCartney, Laboratory control of antimicrobial therapy, Chapter8; Practical Medical Microbiology, 14th edition 1996; 151-177.

55. Maria Fe P. Raymundo, and Myrna T. Mendoza,The Microbiologic Features and Clinical Outcome of Diabetic Foot Infections among Patients Admitted at UP-PGH*

56. Mario Cruciani, , Benjamin A. Lipsky, , Carlo Mengoli, and Fausto de Lalla, Are Granulocyte Colony–Stimulating Factors Beneficial in Treating Diabetic Foot Infections? Diabetes Care 28:454-460, 2005.

57. Morbach S,Lutale JK,Vishwanathan V,Mollenberg J,Rajashekar S,Ramachandran A,Abbas ZG.Regional differences in risk factors and clinical presentation of diabetic foot lesions.Diabet Med 2004 Jan; 21(1):91-5.

58. Morton M Schwartz.Diabetic foot and other chronic superficial skin ulcers,Skin and soft tissue infections,Mandell,Douglas and Bennett's Principles and Practice of Infectious diseases,5th edition; Chapter 78:1046.

59. Murdoch, D. A. Clinical Microbiology Reviews, January 1998, , Vol. 11, No. 1/ p. 81-120 0893-8512/98.

60. Nidal a Younis, Abla M Albsoul the DEPA scoring system and its correlation with the healing rate of diabetic foot ulcers. The journal of foot and ankle surgery, July 2004 Volume 43, Issue 4, Pages 209-213 .

61. Nirmal Joshi, Gregory M. Caputo, Michael R. Weitekamp, and A.W. Karchmer, Infections in Patients with Diabetes Mellitus New England Journal of Medicine, December 16, 1999: Number 25, Volume 341:1906-1912.

62. Pınar Zarakolu, Nilay Çöplü, Ahmet Arslantürk, Belkıs Levent, Engin Güvener; Comparison of Inhibitory and Bactericidal Activities of Cefoperazone + Sulbactam Against Pseudomonas aeruginosa Strains, from 1992 to 1994. Tr. J. of Medical Sciences 29 (1999) 607-609.

63. Raghavendra Rao M, Anuradha K, Vijayakumar G.S. Department of Microbiology, J.S.S Medical College, Mysore. Detection of ESBL among Enterobacteriaceae isolated from pus samples.Poster presentation in IAMM Microcon 2005.

64. Robert G. Frykberg, Des Moines University, Des Moines, Iowa

American Family Physicians, november1, 2002;66:no.9:1655-62.

65. Samson O. Oyibo, Edward B. Jude, Ibrahim Tarawneh, Hienvu C. Nguyen, Lawrence B. Harkless,and Andrew J.M. Boulton, A Comparison of Two Diabetic Foot Ulcer Classification Systems The Wagner and the University of Texas wound classification systems, Diabetes Care, 2001: 24:84-88.

66. Sapico FL, Witte JL, Canawati HN, et al. The infected foot of the diabetic patient: quantitative microbiology and analysis of clinical features. Rev Infect Dis 1984;6 Suppl 1:S171-6.

67. Sapico FL. Canawati HN. Witte JL. Montgomerie JZ. Wagner FW Jr. Quantitative aerobic and anaerobic bacteriology of infected diabetic feet Journal of Clinical Microbiology. 1980 Sep, 12(3):413-20.

68. Scher KS. Steele FJ. The septic foot in patients with diabetes. Surgery. [JC:vc3] 104(4):661-6, 1988 Oct.

69. Sharp CS. Bessman AN. Wagner FW Jr. Garland D. Microbiology of deep tissue in diabetic gangrene Diabetes Care. Sep-Oct 1978,1(5):289-92.

70. Sheila A. Ward, Diabetes, Exercise, and Foot Care Minimizing Risks in Patients Who Have Neuropathy The Physician And Sportsmedicine - August 2005 VOL 33 - NO. 8 - 49.

71. Singhal S,Mathur T, Khan S,Rattan A,Evaluation of methods for AmpC Beta lactamases in Gram Negative Clinical isolates from Tertiary Care

Hospitals. Indian Journal of Medical Microbiology,2005;Vol 23(2):120-124.

72. Styrt B and Gorhach SL," Recent Developments in the Understanding of the Pathogenesis and Treatment of Anaerobic Infections," N England Journal of Medicine, 1989; 321:240-6.

73. Tentolouris N, Petrikkos G, Vallianou N, Zachos C, Daikos GL, Tsapogas P, Markou G, Katsilambros N. Prevalence of methicillin-resistant Staphylococcus aureus in infected and uninfected diabetic foot ulcers. Clin Microbiol Infect. 2006 Feb;12(2):186-9.

74. Thanh L. Dinh and Aristidis Veves: International Textbook Of Diabetes Mellitus, Edited By R.A.Defronzo,E.Ferrannini,H.Keen,And P.Zimmet ,Third Dition;1315-1329.

75. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med. 1993 Sep 30;329(14):977-86.

76. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: Ukpds 38 ;Bmj, September 1998; Volume 317 12:703–13.

77. Uday Kelkar, Anju Kagal Bacteriology of diabetic ulcers: effect of sample collection method Diabetic Foot, The, Autumn, 2004.

78. Unachukwu CN, Obunge OK, Odia OJ.The bacteriology of diabetic foot

ulcers in Port Harcourt, Nigeria. Niger J Med. 2005 Apr-Jun;14(2):173-6.

79. Veves A, Murray H. J., Young M. J.and Boulton A. J. M. The risk of foot ulceration in diabetic patients with high foot pressure: a prospective study Diabetalogia vol 35 number 7 .July 1992;660-663.

80. Vishnevsky A.V, Svetukhin AM, Prokudina MV, Pavlova MV, Puchkova LS, Zemlianoi. Institute of Surgery, Russian Academy of Medical Sciences, Moscow.Microflora of purulent necrotic leisions of the foot in patients with diabetes mellitus. Antibiot Khimioter. 1995 Aug;40(8):40-2.

81. Wagner FW.The dysvascular foot ,a system for diagnosis and treatment.Foot Ankle 1981;2:64.

82 Wheat LJ, Allen SD, Henry M, et al. Diabetic foot infections: bacteriologic analysis. Arch Intern Med 1986;146(10):1935-40.

83. William C.Coleman ,Foot Care and lower extremity problems of diabetes mellitus;Management of Diabetes mellitus perspective of care across lifespan,Mosby.

84. Young Matthew J.Textbook Of Diabetes: 3rd Edition; Pickup and Williams; Blackwell Publishing; Chapter 57,Foot Problems In Diabetes 57.1 To 57.19.

85. Young Matthew J.Textbook Of Diabetes: 3rd Edition; Pickup and Williams; Blackwell Publishing; Chapter 1.The History Of Diabetes; 1.1

To 1.8.