EVALUATION OF SUPERFICIAL AND DEEP SPECIMENS FOR ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES FROM DIABETIC FOOT INFECTIONS

By

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Dissertation submitted to the
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In partial fulfilment of the requirements for the degree of

M.D IN MICROBIOLOGY

Under the guidance of

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Savitha.N

LIST OF ABBREVIATIONS

DFI	Diabetic Foot Infections
VRE	Vancomycin resistant Enterococci
MSSA	Methicillin sensitive staphylococcus aureus
MRSA	Methicillin resistant staphylococcus aureus
BH Streptococci	Beta hemolytic Streptococci
BBA	Brucella blood agar
AA	Anaerobic agar
ABA	Anaerobic basal broth
RCM	Robertson's cooked meat medium
MHA	Muller Hinton agar
IDSA	The Infectious Diseases Society of America's foot infection classification system
IWGDFC	International working group on Diabetic foot Classification
CLSI	Clinical and Laboratory Standards Institute

ABSTRACT

EVALUATION OF BACTERIAL ISOLATES FROM SUPERFICIAL AND DEEP SPECIMENS FROM DIABETIC FOOT INFECTIONS

Introduction:

Diabetes is a worldwide problem affecting millions of people. Diabetic foot infection is a major complication of diabetes often warranting amputation. The identification of specific etiologic agents responsible for Diabetic foot infections helps in alleviating the infection by institution of appropriate antimicrobial therapy. This may sometimes be lifesaving and also may avoid amputation there by giving the patient a better quality of life. Often superficial swab specimens are collected to isolate the pathogen. This may show only the superficial contaminants with treatment failure. Hence appropriate deeper tissues, Pus or curettage specimen should be collected to isolate the actual pathogens and to institute appropriate therapy there by reducing morbidity and mortality.

Objectives:

1.To isolate the specific bacterial pathogens causing the diabetic foot infections

2.To compare the bacterial isolates of superficial swab and punch biopsy/pus specimens in diabetic foot infections.

3.To evaluate and assess the antimicrobial sensitivity pattern of the infecting and colonizing organisms from same patients.

4.To help the treating consultant to choose an appropriate antibiotic and to assess the response.

Material and methods:

2 superficial specimens and 4 to 5 deep tissue bits were simultaneously collected from 50 patients. Gram stained smear was studied on one of the samples from superficial and

deep samples. The other samples were inoculated on to anaerobic media in gas pak jar and incubated at 37^0c for 48 hrs. The samples were also inoculated for aerobic study. The colonies were processed according to standard methods and antibiotic susceptibility of the identified aerobic organisms was performed according to the CLSI guidelines. The anerobes were identified by using the Anident discs (Oxoid, USA). The isolated organisms from the superficial and deep samples were compared and the sensitivity of the deep samples were given to the patient and the treatment altered accordingly and reponse of the patient was followed up.

Results: In superficial swab specimens, a total of 89 organisms were isolated whereas in deep tissue biopsies 259 organisms were isolated including anaerobes. In this study in deep tissue samples out of a total of 259 organisms isolated, aerobes were 135 and anaerobes were 124. Superficial swabs are not useful for anaerobic cultures. Deep tissue specimen show the actual infecting organisms and the treatment should be based on these. DFIs usually are polymicrobial infections and many times multi drug resistant organisms are isolated. This study also highlights the importance of anaerobes in DFIs.

Conclusion:

Collection of superficial swabs for the diagnosis of DFIs should not be undertaken. Deep tissue samples are better indicators of infection. Appropriate antibiotic therapy instituted promptly will save further complications and sometimes amputations also. Good glycemic control is also very important in controlling the infections along with antibiotic therapy.

Keywords: Diabetic foot infections, Deep tissue biopsy, Microorganisms of DFIs, Antibiotic therapy in DFIs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

1.Introduction:

The lifetime risk of a person with diabetes developing foot ulceration is reported to be as high as 25% . They cause substantial morbidity and frequent visits to health care professionals and may lead to amputation of a lower extremity.[1]It is estimated that more than a million people with diabetes require limb amputation each year, suggesting that one major amputation is performed Worldwide every 30 seconds[1].

The most feared and costly complication of diabetic foot disease is **Amputation,**[64]which occurs 10-30 times more often in diabetics than in general population.[9,10] Diabetes accounts for up to 80% of non-traumatic amputations, with 85% of these being preceded by foot ulcer.[11] Amputation carries with it a significantly elevated mortality at follow-up, ranging from 13% to 40% at 1 year to 39-80% at 5 years.[12]

Amputationis associated with significant morbidity and mortality, besides having immense social, psychological and financial consequences.[1]Infections in patients with diabetes are difficult to treat because these individuals have impaired micro vascular circulation, which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues.[13,14]

In general, foot infections in persons with diabetes become more severe and take longer to cure than similar infections do in persons without diabetes.[2]Once the protective layer of skin is breached, underlying tissues are exposed to bacterial colonization. This wound may progress to become actively infected, and by contiguous extension, the infection can involve deeper tissues. This sequence of events can be rapid, even hours or may occur over days, especially in an ischemic limb.

Various poorly characterized immunologic disturbances, especially those that involve polymorphonuclear leukocytes, may affect diabetic patients, and these are likely to increase the risk and severity of foot infections.[4]

Likelihood of presence of a particular pathogen in diabetic foot infection is dependent on factors like chronicity of the wound, depth of the wound, necrosis and prior antimicrobial therapy.[3]Wound cultures may suggest but do not prove the presence of infection, as all open chronic wounds are covered by colonizing flora.

Appropriate and adequate antibiotic treatment requires antibiotic susceptibility testing to be performed on cultures from the wound. However the accuracy of bacteriological results depends, on obtaining an appropriate sample/specimen from clinically infected patients.[3]

Often in most hospitals the collection of sample from diabetic foot ulcer is a superficial swab specimen, the deeper tissue is generally collected only when osteomyelitis is suspected.

The superficial swabs mostly yield surface contaminants which may not be actual pathogens. The deeper tissues actually harbour the real pathogens. Deep tissue cultures obtained by punch biopsy, ulcer curettage, or aspiration of pus, is reported to provide the most reliable bacteriologic information which reflect the actual pathogens in DFIs.[5] These need to be studied and reported to the treating clinician so that appropriate antimicrobial therapy could be instituted to the patient to prevent further complications.

Kolar being a rural area with plenty of diabetic patients with foot ulcers, a study of the microorganisms infecting the foot would help the attending clinician in treating the cases.

A comparative study of the clinical specimens of the superficial swabs and biopsy/pus aspiration would exclude the environmental contaminants and will help in the isolation of the infecting pathogen in the deeper tissues.This to a great extent will help in appropriate use of antibiotics, targeting thespecific pathogen instead of their indiscriminate use to treat the surface contaminants or the colonizers. This also is crucial in reduction in selection of multi drug resistant mutants.

2. Objectives of the study:

1. To isolate the specific bacterial pathogens causing the diabetic foot infections

2. To compare the bacterial isolates of superficial swab and punch biopsy/pus specimens in diabetic foot infections.

3. To evaluate and assess the antimicrobial sensitivity pattern of the infecting and colonizing organisms from same patients.

4. To help the treating consultant to choose an appropriate antibiotic and to assess the response.

3.REVIEW OF LITERATURE:

3.1: History of diabetes and diabetic foot

Diabetes is one of the oldest diseases known to mankind. The history of diabetes has its beginning in antiquity. This disease has apparently plagued man for a very long time, since the writings from the earliest civilizations (Asia Minor, China, Egypt, and India) refer to boils and infections, excessive thirst, loss of weight, and the passing of large quantities of a honeysweet urine which often drew ants and flies.[6]

There is a reference in the Ebers Papyrus(dating back to 1500 BC and discovered by the Egyptologist Georg Ebers in Thebes in 1872). This recommended that those afflicted with the malady go on a diet of fruits, grains, and honey, which was reputed to stifle the excessive urination.[6]Indian writings from the same era attributed the disease to overindulgence in food and drink. Other later Egyptian medical papyri [Hearst and Berlin papyrus] also give recipes against polyuria. [6]

The first known clinical description of diabetes appears to have been made by Aulus Cornelius Celsus (c.30BC - 50AD), but Aretaeus of Cappadocia(2nd century AD) provided a detailed and accurate account and introduced the name 'diabetes' from the Greek word for 'siphon'.[6]The Hindu physicians, Charak and Sushrut, who wrote between 400 and 500 BC, were probably the first to recognize the sweetness of diabetic urine Indeed, the diagnosis was made by tasting the urine or noting that ants congregated round it.[7]

However, the history of gangrene of the foot goes back to Biblical time, when, in Chronicles II, the first case of gangrene of the feet, perhaps due to diabetes, is described.[7]

The relationship between diabetic neuropathy, the insensitive foot, and foot ulceration was recognized by Pryce, a British surgeon, over a century ago. He stated that, "It was abundantly evident that the actual cause of the perforating ulcer was peripheral nerve degeneration and that diabetes itself played an active part in the causation of the perforating ulcer" [7]

3.2: Structure of foot

The foot is truly a mechanical marvel in humans, the only two legged mammal and consists of 29 joints (8 major and 21 minor), 26 bones and 42 muscles, forming the functioning foot unit.[8]A meta-tarsal bone is about the diameter of a pencil-an individual meta-tarsal shaft can be snapped into half by the bare hands.

The anatomical and functional provisions for keeping the foot undamaged would still be inadequate, were it not for one more important factor, namely the sensory feed-back. The skin of the dorsum of the foot is totally different in structure from the skin of the sole of the foot. [8]

The skin of the sole of the foot has the highest thickness of keratin. On the soles, thick calluses act as foreign bodies.In the foot the tendons, the vessels and nerves are all so tightly packed, that once they are released they cannot be easily put back into their appropriate places.The plantar skin is 4 to 5 mm thick with the thickest area covering the heal and the distal meta-tarsals. It is richly innervated. It has no hair follicles or sebaceous glands but has numerous sweat glands.[8]

The foot does not grow very much along with the body growth. Adult foot size remains constant except in some rare instances, like acromegaly and local gigantism. Although the

thighs and legs can share the 'obesity design', increase in shoe size does not occur after certain age. While standing, the body weight is transmitted through the tibia to the talus and then distributed to the calcanium. [8]

The Talo-Navicular joint is the first and most vulnerable joint involved in the 'Diabetic Foot Syndrome'. One needs to assess neuropathic, vascular, infective & mechanical aspects. Of these four elements, the neuropathy is the 'starter' and others are 'chasers'.[8]

The feet that can sweat normally, rarely get ulcerated. Neuroarthropathy of the foot in diabetes is clinically silent since primarily it is caused by lack of sensation in the foot. Veins generally do not undergo atherosclerosis probably due to increased prostaglandin content (8 times more) in the vessel wall. The extensor tendons are not encased in sheaths but lie loose in areolar tissue on the dorsum of the foot, unlike the plantar tendons on the sole.

There is no doubt that 'foot care' is even more important than 'facial care' in the diabetics.
The progression of an ischaemic pain is often called rest pain. 'Foot angina' is a familiar name in intermittent claudication. Walking on a thick callus may be compared to walking on a stone in a shoe. The insensitive foot does not detect the hard pressure point. For every million diabetics there are ten million toes that are potentially troublesome.[8]

3.3: Diabetic foot

Diabetic foot is one problem which cannot be studied in experimental animals (as compared to retinopathy, neuropathy and nephropathy) under any circumstances since diabetic foot syndrome cannot be spontaneously or experimentally reproduced for study purpose in animals.[8]

Diabetes by virtues of its other complications like neuropathy and vasculopathy and other factors alter the musculoskeletal and soft tissue mechanics in a manner that elevates plantar pressure and makes tissue damage more likely, causing non resolving neuro-ischemic ulcers at the weight bearing sites. This is why most of the skin injuries in diabetics are seen on the planter surface, frequently at the site of highest pressure under the foot.Local trauma and/or pressure often in association with lack of sensation because of neuropathy, in addition to micro vascular disease, may result in various diabetic foot infections that run the spectrum from simple, superficial cellulitis to chronic osteomyelitis.[2]

3.4: Epidemiology of diabetic foot

Foot lesions are perhaps the most common mismanaged problem in patients with type 1 and 2 diabetes, which constitute 15% both in Europe and in United states diabetic population. [15]

In a study in Germany, 34% of diabetic foot lesions were due to neuropathic, 21% to ischaemic, and 40% to combined neuropathic and ischemic lesions.[15]WHO predicts that developing countries will bear the brunt of this epidemic in the 21st century. Currently, more than 70% of people with diabetes live in low- and middle income countries.[15]

Diabetic foot is one of the most devastating complications and leads to suffering, disability, loss of time from work, hospitalisation and great expense to both the patient and the community. There are few data on the prevalence of diabetic foot problems, even in developed countries. Diabetic foot complications are more frequent in males and individuals aged over 60 years. Based on recent studies, the annual incidence for diabetic foot ulcers is 1-4%, with a prevalence of 4-10%. In India, the prevalence of diabetic foot ulcers in the clinic population is 3.6%.[15]

An estimated 285 million people, corresponding to 6.4% of the world's adult population, are living with diabetes in 2010. The number is expected to grow to 438 million by 2030, corresponding to 7.8% of the adult population. [15]

70% of the current cases of diabetes occur in low- and middle income countries. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetes population, followed by China with 43.2 million. [15]

The largest age group currently affected by diabetes is between 40-59 years. By 2030 this 'record' is expected to move to the 60-79 age group with 196 million cases.[15]

3.5: Indian scenario

The prevalence of diabetes has reached epidemic proportions. Diabetes in India has long passed the stage of an epidemic and numbers have given the country the dubious distinction of **diabetes capital** of the world.[16,17]India is a home of nearly 33 million diabetics, which is highest in the world out of which; nearly 15% suffer from the dreaded sequel of diabetic foot. The fact that India has more people with Diabetes than any other country and incidence of foot problems and amputations remaining high, accounting for up to 20% of diabetes related hospital admissions is alarming.[19,20]

Sociocultural practices such as barefoot walking, religious practices like walking on fire, use of improper footwear and lack of knowledge regarding foot-care attributes towards increase in the prevalence of foot complications in India.[61,62] A retrospective study to evaluate the clinical profile of diabetic foot infection showed that the recurrence of foot infection was common among South Indian type 2 diabetic patients and was related to the presence of Peripheral Vascular Disease and neuropathy. [69]There is a need for improvement in footwear and foot care education.[19,20]

In a study from Southern India, it was found that patients without foot problems spent 9.3% of the total income, while patients with foot problem had to spend 32.3% of the total income towards treatment.This huge challenge imposed by diabetic foot problem calls for prevention and effective management at initial stages of complication.In India, the choice of empirical

antimicrobials is extrapolated from data available from western countries, which may or may not be appropriate for Indian patients.[21,22]

A majority of patients who enter the hospital because of diabetic foot infection is simply defined as suspected or documented infection of the tissues that comprise the foot of a diabetic patient.

Diabetic foot infection is often caused by introduction of an infection into the otherwise sterile soft tissues of the foot through a minor skin break down. They may be mild usually restricted to the uppermost layers of the skin, moderate extending down to the soft tissues of the foot or severe infection associated with systemic toxicity or metabolic instability.

The diagnosis of DFI should be suspected at an early stage based on the presence of local signs of inflammation with or without systemic signs of toxicity or metabolic instability such as development of swelling, skin discoloration, pain, discharge, or ulceration in patients presenting with systemic signs such as fever, malaise or poor glycemic control even if the local signs are less severe than might be expected.

DFI s are usually associated with prolonged hospital stay, high financial costs and can cause long term morbidity and even mortality.

The management of these patients requires a likeminded, multi-disciplinary team strategy for medical stabilization and infection control via adequate surgical debridement, accurate identification of pathogens, antibiotic selection and delayed reconstruction to achieve functional limb salvage.[23]

Many physicians believe that culturing a diabetic foot wound is not useful since it does not give useful results.[3] Only a proper culture specimen can give useful culture results. Good tissue specimen can be obtained after debriding the wound. If an abscess/ pus is present, aspiration of the pus with aseptic techniques can be done. Dermal curette or scalpel blade can be used for debriding the wound and to get tissue specimen. The tissue obtained has to be transferred to a sterile container and sent to the microbiology lab. [3]

Inappropriate method of obtaining culture specimens is commonly observed in hospitals.
To avoid the isolation of colonizing (rather than pathogenic) flora, the investigators were instructed to first clean and debride all foot wounds and to obtain specimens by tissue biopsy, wound curettage, or aspiration rather than swab techniques.[22]

Collecting specimen with a cotton swab has following disadvantages:[3]

1. The pathogens responsible for infection live and colonize underneath the Escher and the cotton swab cannot reach deep into the wound.

2 .Since there is air inside the cotton swab, anaerobic and fastidious organisms do not survive in cotton swab and microbiology report may be negative for these organisms.[3]

Obtaining tissue specimen from Diabetic patients is a painless procedure since they have sensory neuropathy. A 4-mm punch biopsies which do not inhibit the wound healing is a useful procedure.[3]

Wound cultures may suggest but do not prove the presence of infection, as all open chronic wounds are covered by colonizing flora. Most of diabetic foot infections are poly-microbial in nature and mixed organisms are frequently encountered. The spectrum of microorganisms

depends mainly on microbial flora of the lower limb, metabolic factors, foot hygiene and use of antibiotics.

Unless strict criteria are implemented for diagnosis of DFIs, overestimation may be of major concern, leading to misuse of anti-microbial agents with potential adverse effects and possible development of antibiotic resistance, as well as wasting money. In order to select appropriate antibiotic therapy it is important to know the microbiology of DFI.

3.6: Microbiology of DFIs

A bacteriological evaluation of diabetic foot ulcer infections is necessary to identify those agents that contribute to degeneration and deterioration of these lesions.An understanding of the bacteriology of DFI is also important in guiding antibiotic selection and correlating culture results with appropriate definitive therapy that will assist health care professionals to manage diabetic patients and prevent the loss of the lower extremity. [55,56,57]

While Staphylococcus aureus and beta-hemolytic streptococci are widely recognized as pathogens in early DFIs, the role of other frequently isolated organisms is less clear to both the clinician and the microbiology laboratory.

Some studies suggest that the interactions of organisms within these polymicrobial mixtures lead to the production of virulence factors, such as hemolysins, proteases, and collagenases, as well as short chain fatty acids, that cause inflammation, impede wound healing, and contribute to the chronicity of the infection.[22]

In such mixtures, biofilms that impede the penetration of antimicrobial agents into the infected site may also form. Thus the presence of multiple species can have important clinical implications that should not be overlooked.[22,23,24]

If the patient has no history of treatment for acute onset infections, the infection is almost always caused by gram positive cocci – Streptococci and Staphylococci. If the wound is chronic or if patient has had prior antimicrobial therapy, then gram negative rods are often observed**.** [25,26,27]

If the patients present late, having taken `home remedies' or over the counter antibiotics, mixed flora with an average of 5 or 6 different microorganisms is likely to be seen in culture results, most probably because of the selection of the resistant mutants.

Aerobic gram positive cocci are the predominant microorganisms that colonize and acutely infect breaks in the skin. S.aureus and the beta-hemolytic streptococci (especially group A,B,C & G) are the most commonly isolated pathogens. [58,59,66]

Chronic wounds develop a more complex colonizing flora, including Enterococci, various Enterobacteiaceae, obligate anaerobes Pseudomonas aeruginosa, and, sometimes, other non-fermentative gram negative organisms.

Hospitalization, surgical procedures, and, especially, prolonged or broad spectrum antibiotic therapy may predispose patients to colonization and /or infection with antibiotic resistant organisms (e.g., MRSA or VRE)[31,32] Although MRSA strains have previously been isolated

mainly from hospitalized patients, community associated cases are now becoming common and are associated with worse outcomes in patients with DFIs.[32,32]

Vancomycin(or glycopeptide) intermediate Staphylococcus aureus has been isolated in several countries. Of note, the first 2 reported cases of Vancomycin resistant Staphylococcus .aureus each involved a diabetic patient with a foot infection.[4,31,32] The impaired host defences around necrotic soft tissue or bone may allow low virulence colonizers, such as coagulase negative Staphylococci and Corynebacterium species (diphtheroids), to assume a pathogenic role.[63,65]

Acute infections in patients who have not recently recieved antimicrobials are often monomicrobial (almost always with an aerobic gram positive coccus), whereas chronic infections are often poly microbial, yield 3-5 isolates, including gram positive and gram negative aerobes and anaerobes.[2,4,16,21]

Some of the better DFI studies have been conducted in India.[2,16,21] The microbiology of DFI in India is different from that in western countries where gram positive infections are common.[2]Gram negative pathogens such as Pseudomonas are more common in southern warmer climates. It is probably related to warm climate and wearing of footwear which absorbs perspiration.[2]

Pseudomonas can live in footwear and can enter the wound through a break in the skin.

Anaerobes are likely to grow if the patient has ischaemic limbs and necrotic tissue. The role of anaerobes is particularly unclear, because in many studies specimens were not collected or

cultured properly to recover these organisms. Some report that anaerobes play a minimal role, while others suggest that Bacteroides fragilis is the predominant anaerobe isolated.[22,33,34,35]

These discrepancies could be partly due to differences in the causative organisms occurring over time, geographical variations, or types and severity of infection included in the studies. Also, laboratory processing of the samples may have been inadequate to grow anaerobes or fastidious organisms, and protocols that classify potential pathogens (e.g., coagulase-negative staphylococci [CoNS] or Corynebacterium species) as colonizers may have been used.[22]

Antibiotic resistance in aerobic bacteria is of global concern; however, antibiotic resistance in anaerobes is often overlooked. With reports of resistance to anaerobic microbials, and variable antimicrobial resistance amongst anaerobic genera, continued surveillance of anaerobic susceptibility patterns is vital to determine current and future trends.[36,37,38,39]However this may be difficult in many diagnostic laboratories where anaerobic cultures are routinely not done.[22,36,37,38,39]

The clinical characteristics of patients with definite anaerobic foot infections do not differ significantly from those presented by patients without anaerobic infection, except that more patients with Wagner V infections had anaerobes. Thus, a high index of suspicion should be practised by the physician, especially in cases of DFIs classified under Wagner IV and Wagner V.[40]

Diabetic foot ulcers were graded using Wagner's classification prior to 1999 and by the University of Texas classification after 1999.[41,42]

Wagner's classification:[41,42,43]

Grade 0: High risk of foot and no ulcer.

Grade I: Superficial ulcer involving the full skin thickness but not underlying tissues.

Grade II: Deep ulcer, penetrating down to ligaments and muscle, but no bone involvement or abscess formation.

Grade III: Deep ulcer with cellulitis or abscess formation, often with osteomyelitis.

Grade IV: Localized gangrene..

Grade V: Extensive gangrene involving the whole foot.

University of Texas Wound Classification System of Diabetic Foot Ulcers

Grade I-A: non-infected, non-ischemic superficial ulceration

Grade I-B: infected, non-ischemic superficial ulceration

Grade I-C: ischemic, non-infected superficial ulceration

Grade I-D: ischemic and infected superficial ulceration

Grade II-A: non-infected, non-ischemic ulcer that penetrates to capsule or bone

Grade II-B: infected, non-ischemic ulcer that penetrates to capsule or bone

Grade II-C: ischemic, non-infected ulcer that penetrates to capsule or bone

Grade II-D: ischemic and infected ulcer that penetrates to capsule or bone

Grade III-A: non-infected, non-ischemic ulcer that penetrates to bone or a deep abscess

Grade III-B: infected, non-ischemic ulcer that penetrates to bone or a deep abscess

Grade III-C: ischemic, non-infected ulcer that penetrates to bone or a deep abscess

Grade III-D: ischemic and infected ulcer that penetrates to bone or a deep abscess

International working group on DFI (IWGDF) developed a classification scheme with the acronym PEDIS, the latin word for foot (P-perfusion, E-extentof size, D-death or tissue loss,I-infection and S-sensation or neuropathy). each subcategory is defined according to strict criteria, which are applicable worldwide. [2,44]

The international working group classified infections into 4 grades, from no signs of infection to systemic inflammatory response. The IDSA (The Infectious Diseases Society of America's foot infection classification system) classified the wound as uninfected, mildly infected, moderately infected and severely infected.[2,45,67,70]

IWGDF -PEDIS Clinical classification of DFI. [2,44]

Grade I: No purulence, inflammation, erythema, pain, warmth, tenderness
(uninfected wound) or induration

Grade II: Erythema < 2 cms, infection not deeper than subcutaneous tissue (mild infection)

Grade III: Presence of one sign or symptom of inflammation.
(moderate infection) Inflammation >2cms, spread deep into fascia, muscle joint/bone

GradeIV: Patients with systemic toxicity with continuous fever,
(severe infection) leucocytosis, chills, severe metabolic abnormalities

4. MATERIALS AND METHODS:

This is a prospective study carried out at R.L Jalappa Hospital & Research Centre, attached to Sri DevrajUrs Medical College, Tamaka, Kolar, between February 2010 to march 2011.

A total of 50 patients were studied with informed consent.The specimens included superficial wound swabs, punch biopsy tissues & aspirated pus. The inclusion criteria were patients with clinically diagnosed infected diabetic foot with ulcer/wound/osteomyelitis or previous amputated stump reinfected. The diabetic foot ulcers were graded using IWGDF - PEDIS classification, Grade III & Grade IV were included.

Two surface swab specimens and 4 to5 bits of deep tissue samples from punch biopsy were simultaneously obtained from each foot ulcer (Ref:flow chart below).Tissue samples were immediately smeared on to the plating media and inoculated in to the liquid media. Grinding of the tissue samples yielded more contaminants in the pilot study and therefore avoided.

One of the samples from superficial swab & deep tissue were inoculated on to Brucella blood agar, Anaerobic Hi veg agar & Anaerobic basal broth(Himedia laboratories), Robertson's cooked meat broth and incubated at 35^0c in a gaspak jar for 5-7 days for anaerobic study. The deep tissue samples were also inoculated on to Blood agar, Mac Conkey's agar and Thioglycollate medium for aerobic study.

The 2^{nd} sample from superficial swab & deep tissue were subjected to Gram's stain. Colonies on the Blood agar, Mac conkey's agar were processed & monitored daily according to standard methods and Thioglycollate broth was subcultured on to Blood agar and Mac conkey's agar. Bacterial colonies appearing on these plates were then studied and categorized as cocci and rods. Cocci that fermented Mannitol were considered Staphylococci and

confirmed as Staphylococcus aureus by the isolate's ability to produce coagulase both on slide and test tubes using human pooled plasma. Those Staphylococci that did not produce coagulase were deemed coagulase negative (CONS). Rods that grew on Mac conkey's agar plates were categorized as lactose fermenters & non lactose fermenters. Each colony of bacteria was further tested on conventional media, such as Citrate, Urea, Triple sugar iron agar, Lysine iron agar, Mannitol motility medium and peptone water for Indole.Antibiotic susceptibility of the identified organism was carried out according to the CLSI guidelines. [46]

Four to five well isolated colonies of 18- 24 h agar plate of the same morphological type were selected by touching the tip of each colony with a wire loop and transferring them to a tube containing 4-5 ml of peptone water. Such tubes was then incubated at 35^0C for 2-5 hrs to produce moderately cloudy suspension that was standardized by visually equivalent to the McFarland standard 0.5 (a turbidity standard prepared by adding 0.5 ml of 1% Barium chloride solution to 99.5 of 1% Sulphuric acid) This equates to approximately 10^8organisms per milliliter.[46]

A sterile cotton tipped applicator was dipped onto the adjusted suspension and inoculated onto a dried Mueller–Hinton agar (MHA) plate by streaking the swab over the entire agar surface.

The antibiotic discs used for **GRAM POSITIVE COCCI**:

Staphylococcus : Penicillin(P)30µg, Ampicillin(A)10µg, Cefoxitin(CN)30µg (To look for Methicillin resistance), Vancomycin(Va)30µg, Gentamicin(G)10µg, Erythromycin(E)15µg,Tetracycline(T), Chloramphenicol(C)30µg, Clindamycin(C)2µg, Linezolid(LZ)30µg, Trimethoprim/sulfamethoxazole (tmp/smx) 25µg, Ciprofloxacin(Cf)5µg

Enterococcus: Penicillin(P)30µg, Ampicillin(A)10µg, Vancomycin(Va)30µg, Linezolid(LZ)30µg (High level gentamicin resistance not measured as discs were not available).

B- H Streptococcus: Penicillin(P)30µg, Ampicillin(A)10µg, Tetracycline(T), Chloramphenicol(C)30µg, Gentamicin(G)10µg, Vancomycin(Va)30µg, Linezolid(LZ)30µg.

Antibiotic discs used for **GRAM NEGATIVE BACILLI**

Family Enterobacteriaceae : Ampicillin(A)10µg,Amoxyclav(AC)30µg, Piperacillin(P)100µg,Piperacillin-tazobactam(PT)100µg,Cephalothin(CH)30µg, Cefoxitin(Cn)30µg, Cefuroxime(Cu)30µg, Cefotaxime(Ce)30µg, Ceftriaxone(Ci)30µg, Ceftazidime(Ca)30µg, Aztreonam(Ao), Imipenem(I)10µg, Meropenem(Mr)10µg, Gentamicin(G)10µg, Amikacin(Ak)30µg, Tobramycin(Tb)10µg, Ciprofloxacin(Cf)5µg, Levofloxacin(Le)5µg, Tetracycline(T)30µg, Trimethoprim-sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,

Pseudomonas aeruginosa : Piperacillin(P)100µg, Piperacillin-tazobactam(PT)100µg, Ceftazidime(Ca)30µg, Cefepime(Cpm)30µg, Aztreonam(Ao), Imipenem(I)10µg, Meropenem(Mr)10µg, Gentamicin(G)10µg, Amikacin(Ak)30µg,Tobramycin(Tb)10µg,Ciprofloxacin(Cf)5µg, Levofloxacin(Le)5µg, Tetracycline(T)30µg, Trimethoprim-sulfamethoxazole (TMP/SMX)25µg, Polymyxin-B(300U), Colistin(10µg).

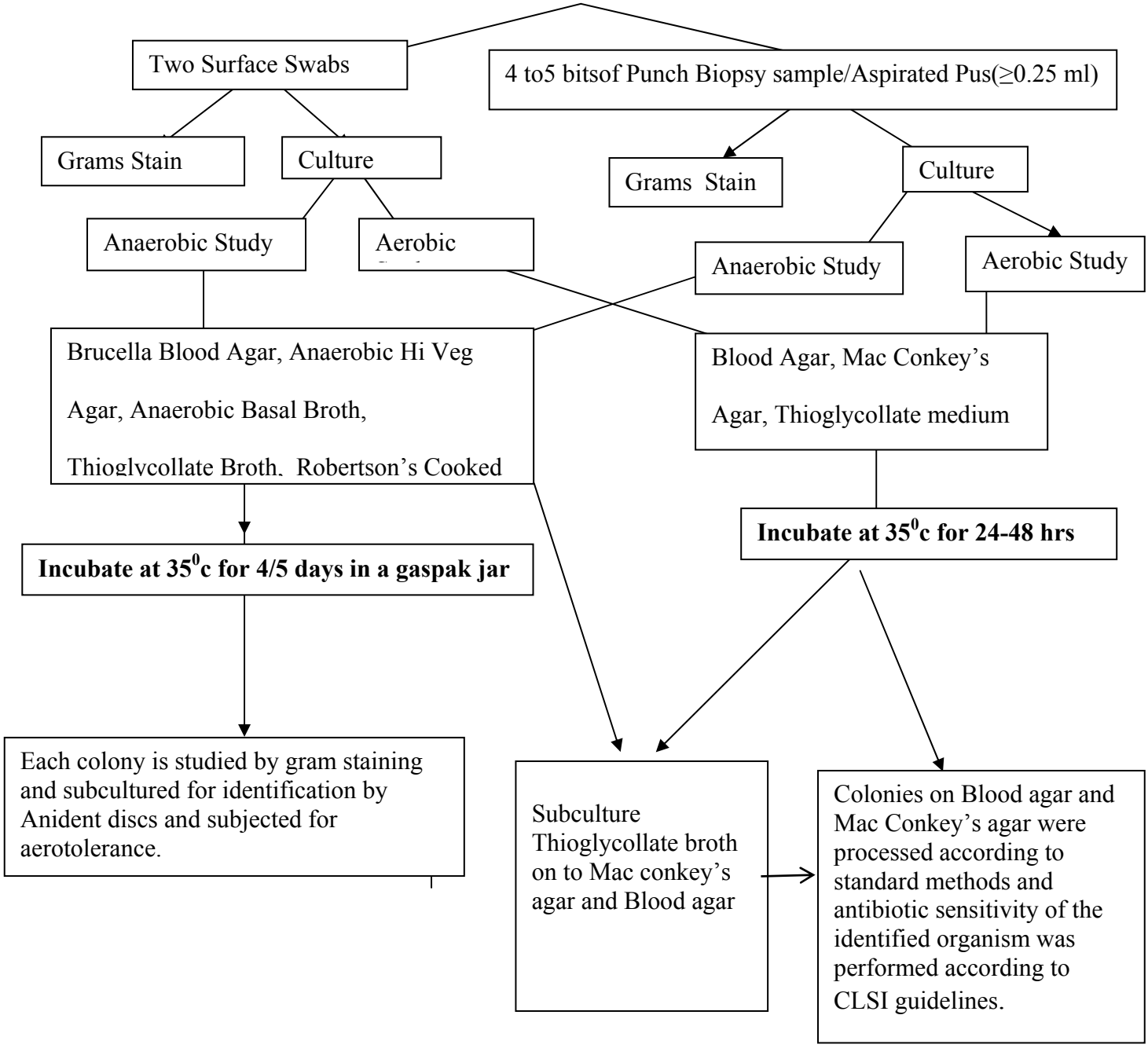
Acinetobacter: Piperacillin(P)100µg, Piperacillin-tazobactam(PT)100µg, Ceftazidime(Ca)30µg, Cefepime(Cpm)30µg, Aztreonam(AO), Imipenem(I)10µg, Meropenem(Mr)10µg, Gentamicin(G)10µg, Amikacin(Ak)30µg,Tobramycin(Tb)10µg, Ciprofloxacin(Cf)5µg, Levofloxacin(Le)5µg, Tetracycline(T)30µg, Trimethoprim-sulfamethoxazole (TMP/SMX)25µg, Polymyxin-B(300U), Colistin(10µg).

The plate was then inverted and placed in the incubator at 37^0C for 24 hrs and thereafter examined. The diameter of growth inhibition was then measured with a transparent ruler and recorded. The zone of inhibition was interpreted by referring to manufacturer's provided standard table and the isolate was scored susceptible or resistant.Staphylococcusaureus ATCC 25923 was employed as a control organism.

Anaerobes were identified using the Anident discs (Oxoid, USA).

FLOW CHART SHOWING THE METHOD FOLLOWED

SPECIMENS COLLECTED

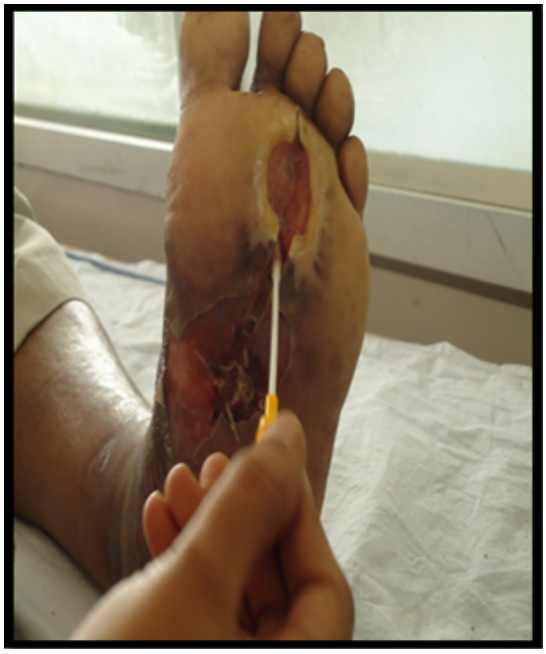


Identification Of Anaerobes

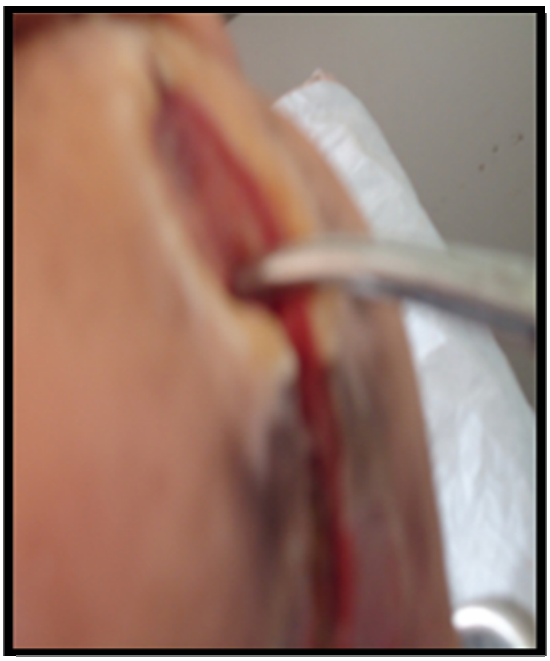
Anaerobes were identified using the following chart:

Bacteria	Erythromycin 60µg	Rifampicin 15µg	Colistin 10µg	Penicillin 2U	Kanamycin 1000µg	Vancomycin 5µg
Bacteroides fragilis	S	S	R	R	R	R
Prevotella melaninogenica	S	S	V	S	R	R
Bacteroides oralis	S	S	S	S	R	R
Bacteroides ureolyticus	S	S	S	S	S	R
Fusobacterium species	R	R	S	S	S	R
Gram positive cocci	S	S	R	S	S	S
Gram negative cocci	S	S	S	S	S	R
S=Sensitive, R=Resistant, V=Variable						

Photos



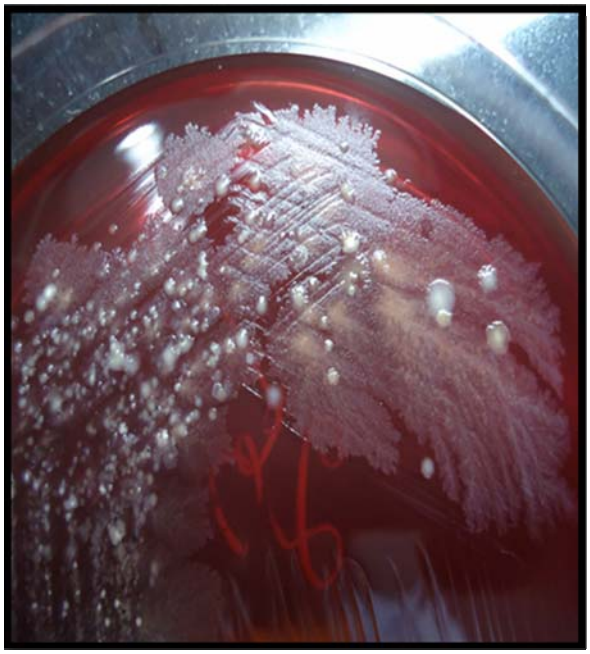
Picture 1:Superficial swab sampling



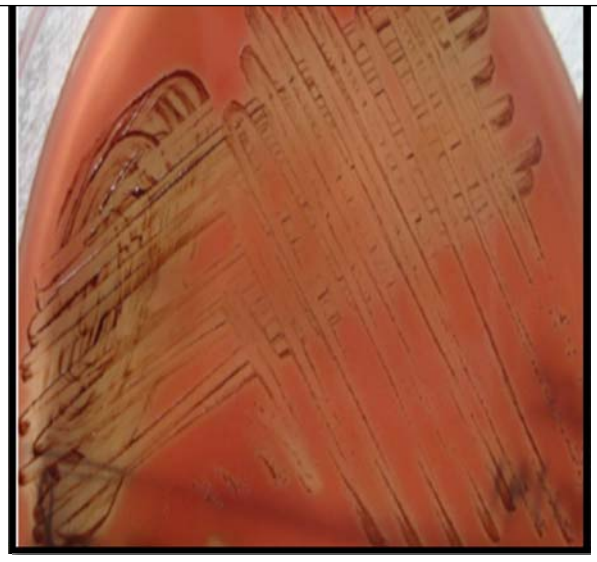
Picture 2:Deep tissue sampling

Picture 3:Gas pak anaerobic jar

Picture 4: Anident discs (Oxoid, USA)

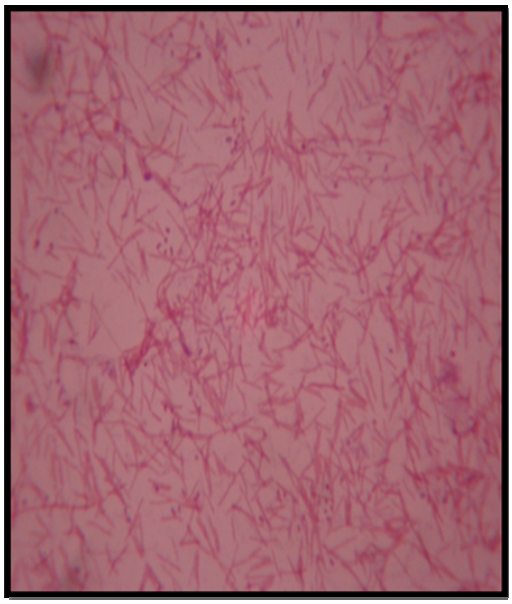


Picture 5:Brucella Blood agar **Picture 6:Anaerobic hi veg**

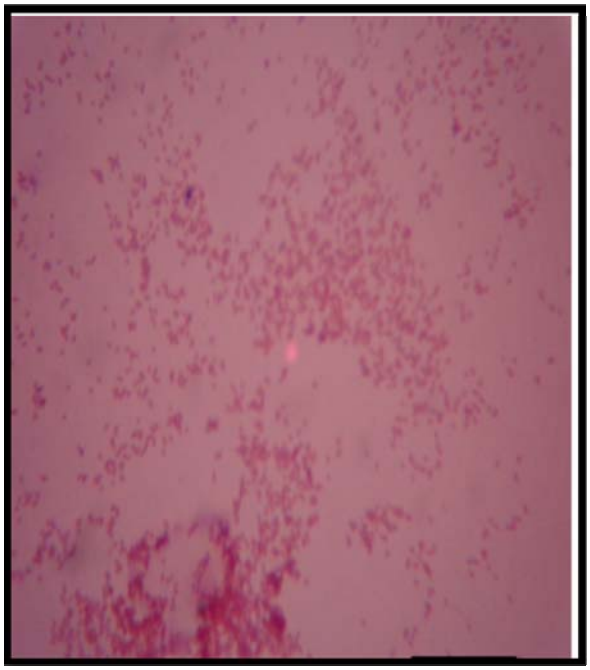


Picture 7: Swarming Clostridial colony With Prevotella melaninogenica

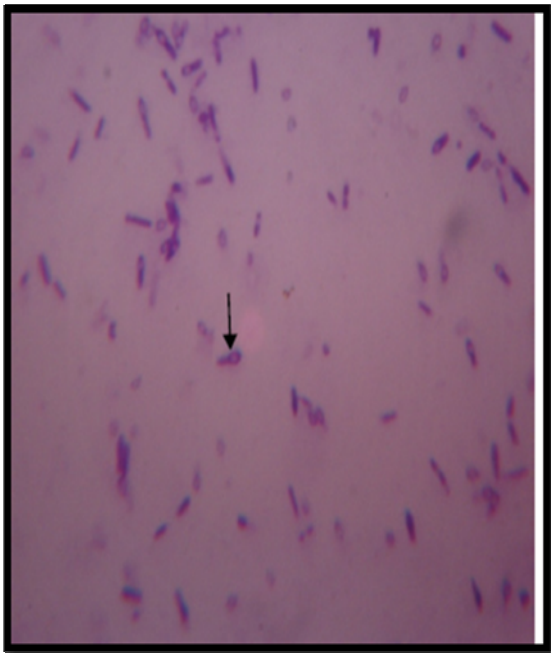
Picture 8: Prevotella melaninogenica



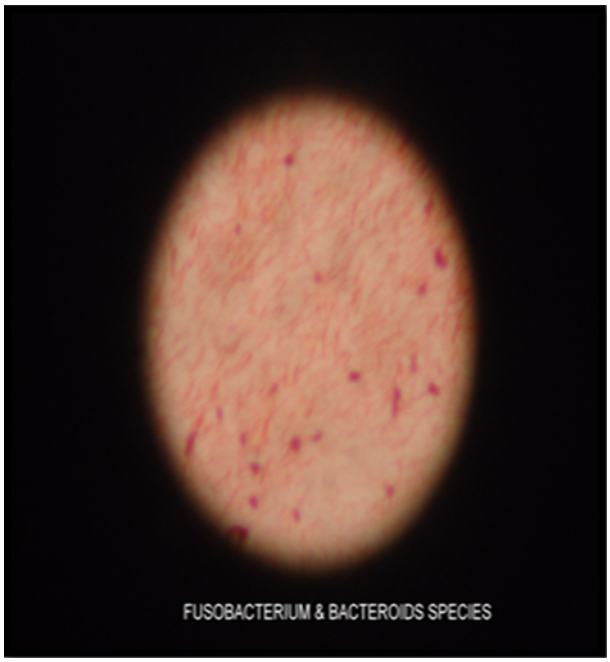
Picture 9: Fusobacterium species



Picture 10: Bacteroides species

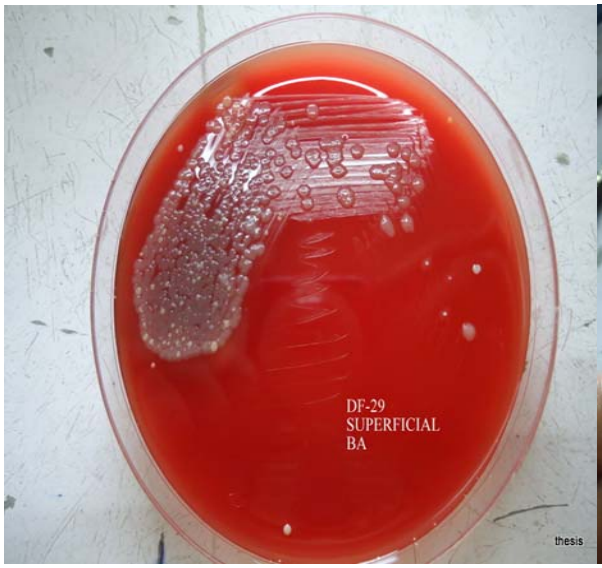


Picture 11: Clostridium species



Picture 12:
Fusobacterium sp. & Bacteroides species

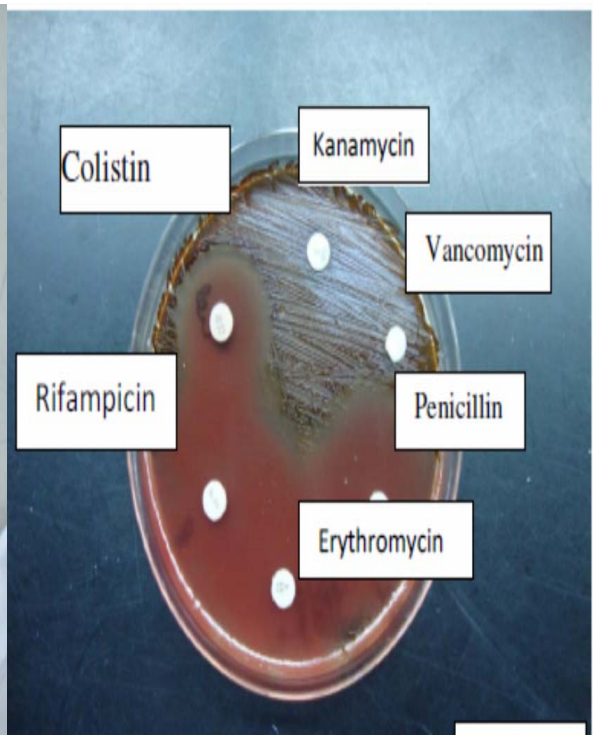
Difference in the organisms of superficial and deep samples seen on blood plate



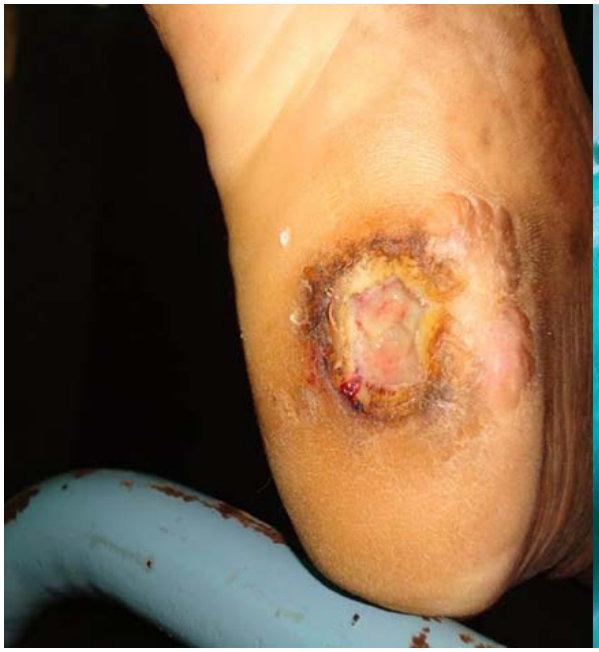
Picture 13: DF-29 Superficial sample

Picture 14: DF-29: Deep sample

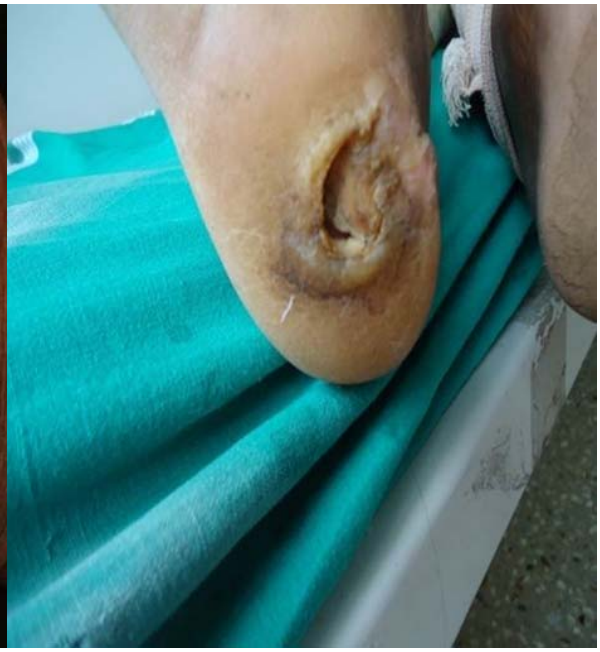
Picture 15: Aerotolerance test



Picture 16: Antibiotic sensitivity of anaerobes

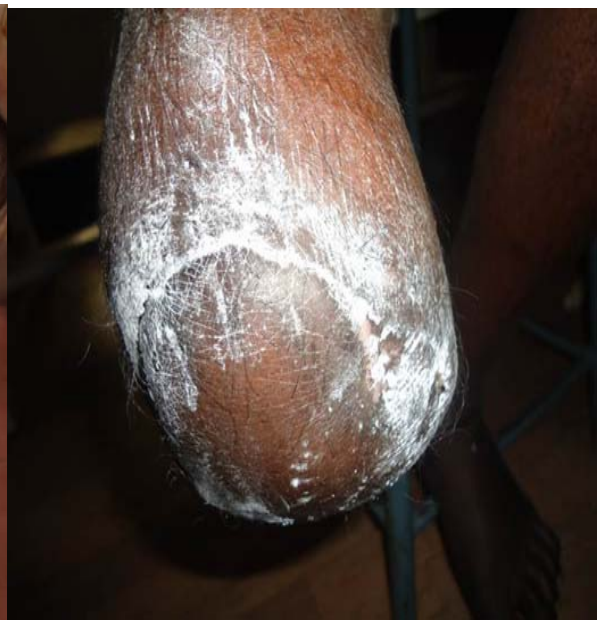


Picture 17: DF-26 At Admission



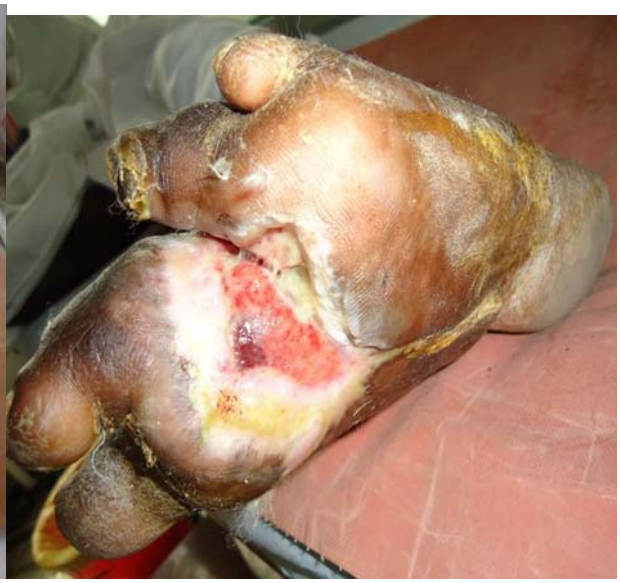
Picture 18: DF-26 After Treatment

Picture 19: DF-21- Infected amputated stump

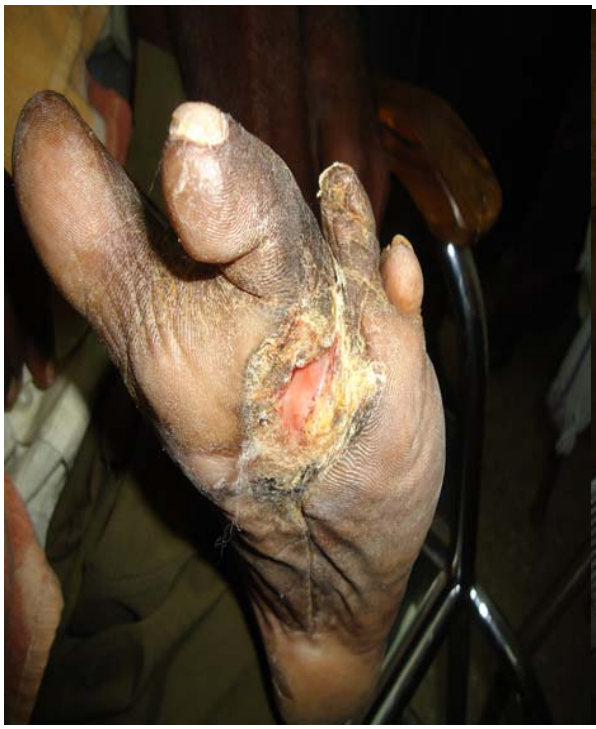


Picture 20: DF-21-After treatment

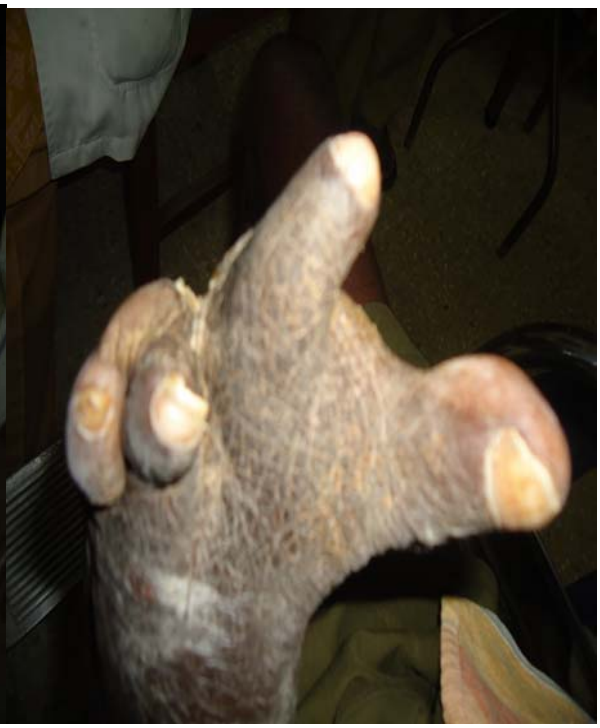
Picture 21: DF-27 - at Admission



Picture 22: DF-27 -at 2nd month of treatment



Picture 23: DF-27 at 6 th month of treatment



Picture 24: DF-27 at 6 th month of treatment

RESULTS:

A total of 50 patients with diabetic foot ulcers participated in this study. All patients came from various parts of Kolar and Chikballapur districts had type II diabetes mellitus. The median age group of the patients was 55 yrs, though the duration of diabetes mellitus ranged from 2 days to 20 years. The duration of foot ulcer ranged from 2 days to 1 year with 76% give a history of poor glycaemic control. The incidence of diabetic foot ulcers among our male subjects was 86% against females 14% was similar to reports of other investigators of male preponderance of this condition in general, indicates high level of activity among males compared to females. There were 42(84%) inpatients and 8(16%) out patients which could account for multiple drug resistant nature of bacterial isolates observed. (Table1)

Table 1: Clinical characteristics of the patients with diabetic foot ulcer

Parameters		Results
Age Range		35 – 76 yrs
Gender Number (%)	males	43 (86)
	females	07 (14)
Residence		Kolar & Chikballapur districs
Total No. of Inpatients (%)		42 (84)
Total No. of Out patients (%)		08 (16)
Duration of diabetes mellitus		2 Days To 20 Yrs
Duration of foot infection		2 Days To 1 Year
No. of patients with good glycemic control (%)		12 (24)
No. of patients with poor glycemic control (%)		38 (76)

ISOLATES AND THEIR ANTIBIOTIC SENSITIVITY OF SUPERFICIAL SAMPLES

TABLE : 2 Patients showing Gram positive cocci in superficial samples

Organism	Total Isolates	Superficial samples- Gram Positive Cocci- (Numbers sensitive)											
		P	A	CN	VA	G	E	T	C	CD	LZ	TMP/ SMX	CF
E.faecalis	10	2	9	NT	10	NT	NT	NT	NT	NT	10	NT	NT
MSSA	06	0	0	6	6	3	1	4	4	3	6	0	3
MRSA	01	0	0	0	1	0	0	0	1	1	1	0	0
BH Streptococci	05	5	5	NT	5	5	NT	2	4	NT	NT	NT	NT
Total	22	Penicillin(P)30µg, Ampicillin(A)10µg, Cefoxitin(CN)30µg , Vancomycin(VA)30µg, Gentamicin(G)10µg, Erythromycin(E)15µg, Tetracycline(T), Chloramphenicol(C)30µg, Clindamycin(CD)2µg, Linezolid(LZ)30µg, Trimethoprim -sulfamethoxazole (TMP/SMX) 25µg, Ciprofloxacin(CF)5µg, NT- not tested											

Figure 1: Percentage of Gram positive cocci in superficial samples

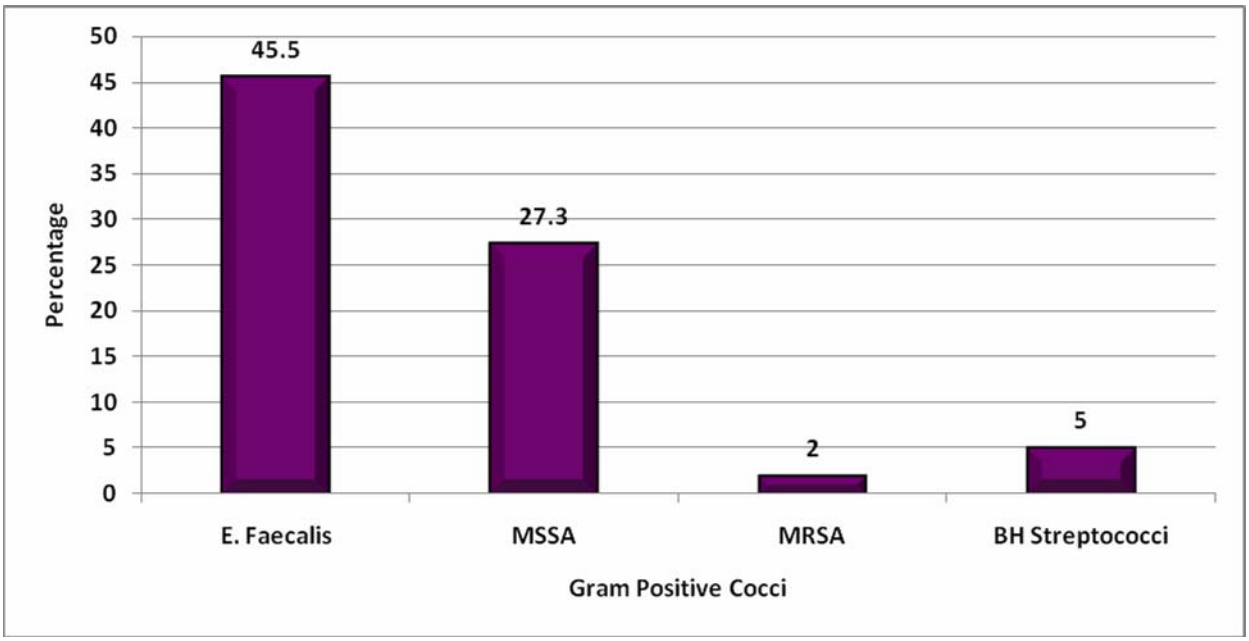


Figure 2: Percentage of Enterococci of superficial samples sensitive to antibiotics

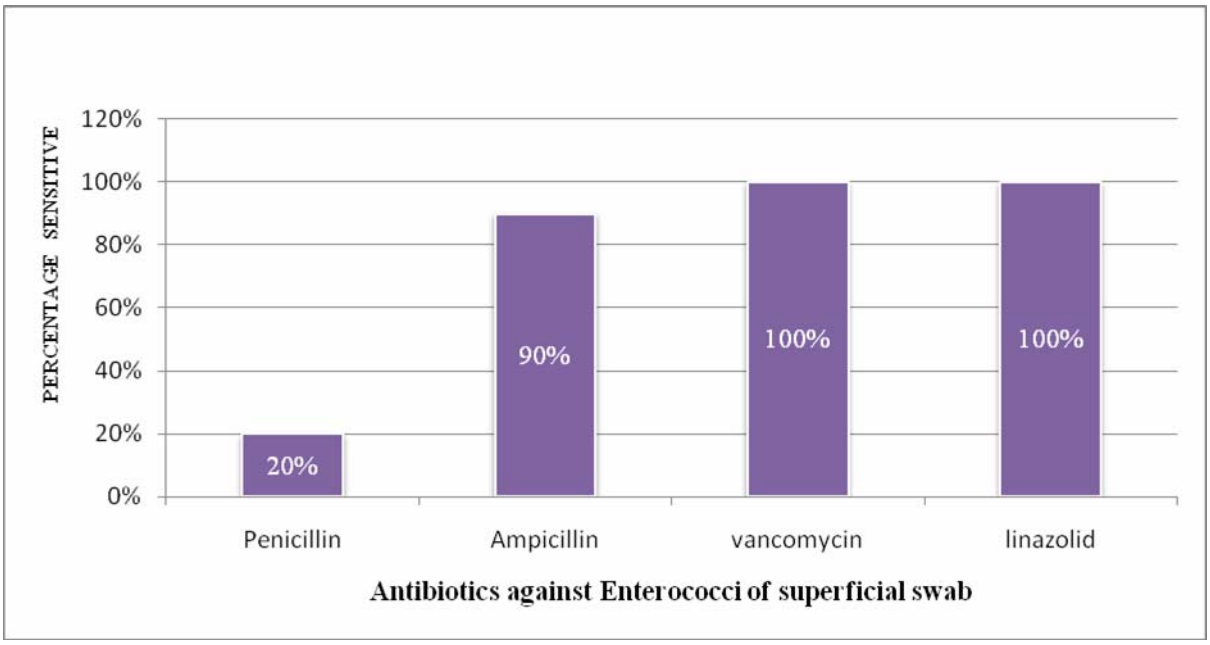
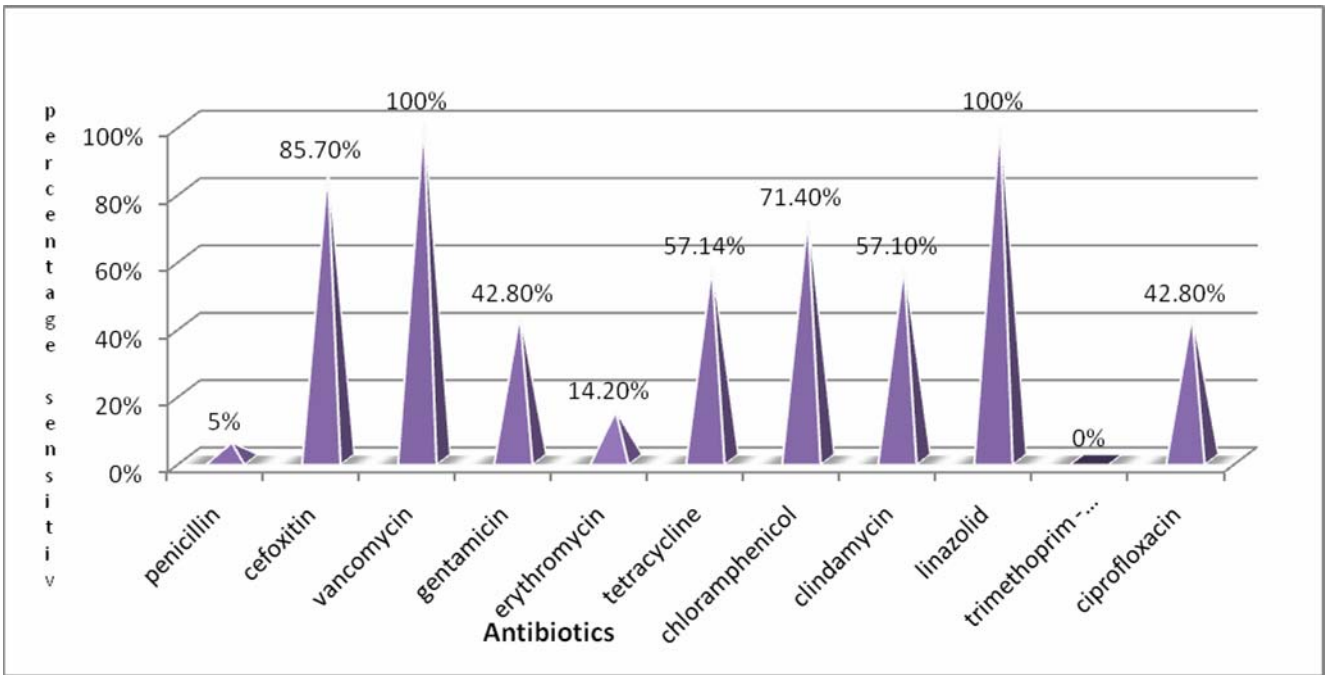


Figure 3: Antibiotic sensitivity of Staphylococci of superficial samples



Gram positive cocci of superficial samples

Out of the 22 gram positive cocci isolated from superficial samples, Enterococcus species was found to be the most prevalent. It comprised 45.55%. MSSA comprised of 27.27%, MRSA with 2% and Beta haemolytic streptococci with 5% of the total gram positive coccci isolated in the Superficial samples. (Table 1) &(Fig 1). (All of them belonged to Group A).

Only 20% of Enterococci isolated from superficial samples were sensitive to Penicillin and 90% sensitive to Ampicillin. However, 100% of Enterococci were sensitive to Vancomycin and Linezolid. (Fig 2)

While 100% of Staphylococcal isolates were sensitive to Vancomycin and Linezolid, 85% were sensitive to Cefoxitin. 71 % were sensitive to Chloramphenicol. 57% sensitive to Clindamycin and Tetracycline. 42 % were sensitive to Gentamicin and Ciprofloxacin. 14% were sensitive to Erythromycin and 5% to Penicillin. However 42 % of isolates showed inducible resistance to Clindamycin. (Fig 3)

Superficial specimens - Enterobacteriaceae

The total isolates of Superficial specimens identified to be belonging to the family Enterobacteriaceae were 40. (Table 3a & 3b)

Escherichia coli was the most prevalent comprising 27.5% , followed by Klebsiella pneumoniae with 17.5%, Enterobacter species comprising 15% and Proteus vulgaris and Morganella morganii comprising 10% each of the total Enterobacteriaceae isolated. While 100% of the isolates were sensitive to the Carbapenems i.e., Imipenem and Meropenem, 80% were sensitive to Amikacin. 57.5% sensitivity was seen for Piperacillin-tazobactam.

While the 40% of isolates were sensitive to other drugs like Piperacillin, Cefotaxime, Ceftriaxone, Gentamicin, Ciprofloxacin, Trimethoprim-sulfamethoxazole and Chloramphenicol. (Table 4).Only 5% were sensitive to Ampicillin, 15% to Amoxyclav and Tetracycline and 25% to Aztreonam. (Fig 4)

Superficial samples : Pseudomonas

Out of 10 isolates of Pseudomonas in the Superficial samples, 100% were sensitive to Imipenem, Meropenem, Polymixin–B and Colistin. 40% of the isolates were sensitive to Amikacin and Gentamicin. 30% were sensitive to Piperacillin-tazobactam and Cefepime. Only 20% sensitive to Ceftazidime, Piperacillin, Tobramycin and Levofloxacin.(Table 5 & Fig 5)

Superficial samples: Acinetobacter

Out of the 2 isolates of Acinetobacter from the Superficial samples, both were sensitive to Polymixin-B and Colistin. While both were resistant to the Carbapenems, only 1 isolate was sensitive to Piperacillin-tazobactam and Tobramycin**.** (Table 6 & Fig 6)

Table 3a: Enterobacteriaceae isolated from superficial samples

ORGANISM	TOTAL ISOLATES	SUPERFICIAL SAMPLES - FAMILY ENTEROBACTERIACEAE (numbers sensitive)																				
		A	AC	PC	PT	CH	CN	CU	CE	CI	CA	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	C
Escherichia coli	**11**	**0**	**2**	**2**	**4**	**0**	**7**	**3**	**4**	**4**	**3**	**3**	**11**	**11**	**4**	**9**	**3**	**3**	**4**	**1**	**5**	**5**
Klebsiella pneumoniae	**07**	**0**	**0**	**4**	**4**	**0**	**5**	**3**	**3**	**3**	**3**	**2**	**7**	**7**	**5**	**6**	**4**	**3**	**4**	**3**	**4**	**5**
Enterobacter species	**06**	**0**	**0**	**1**	**2**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**6**	**6**	**2**	**5**	**1**	**2**	**3**	**0**	**1**	**1**
Citrobacter diversus	**02**	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**2**	**2**	**1**	**2**	**1**	**2**	**2**	**0**	**1**	**1**
TOTAL	**26**	Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(CU)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(Ao), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,																				

Table 3b: Enterobacteriaceae isolated from superficial samples

ORGANISM	TOTAL ISOLATES	SUPERFICIAL SAMPLES - FAMILY ENTEROBACTERIACEAE (numbers sensitive)																				
		A	AC	PC	PT	CH	CN	CU	CE	CI	CA	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	C
Proteus mirabilis	**03**	**1**	**1**	**2**	**2**	**0**	**2**	**1**	**2**	**2**	**2**	**1**	**3**	**3**	**2**	**2**	**1**	**1**	**2**	**0**	**3**	**2**
Proteus vulgaris	**04**	**1**	**3**	**3**	**3**	**1**	**2**	**1**	**2**	**2**	**3**	**1**	**4**	**4**	**2**	**4**	**2**	**3**	**3**	**1**	**3**	**2**
Morganella morganii	**04**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**2**	**2**	**2**	**0**	**4**	**4**	**1**	**2**	**0**	**2**	**2**	**0**	**2**	**1**
Providencia rettgeri	**03**	**0**	**0**	**2**	**3**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**3**	**3**	**2**	**2**	**2**	**2**	**2**	**1**	**0**	**0**
TOTAL	**14**	Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(CU)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(Ao), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,																				

Table 4: The antibiotic sensitivity of Enterobacteriaceae of the Superficial samples

Enterobacteriaceae of Superficial samples Total Isolates : 4O / 50 cases																				
Antibiotics	**A**	**AC**	**PC**	**PT**	**CH**	**CN**	**CU**	**CE**	**CI**	**CA**	**AO**	**I**	**MR**	**G**	**AK**	**TB**	**CF**	**LE**	**T**	**TMP/ SMX**	**C**
Numbers sensitive	2	6	17	23	01	17	10	16	16	15	10	40	40	19	32	14	18	22	6	19	17
Percentage	**5**	**15**	**42.5**	**57.5**	**2.5**	**42.5**	**25**	**40**	**40**	**37.5**	**25**	**100**	**100**	**47.5**	**80**	**35**	**45**	**55**	**15**	**47.5**	**42.5**
Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(CU)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(Ao), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,																				

Figure 4: Percentage of Enterobacteriaceae sensitive to antibiotics

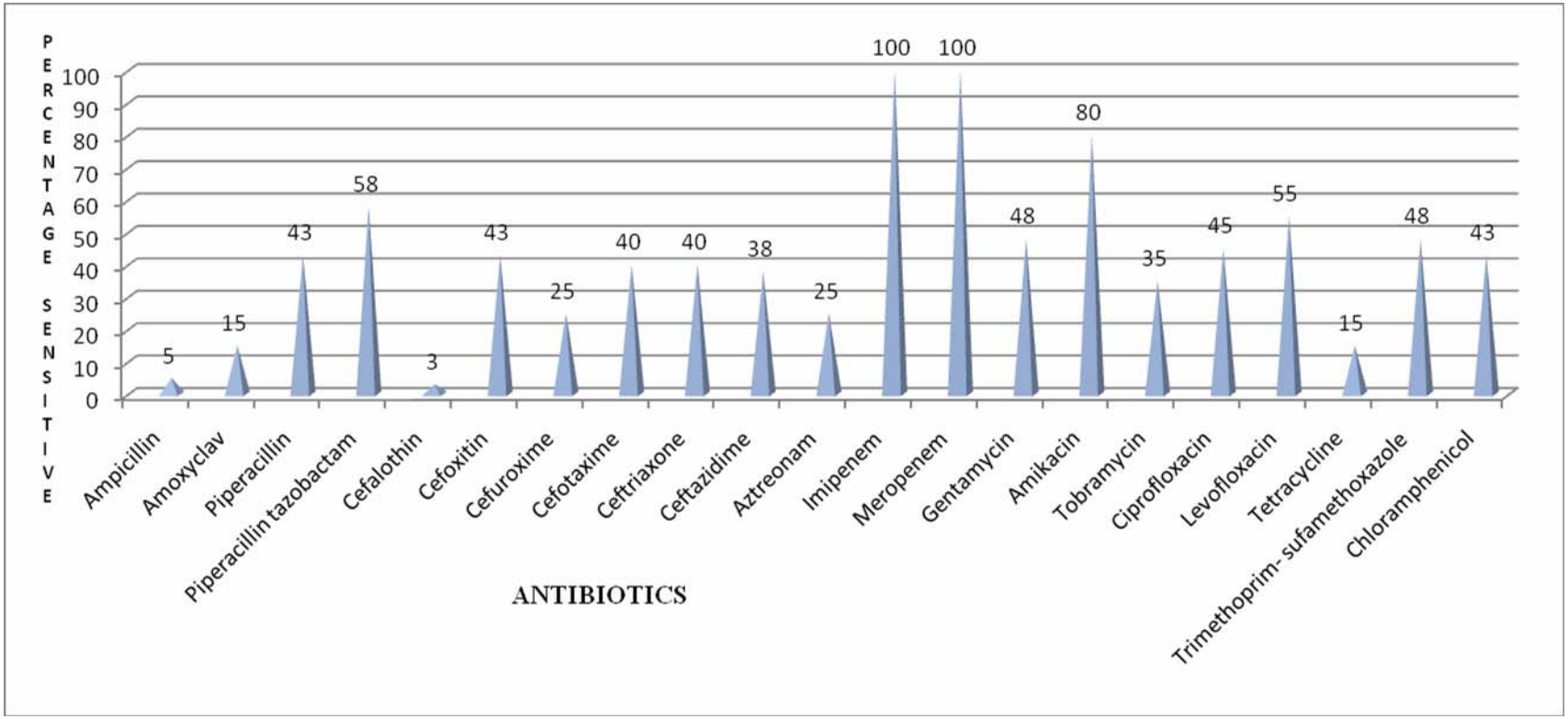


Table 5: Pseudomonas in the superficial samples

TOTAL ISOLATES	superficial samples – pseudomonas(numbers sensitive)													
	PC	PT	CA	CPM	AO	I	MR	G	AK	TB	CF	LE	PB	CL
Frequency	2	3	2	3	1	10	10	4	4	2	1	2	10	10
Percentage	20	30	20	30	10	100	100	40	40	20	10	20	100	100
Piperacillin(PC)100µg, Piperacillin-tazobactam(PT)100µg, Ceftazidime(CA)30µg, Cefepime(CPM)30µg, Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Polymyxin-B(PB)300U, Colistin(CL)10µg.														

Figure 5: Percentage of Pseudomonas sensitive to antibiotics

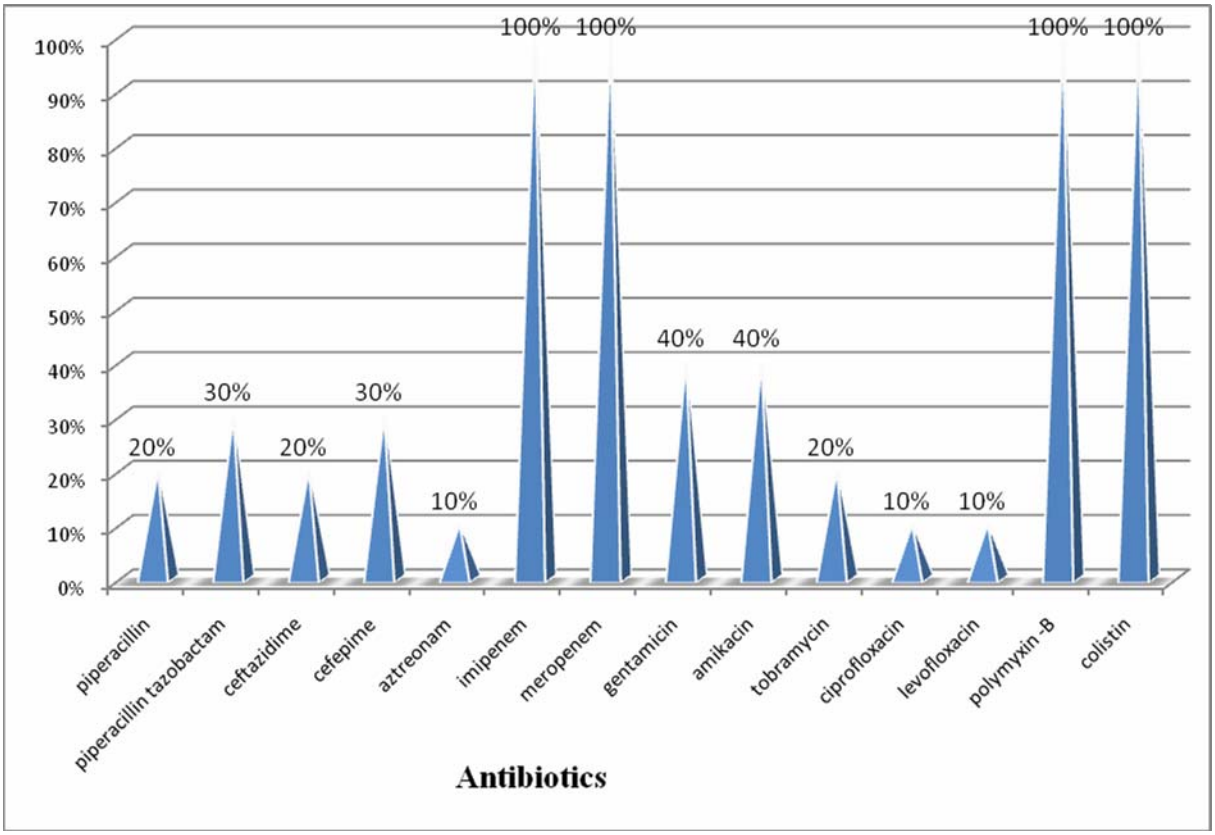
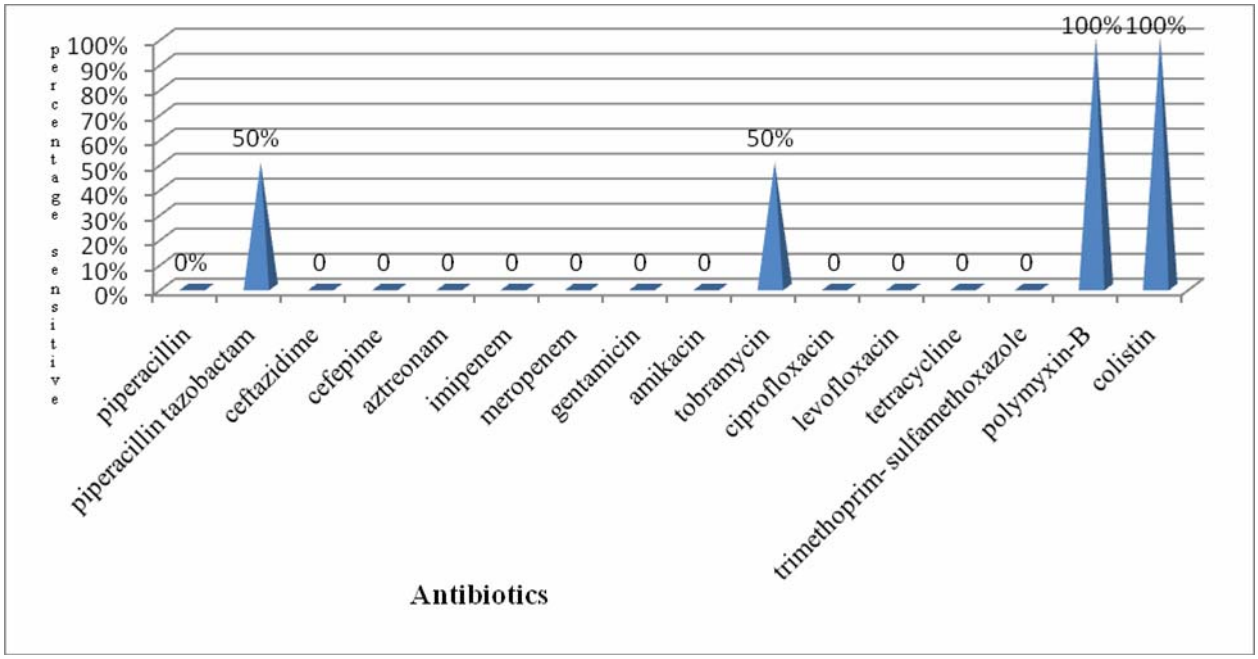


Table 6 : Acinetobacter of Superficial samples

Total Isolates	Superficial samples- Acinetobacter (Numbers sensitive)															
	PC	PT	CA	CPM	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	PB	CL
Frequency	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**2**
Percentage	**0**	50	**0**	**0**	**0**	**0**	**0**	**0**	**0**	50	**0**	**0**	**0**	**0**	100	100
Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Ceftazidime(CA)30µg, Cefepime(CPM)30µg, Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg,Trimethoprim-sulfamethoxazole(TMP/SMX)25µg, Polymyxin-B(PB)300U, Colistin(CL)10µg															

Figure 6: Percentage of Acinetobacter of superficial samples sensitive to antibiotics

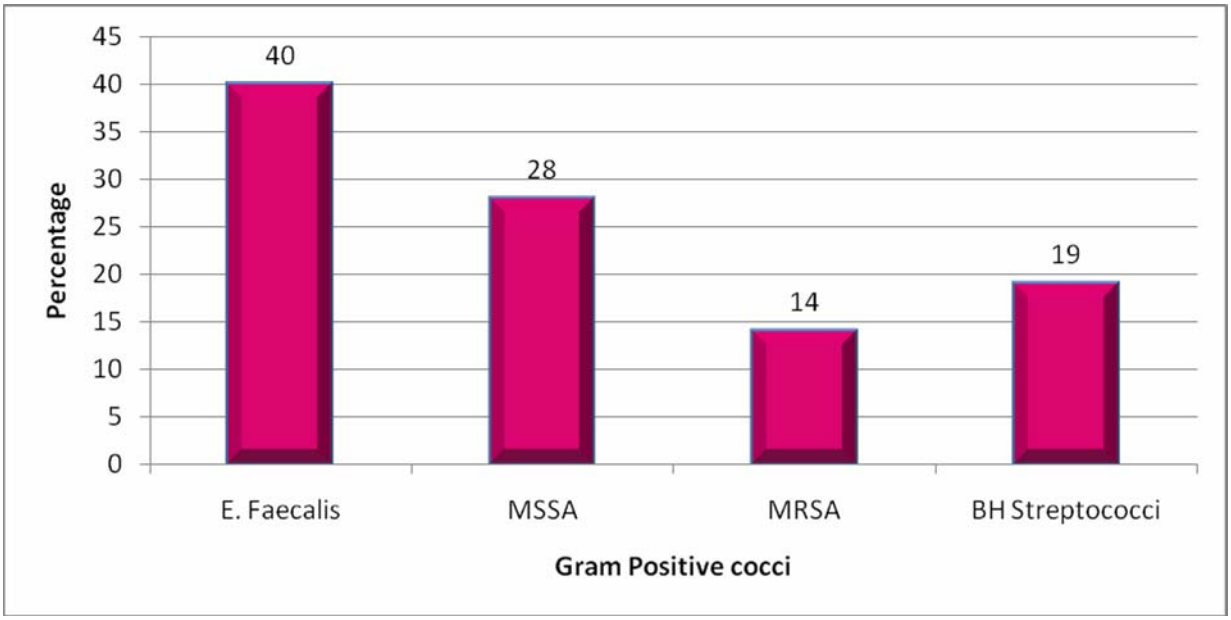


ISOLATES AND THEIR ANTIBIOTIC SENSITIVITY OF DEEP TISSUE SAMPLES

Table 7: Total Gram positive cocci in deep tissue samples

Organism	Total Isolates	Deep samples- Gram Positive Cocci- (Numbers sensitive)											
		P	A	CN	VA	G	E	T	C	CD	LZ	TMP/ SMX	CF
E.faecalis	23	4	11	NT	23	NT	NT	NT	NT	NT	23	NT	NT
MSSA	16	1	0	16	16	9	5	11	13	13	16	5	6
MRSA	08	0	0	0	08	5	0	4	7	5	8	2	3
BH Streptococci	11	11	11	NT	11	7	NT	4	7	NT	11	NT	NT
TOTAL	58	Penicillin(P)30µg, Ampicillin(A)10µg, Cefoxitin(CN)30µg , Vancomycin(VA)30µg, Gentamicin(G)10µg, Erythromycin(E)15µg, Tetracycline(T), Chloramphenicol(C)30µg, Clindamycin(CD)2µg, Linezolid(LZ)30µg, Trimethoprim -sulfamethoxazole (TMP/SMX) 25µg, Ciprofloxacin(CF)5µg, NT- not tested											

Figure 7: Percentage of Gram positive cocci sensitive to antibiotics



Deep tissue samples: Gram positive cocci

Out of the 58 isolates of gram positive coccci in the Deep tissue samples, again Enterococcus species was most prevalent being 40%. MSSA was found to be 28 % while MRSA being 08% of the total gram positive isolates. Beta haemolytic Streptococci were 19%. (1 isolatebelonged togroupG, while the others were Group A). (Table 7)

All Enterococci were sensitive to Vancomycin and Linezolid whereas only 47.8% were sensitive to Ampicillin and 18% sensitive to Penicillin. (Fig 8) Out of 24 isolates of Staphylococci, 100% were sensitive to Vancomycin and Linezolid. Chloramphenicol and Clindamycin were found to be very effective drugs in our study with isolates showing 84% and 75% sensitivity respectively. About 60% of isolates showed sensitivity to Cefoxitin, Tetracycline and Gentamicin. Only about 30% isolates showed sensitivity to Trimethoprim-sulfamethoxazole and Ciprofloxacin, 21% to Erythromycin and only 5% showed sensitivity to Penicillin. (Fig 9)

Deep tissue samples: Enterobacteriaceae

Out of the 64 total isolates of Deep tissue samples that belong to family Enterobacteriaceae, Escherichia coli was the most prevalent again with 23.43%, followed by Klebsiella pneumoniae with 20.31%. Enterobacter species and Proteus mirabilis comprised to be 12.5% each with Morganella morganii 10.93%. Proteus vulgaris, Citrobacter species and Providencia rettgeri comprised about 7% each. (Table 8a & 8b)

All Enterobacteriaceae isolates were sensitive to the Carbapenems i.e., Imipenem and Meropenem. 72% of the isolates were sensitive to Amikacin. 64% were sensitive to Piperacillin-tazobactam. About 50 % of the isolates were sensitive to Piperacillin, Cefotaxime, Ceftriaxone, Gentamicin, Levofloxacin, Chloramphenicol. 40% of isolates were sensitive to Cefoxitin, Ceftazidime. (Table 9). Only 30% of isolates were sensitive to

Cefuroxime, Aztreonam, Tobramycin, Ciprofloxacin and Trimethoprim/sulfamethoxazole. Whereas only 19% of the isolates were sensitive to Amoxyclav and Tetracycline, 10% were sensitive to Ampicillin. (Fig 10)

Deep tissue samples: Pseudomonas

Out of the 8 Pseudomonas aeruginosa isolated from the Deep tissue samples, 100% were sensitive to Imipenem, Meropenem, Polymixin- B and Colistin. 50% were sensitive to Piperacillin-tazobactam, Ceftazidime, Amikacin. About 37% were sensitive to Piperacillin, Cefepime, Gentamicin, Tobramycin, Ciprofloxacin and Levofloxacin. Only 25% of isolates were sensitive to Aztreonam. (Table 10) & (Fig 11)

Deep tissue samples: Acinetobacter

There were 5 isolates of Acinetobacter. 100% sensitivity to Polymixin-B and Colistin was seen whereas only 40% of isolates showed sensitivity to Imipenem and 20% to Meropenem. 60% of isolates showed sensitivity to Ceftazidime.40% of isolates showed sensitivity to Gentamicin, Tobramycin, Levofloxacin, Tetracycline and Trimethoprim sulfamethoxazole. 20% of isolates showed sensitivity to Piperacillin, Piperacillin-tazobactam, Aztreonam, Amikacin and Ciprofloxacin. (Table 11) & (Fig 12)

Figure 8 : Percentage of Enterococcus species of deep samples sensitive to antibiotics

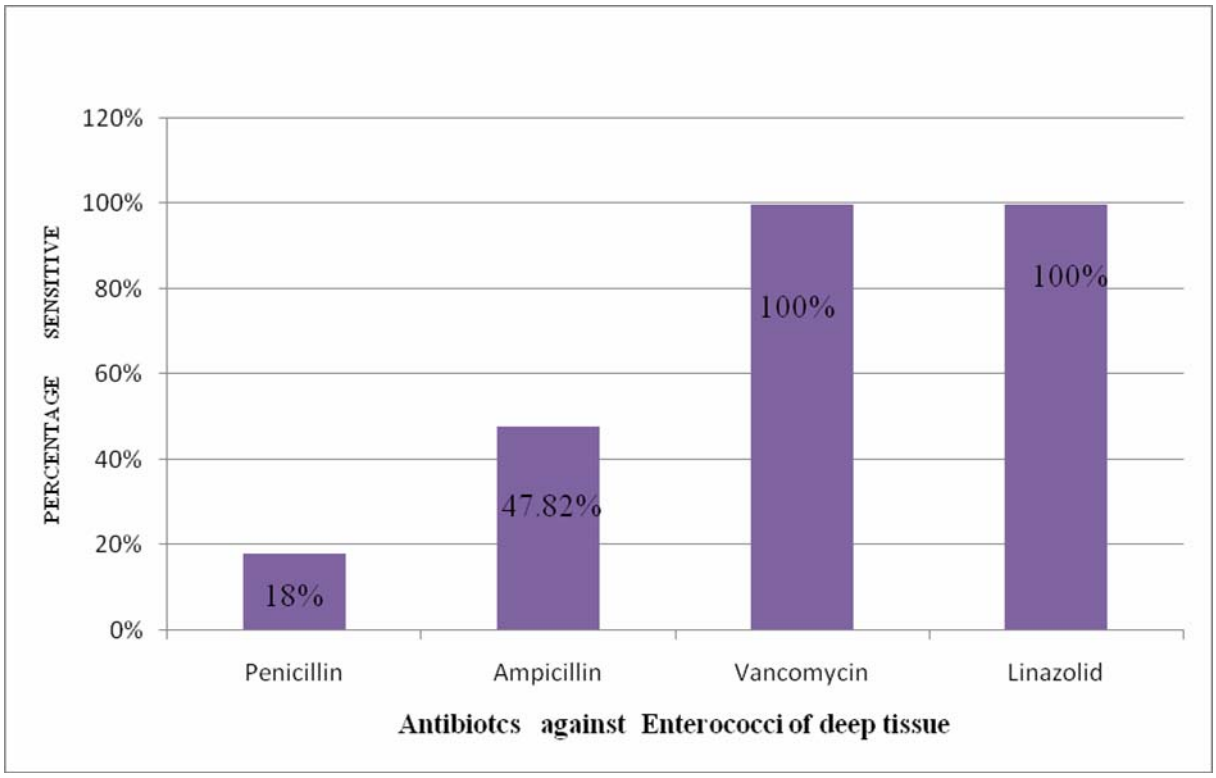


Figure 9 : Percentage of Staphylococci of deep samples sensitive to antibiotics

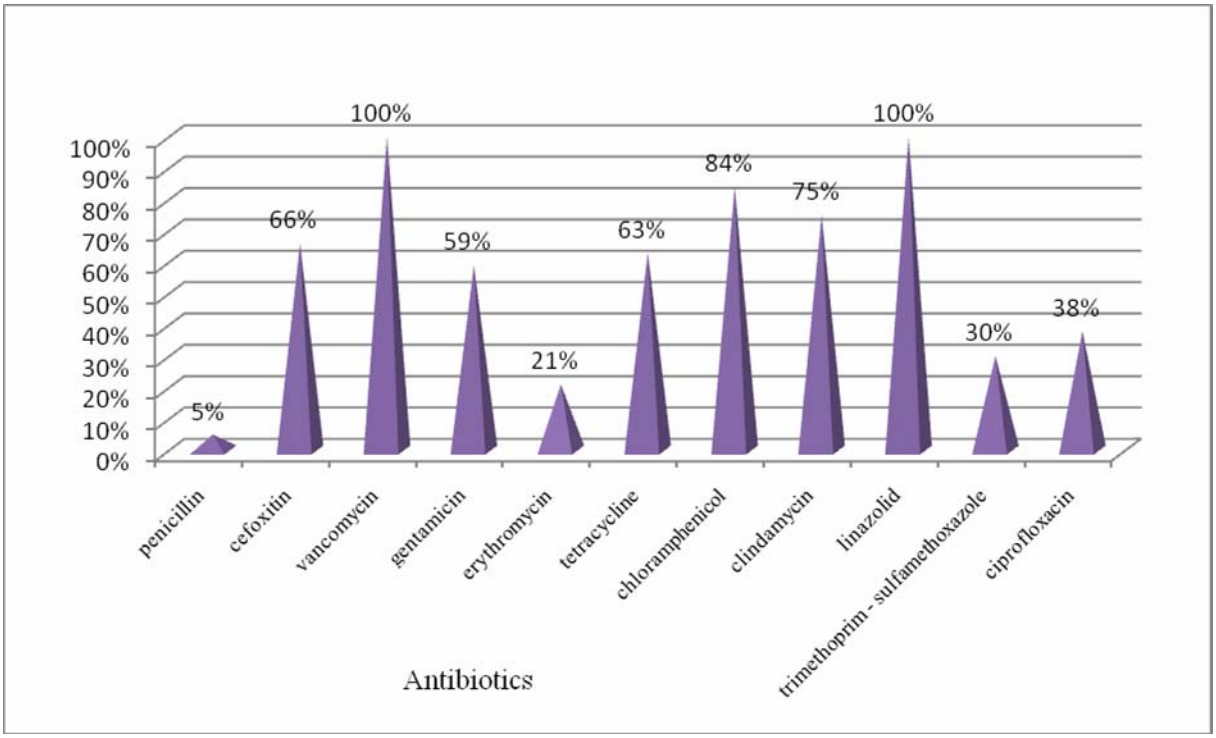


Table 8a: Enterobacteriaceae isolated from deep tissue samples

ORGANISM	TOTAL ISOLATES	DEEP TISSUE SAMPLES - FAMILY ENTEROBACTERIACEAE (numbers sensitive)																			
		A	AC	PC	PT	CH	CN	CU	CE	CI	CA	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	C
Escherichia coli	**15**	**0**	**4**	**7**	**10**	**0**	**8**	**5**	**7**	**6**	**6**	**6**	**15**	**15**	**9**	**14**	**0**	**0**	**6**	**0**	**6**	**10**
Klebsiella pneumoniae	**13**	**0**	**0**	**5**	**5**	**0**	**9**	**5**	**6**	**5**	**5**	**4**	**13**	**13**	**5**	**12**	**3**	**7**	**8**	**7**	**0**	**9**
Enterobacter species	**08**	**1**	**1**	**3**	**4**	**0**	**1**	**3**	**3**	**3**	**3**	**3**	**8**	**8**	**3**	**8**	**4**	**3**	**4**	**2**	**2**	**4**
Citrobacter diversus	**04**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**0**	**1**	**4**	**4**	**2**	**3**	**1**	**2**	**3**	**0**	**1**	**1**
TOTAL	**40**	Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(CU)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,																			

Table 8: Enterobacteriaceae isolated from deep tissue samples

ORGANISM	TOTAL ISOLATES	DEEP TISSUE SAMPLES - FAMILY ENTEROBACTERIACEAE (numbers sensitive)																			
		A	AC	PC	PT	CH	CN	CU	CE	CI	CA	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	C
Proteus mirabilis	**08**	**2**	**3**	**7**	**8**	**2**	**5**	**3**	**5**	**5**	**3**	**3**	**8**	**8**	**5**	**7**	**3**	**2**	**2**	**1**	**3**	**2**
Proteus vulgaris	**05**	**1**	**2**	**5**	**5**	**0**	**3**	**1**	**4**	**4**	**3**	**2**	**5**	**5**	**3**	**5**	**2**	**1**	**3**	**0**	**1**	**2**
Morganella morganii	**07**	**2**	**2**	**5**	**5**	**0**	**2**	**3**	**4**	**4**	**4**	**3**	**7**	**4**	**5**	**5**	**5**	**3**	**5**	**1**	**4**	**3**
Providencia rettgeri	**04**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**2**	**2**	**2**	**0**	**4**	**4**	**1**	**2**	**2**	**1**	**2**	**1**	**1**	**0**
TOTAL	**24**	Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(Cu)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(Le)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg,																			

Table 9 : Enterobacteriaceae of deep tissue samples sensitive to antibiotics

Enterobacteriaceae of Deep tissue samples Total Isolates : 64 / 50 cases																				
Antibiotics	**A**	**AC**	**PC**	**PT**	**CH**	**CN**	**CU**	**CE**	**CI**	**CA**	**AO**	**I**	**MR**	**G**	**AK**	**TB**	**CF**	**LE**	**T**	**TMP/ SMX**	**C**
Numbers sensitive	6	12	35	41	02	28	21	32	30	26	22	64	64	33	46	20	19	33	12	18	31
Percentage	9.4	18	54	64.2	2	43.5	32.8	50	46.8	40.6	34.3	100	100	51.5	71.8	31.2	29.6	51.5	18.7	28.1	48.4
Ampicillin(A)10µg, Amoxyclav(AC)30µg, Piperacillin(P)100µg, Piperacillin tazobactam(PT)100µg, Cephalothin(CH)30µg Cefoxitin(CN)30µg, Cefuroxime(CU)30µg, Cefotaxime(CE)30µg, Ceftriaxone(CI)30µg, Ceftazidime(CA)30µg , Aztreonam(Ao), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg, Trimethoprim- sulfamethoxazole (TMP/SMX)25µg, Chloramphenicol(C)30µg																				

Figure 10: Percentage of Enterobacteriaceae of deep samples sensitive to antibiotics

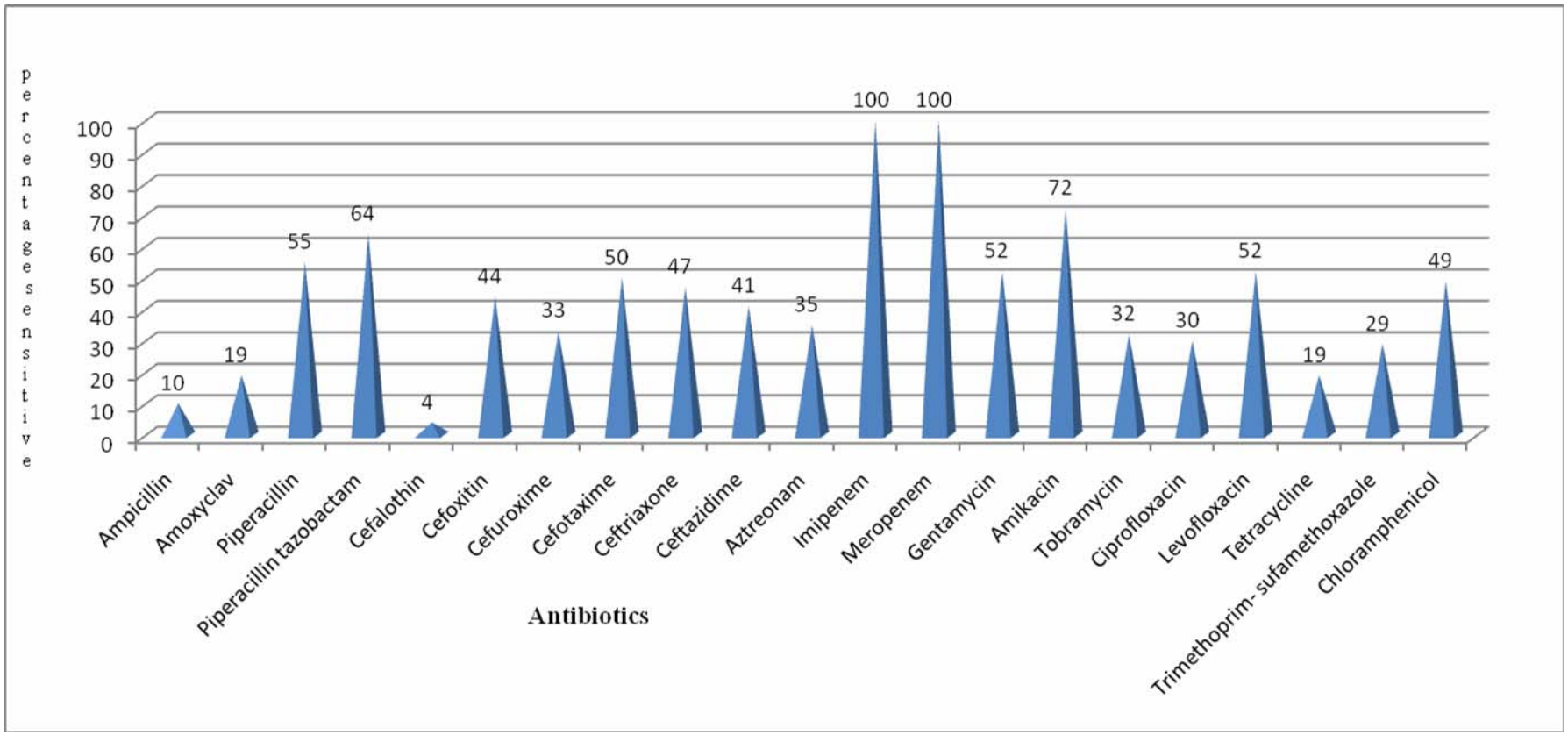


Table 10 : Pseudomonas of deep tissue samples

TOTAL ISOLATESS (08)	DEEP TISSUE – PSEUDOMONAS(Numbers sensitive)													
	PC	PT	CA	CPM	AO	I	MR	G	AK	TB	CF	LE	PB	CL
Frequency	3	4	4	3	2	8	8	3	4	3	3	3	8	8
Percentage	37	50	50	37	25	100	100	37	50	37	37	37	100	100
Piperacillin(PC)100µg, Piperacillin-tazobactam(PT)100µg, Ceftazidime(CA)30µg, Cefepime(CPM)30µg, Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Polymyxin-B(PB)300U, Colistin(CL)10µg.														

Figure 11 : Percentage of Pseudomonas of deep tissue samples sensitive to antibiotics

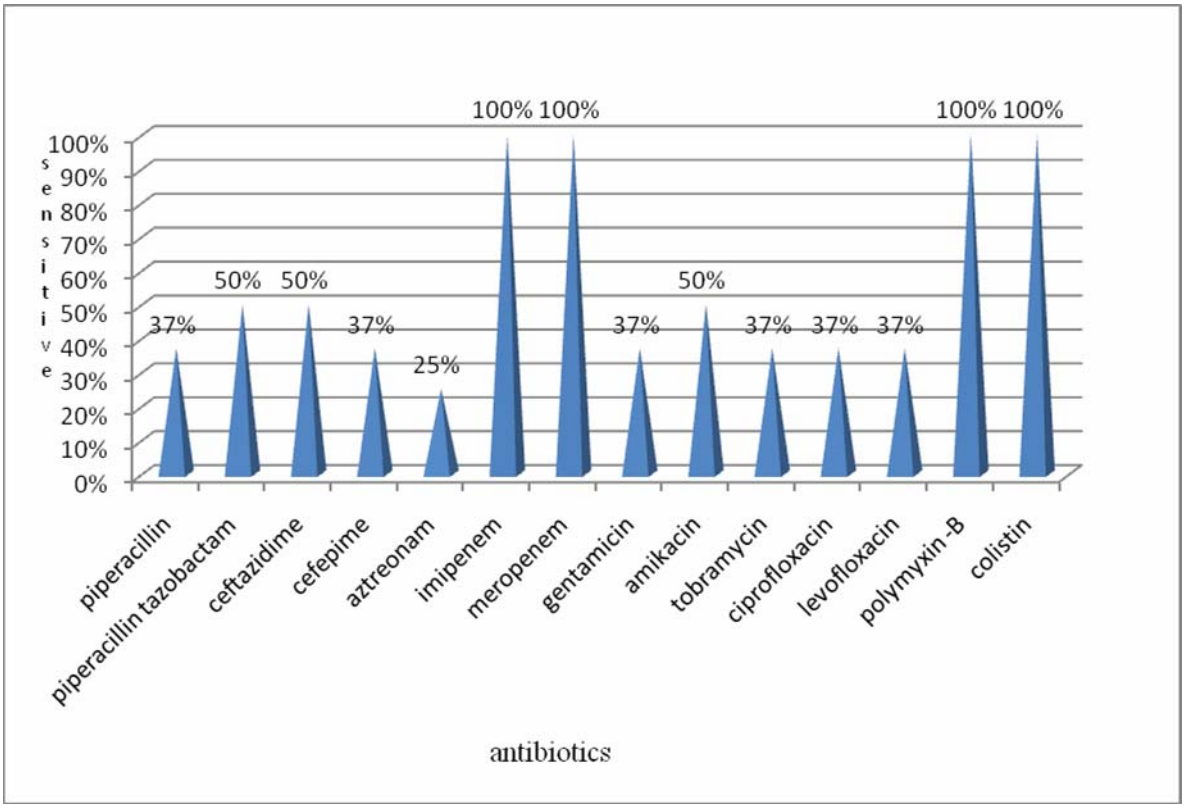
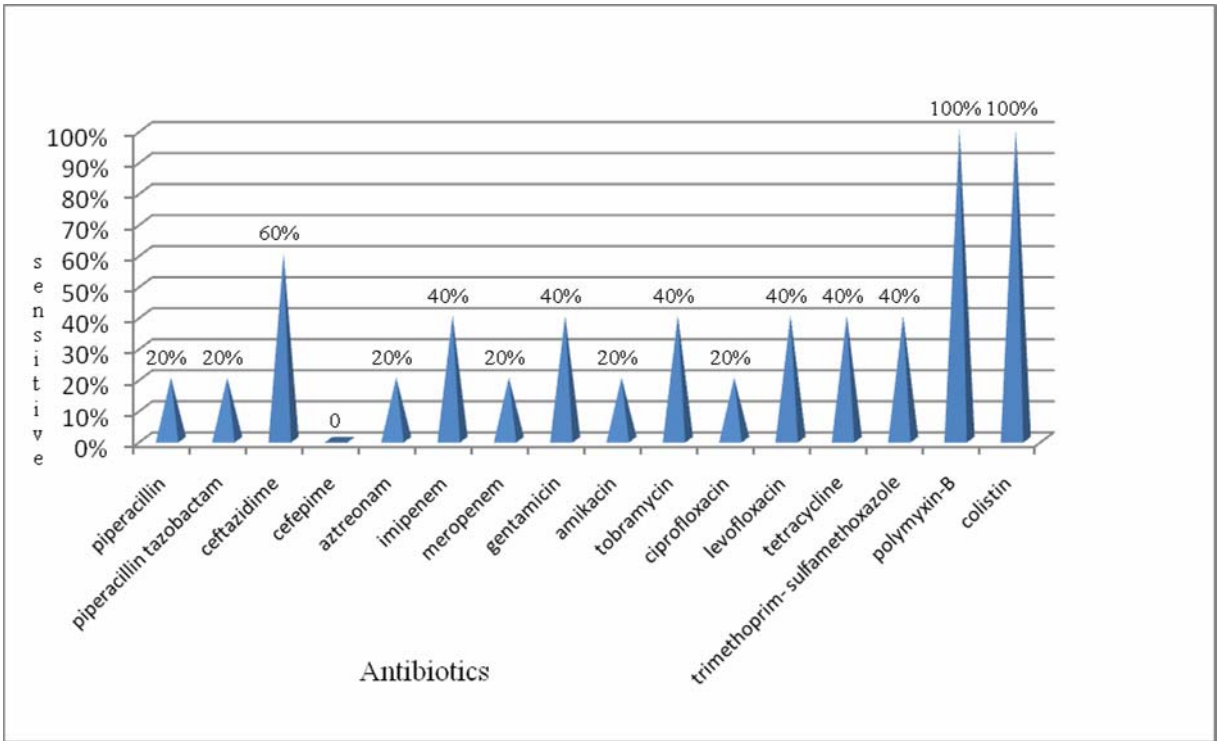


Table 11: Acinetobacter of deep tissue samples

Total Isolates (05)	Deep samples- Acinetobacter (Numbers sensitive)															
	PC	PT	CA	CPM	AO	I	MR	G	AK	TB	CF	LE	T	TMP/ SMX	PB	CL
Frequency	1	1	3	0	1	2	1	2	1	2	1	2	2	2	5	5
Percentage	20	20	60	0	20	40	20	40	20	40	20	40	40	40	100	100
Piperacillin(P)100µg, Piperacillin- tazobactam(PT)100µg, Ceftazidime(CA)30µg, Cefepime(CPM)30µg, Aztreonam(AO), Imipenem(I)10µg, Meropenem(MR)10µg, Gentamicin(G)10µg, Amikacin(AK)30µg, Tobramycin(TB)10µg, Ciprofloxacin(CF)5µg, Levofloxacin(LE)5µg, Tetracycline(T)30µg,Trimethoprim-sulfamethoxazole(TMP/SMX)25µg, Polymyxin-B(PB)300U, Colistin(CL)10µg															

Figure 12 :Percentage of Acinetobacter of deep tissue samples sensitive to antibiotics



COMPARATIVE ANALYSIS OF SUPERFICIAL SAMPLES AND DEEP TISSUE SAMPLES

Table 12: Total aerobic & anaerobic isolates from superficial and deep Samples

Total Isolates-Aerobes		
Sl. No.	**Superficial samples (per 50 cases)**	**Deep samples (per 50 cases)**
1.	Enterococcus faecalis- **10**	Enterococcus faecalis-**23**
2.	MSSA-**6**	MSSA--**16**
3.	MRSA-**1**	MRSA-**8**
4.	Streptococci– **5**	Streptococci– **11**
5.	CONS – **9**	CONS -**0**
6.	Diphtheroids-**6**	Diphtheroids- **0**
7.	Escherichia Coli-**11**	Escherichia coli-**15**
8.	Klebsiella Pneumoniae -**07**	Klebsiella pneumoniae-**13**
9.	Enterobacter Species-**06**	Enterobacter species-**8**
10.	Citrobacter Diversus -**02**	Citrobacter diversus-**4**
11.	Proteus Mirabilis-**3**	Proteus mirabilis-**8**
12.	Proteus Vulgaris-**04**	Proteus vulgaris-**5**
13.	Morganella morganii- **04**	Morganella morganii-**7**
14.	Providencia rettgeri- **03**	Providencia rettgeri-**4**
15.	Pseudomonas aeruginosa -**10**	Pseudomonas aeruginosa-**8**
16.	Acinetobacter species- **2**	Acinetobacter- **5**

Total Isolates-Anaerobes Deep tissue samples of 50 cases (%per 50 cases)		
1.Peptococci 47 (94%)	4.Propionibacterium species 9 (18%)	
2.Peptostreptococc 47(94%)	5.Fusobacterium sp. 5 (10%)	7.Clostridium novyii 1 (2%)
3.Bacteroides sp. 13(26%)	6.Prevotella melaninogenica 2(4%)	

Table 13: Comparison of total isolates from the superficial and the deep from 50 cases studied

TOTAL ISOLATES FOR 50 CASES		
Organisms	**Superficial samples**	**Deep tissue samples**
Gram positive cocci	31	**58**
Diphtheroids	06	**00**
Enterobacteriaceae	40	**64**
Pseudomonas	10	**08**
Acinetobacter	02	**05**
Anaerobes	0	**124**
TOTAL	**89**	**259**

Table 14: Statistical analysis of the superficial and the deep tissue samples

Sl no	Study site	Aerobes	Anaerobes	Mean No. Of Organisms	Standard Deviation	p value
1.	**Superficial**	89(25.57)	0	1.78	0.89	**<0.001****
2.	**Deep**	**135**(38.79)	**124**(35.63)	**5.18**	**1.57**	

**** Very significant**

Figure 13: Comparison of organisms isolated from the superficial and the deep samples

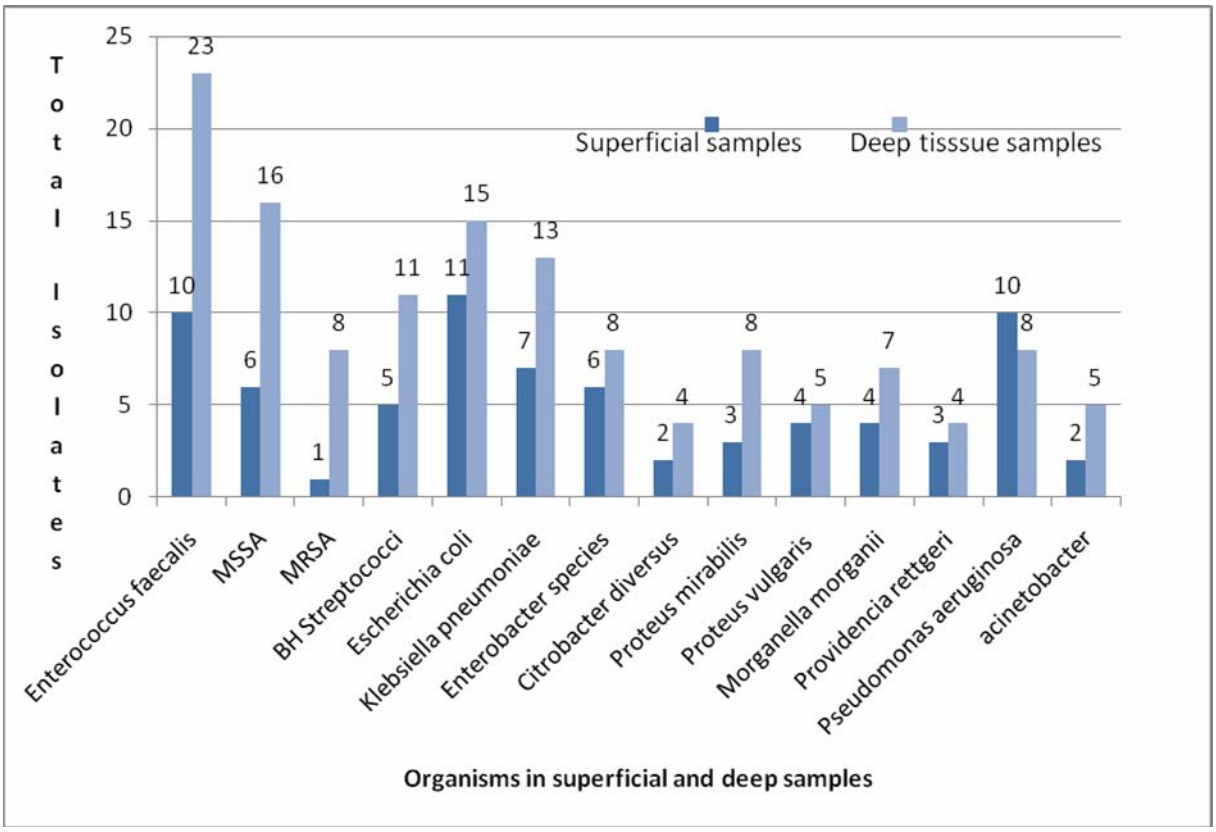


Figure 14: Comparison of Enterobacteriaceae isolated from the superficial and the deep

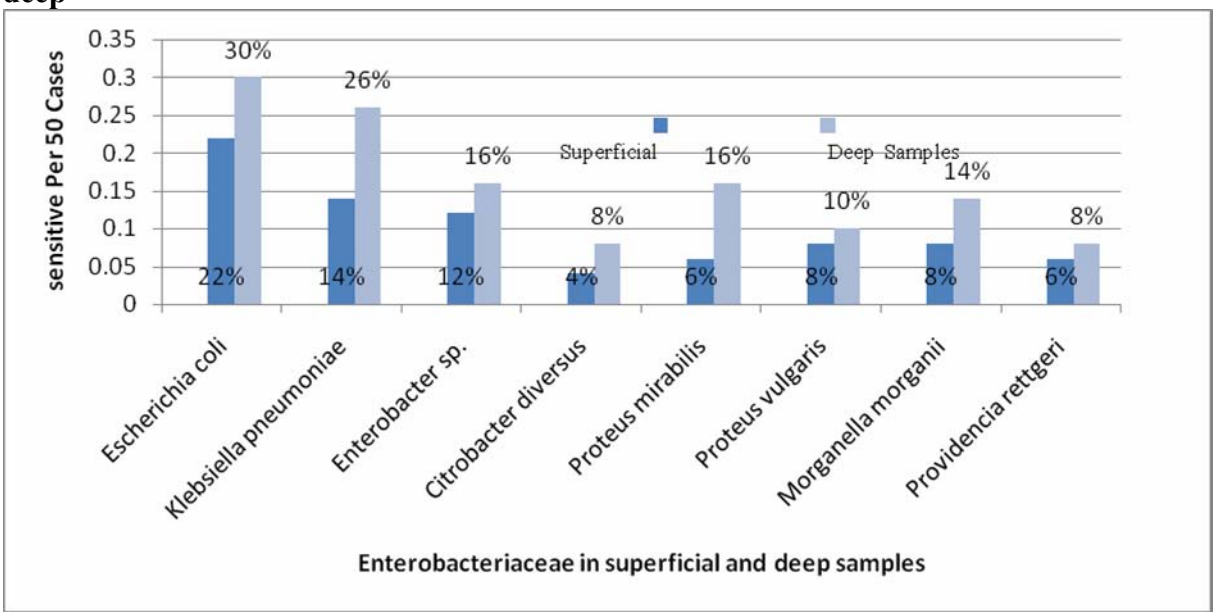


Table 15: Statistical analysis of the superficial and the deep tissue samples

Statistical Analysis				
Serial No.	**Organism**	**Superficial Samples**	**Deep Samples**	**p value**
1.	Enterococcus faecalis	20%	46%	**0.03***
2.	MSSA	12%	32%	0.07
3.	MRSA	2%	16%	**0.023***
4.	Streptococci	10%	22%	**<0.001****
5.	CONS	18%	0	-
6.	Diphtheroids	12%	0	-
7.	Escherichia coli	22%	30%	**0.01***
8.	Klebsiella pneumoniae	14%	26%	**<0.001****
9.	Entrobacter species	12%	16%	**0.04***
10.	Citrobacter diversus	4%	8%	0.15
11.	Proteus mirabilis	6%	16%	**<0.001****
12.	Proteus vulgaris	8%	10%	**0.002****
13.	Morganella morganii	8%	14%	**<0.001****
14.	Providencia rettgeri	6%	8%	**<0.001****
15.	Pseudomonas aeruginosa	20%	16%	**<0.001****
16.	Acinetobacter species	4%	10%	**0.008***
17.	Anaerobes	0	124	-

*** Significant , ** Highly significant**

A comparative analysis of antibiotic sensitivity of isolates of superficial and deep samples

Table 16: Antibiotic Sensitivity of Staphylococci

Sl. No	Antibiotic	Superficial Samples	Deep Tissue Samples
1.	Cefoxitin	**85.71%**	66%
2.	Vancomycin	100%	100%
3.	Gentamicin	42.85%	**59%**
4.	Erythromycin	14.2%	**21%**
5.	Tetracycline	**75.1%**	63%
6.	Chloramphenicol	71.42%	**84%**
7.	Clindamycin	57.1%	**75%**
8.	Linezolid	100%	100%
9.	Tmp/Smx	0%	**30%**
10.	Ciprofloxacin	**42.85%**	38%

Figure 15:**Antibiotic Sensitivity of Staphylococci of superficial & deep samples**

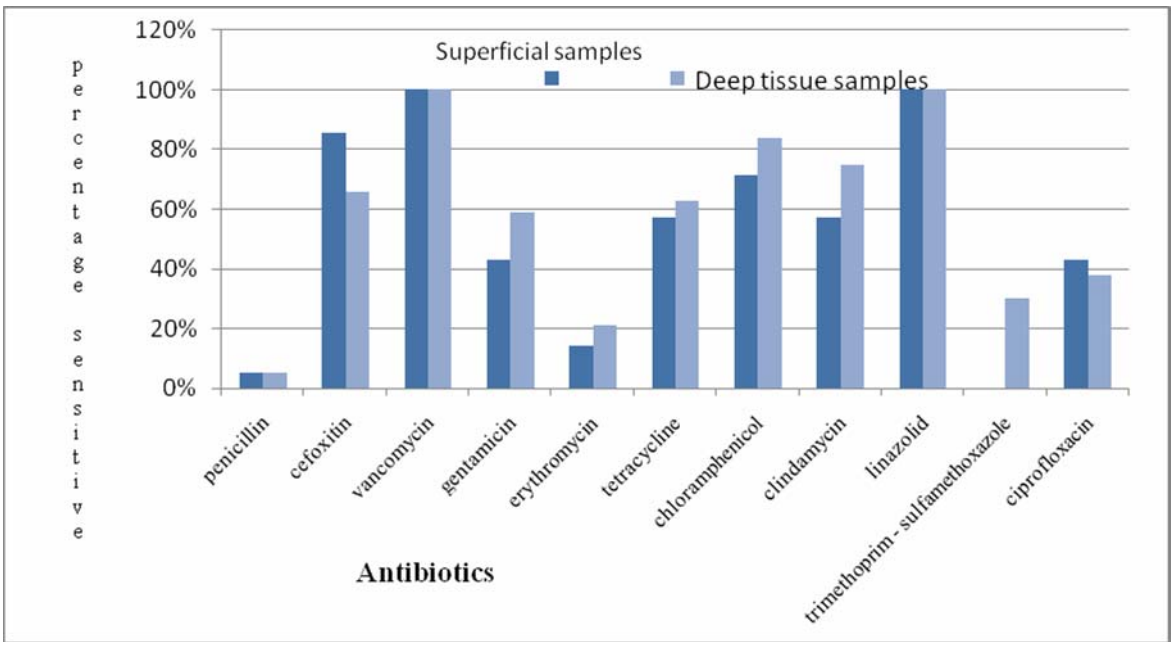


Table 17: Antibiotic sensitivity of Enterobacteriaceae

Sl.No	Antibiotic	Superficial Samples	Deep Tissue Samples
1.	**Ampicillin**	**5**	**10**
2.	Amoxyclav	15	**19**
3.	Piperacillin	43	**55**
4.	Piperacillin- Tazobactam	58	**64**
5.	Cefuroxime	25	**33**
6.	Cefotaxime	40	**50**
7.	Ceftriaxone	40	**47**
8.	Ceftazidime	38	**41**
9.	Aztreonam	25	**35**
10	Imipenem & Meropenem	100	100
11.	Gentamicin	48	52
12.	Amikacin	30	72
13.	Ciprofloxacin	45	30
14.	Levofloxacin	55	52
15.	Tetracycline	15	19
16.	Trimethoprim - sulfamethoxazole	48	29
17.	Chloramphenicol	43	49

Figure 16: Antibiotic sensitivities of Enterobacteriaceae of superficial and deep samples

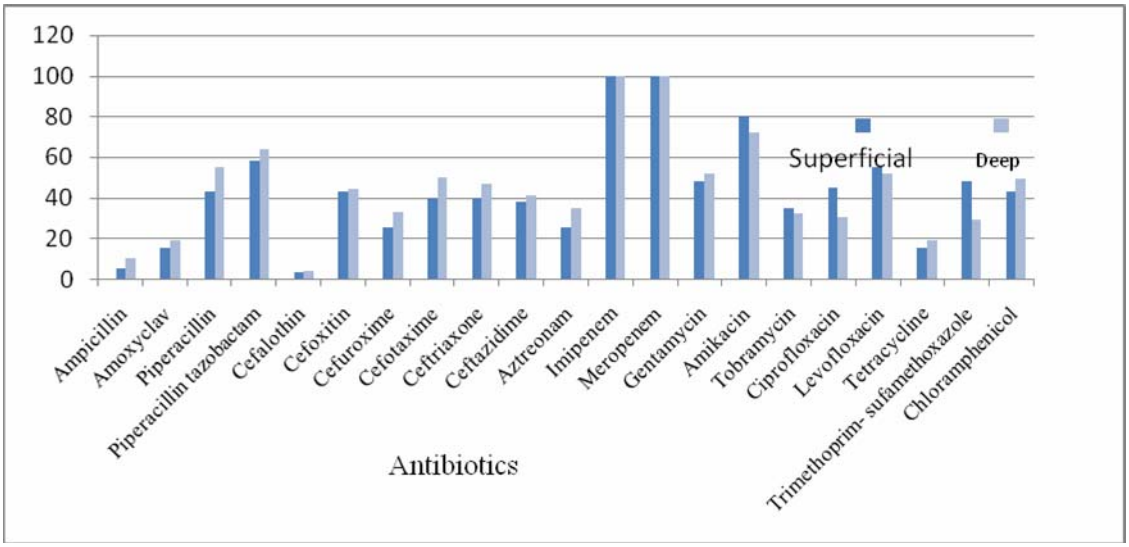


Table 18: Antibiotic sensitivity of Pseudomonas

Sl.No	Antibiotic	Superficial Samples	Deep Tissue Samples
1.	Piperacillin	20%	**37%**
2.	Piperacillin tazobactam	30%	**50%**
3.	Ceftazidime	20%	**50%**
4.	Cefepime	30%	**37%**
5.	Aztreonam	10%	**25%**
6.	Imipenem	100%	100%
7.	Meropenem	100%	100%
8.	Gentamicin	40%	37%
9.	Amikacin	40%	**50%**
10.	Tobramycin	20%	**37%**
11.	Ciprofloxacin	10%	**37%**
12.	Levofloxacin	10%	**37%**
13.	Polymyxin-B	100%	100%
14.	Colistin	100%	100%

Table 19:Antibiotic sensitivity of Acinetobacter species

Sl.No	Antibiotic	Superficial Samples	Deep Tissue Samples
1.	Piperacillin	17	**31**
2.	Piperacillin-Tazobactam	34	**39**
3.	Cefepime	**25**	24
4.	Gentamicin	34	**39**
5.	Amikacin	34	**39**
6.	Ceftazidime	17	**54**
7.	Ciprofloxacin	09	**31**
8.	Levofloxacin	17	**39**
9.	Tobramycin	25	**39**
10.	Polymixin-b & Colistin	100	**100**
11.	Aztreonam	09	**24**
12.	Imipenem	84	**77**
13.	Meropenem	84	**70**

Figure 17 : Antibiotic sensitivity of Pseudomonas of superficial and deep samples

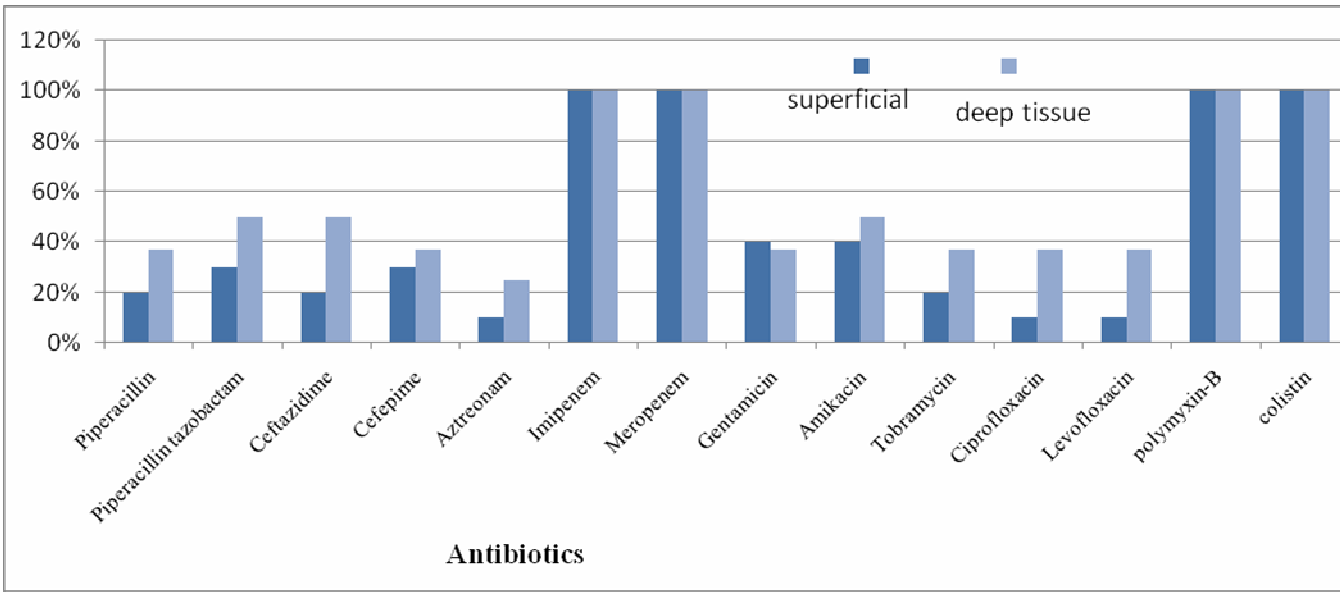
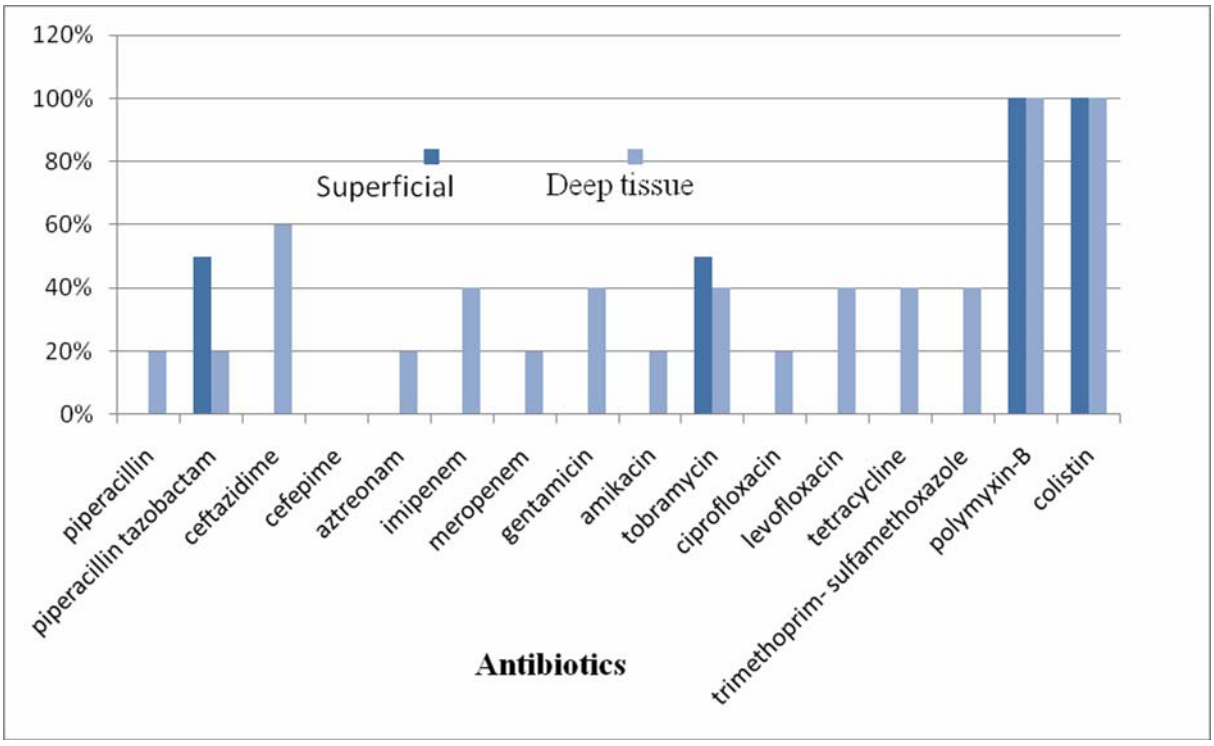


Figure 18: Antibiotic sensitivity of Acinetobacter of superficial and deep samples



DISCUSSION:

This study highlights the importance of appropriate samples to be collected from infected diabetic foot ulcers to isolate the pathogens. The study was undertaken to isolate specific bacterial pathogens causing DFI's and to compare the bacterial isolates of superficial swab and punch biopsy/deep tissue specimens, to evaluate and assess the antimicrobial sensitivity pattern of the infecting and colonizing organisms, and finally to help the treating consultant to choose an appropriate antibiotic and to assess the response.

Previous studies state that superficial swab sample was insufficient for the surgeon to decide on the most appropriate therapy.[47,48] However the study in South-western Nigeria found no difference in the microorganisms of the superficial swab and Deep tissue. [28]

Diabetic foot infections are commonly multimicrobial. Most commonly in most of the hospitals in India, just a swab is collected from the superficial aspect of the foot ulcer and sent for microbiological study to isolate organisms and frequently empiric antibiotics are started and if necessary altered according to those culture results. However this superficial sample may not show the actual pathogen or pathogens and the antibiotic therapy may not be appropriate.

In a study in France from 2003 to 2007, in implementing the guidelines for obtaining specimens for culture from patients with diabetic foot infections, A. Sotto et al have indicated clearly that after 2003, superficial swab collections were stopped and only the deep tissue culturing was done, in identifying the pathogens of DFIs.[49]

As surface swabs of decubitous ulcers, swab samples of encrusted walls of abscesses, mucosal linings, and eschars are not the samples to be processed for anaerobes, according to the standard text books [50,51,53] and references,[52] we did not proceed to look for anaerobes in the superficial samples. However our pilot study did not yield anaerobes from the superficial samples and hence we stopped anaerobic culture of superficial swabs.

Hence., in this study, 2 different samples, superficial swab samples which were routinely sent to our laboratory from the Surgery and Medicine departments and deep tissue biopsy were collected and processed simultaneously from each of 50 subjects.

A total of 348 bacterial isolates were cultured from the 50 cases from both superficial samples and deep samples of DFIs.Of these 89(25.57%) were from superficial and 135(38.79%) from deep. Altogether, 224(64.36%) were aerobes.

Aerobic gram positive bacteria were 95(27.29%) of which 37(10.63%) were from superficial & 58(16.66%) from the deep. Among these, total aerobic gram positive cocci were 80(22.98%) of which, 22(6.32%) from superficial & 58(16.66%) were from the deep. Staphylococci were 40(11.42%) of which S.aureus 31(8.9%) & CONS 9(2.58%), BH Streptococci 16(4.59%), Enterococci 33(9.48%), Corynebacterium species 6 (1.72%) of gram positive isolates.

The total aerobic GNB were 133 isolates, of which 56 (42 %) were from superficial & 77 (57.89%) were from deep. Escherichia coli constituted 26 (19.54%) isolates, of total aerobic Gram negative rods, with 11(8.27%) from superficial &15 (11.27%) from the

Deep. Klebsiella pneumoniae were 20 (15.54%) isolates, with 7 (5.26%) from superficial & 13(9.77%) from the Deep. Enterobacter species 14(10.52%) isolates, of which 6 (4.51%) from superficial & 8 (6%) from deep. Citrobacter diversus 6 (4.5%) isolates, of which 2(1.5%) from superficial & 4(3%) from deep. Proteus species made up of Proteus mirabilis & Proteus vulgaris accounted for 20(15.54%) of aerobic Gram negative rods. Providencia rettgeri 7(5.36%) isolates.

Pseudomonas aeruginosa comprised 18(13.5%) and Acinetobacter 7(5.2%) of the total gram negative bacilli. Pseudomonas were 10(2.8%) & Acinetobacter 2 (4%) in the superficial samples whereas Pseudomonas was 8(16%) & Acinetobacter 5(0.5%) in the deep tissue.

The total isolates belonging to the family Enterobacteriaceae was 104(29.88%), of which 40(11.49%) were from superficial specimens & 64(18.39%) were from deep tissue specimen. Our study showed out of the total 50 cases,14 (30%) superficial specimens were mono microbial while only 2 (8%) of deep tissue samples were mono microbial (Ref: Annexure)

Thus total anaerobes isolated in the deep tissue specimens was 124(35.63). Peptococci and Peptostreptococci comprised 94% of the anerobes were isolated from 47 cases. Bacteroides species comprised 26% of anerobes. While Propionibacterium species comprised 18%, Fusobacterium comprised 10% of anaerobic isolates. In only 1 case Clostridium species was isolated.

In our study, DF-14 showed no growth in the Superficial sample while the Deep tissue sample isolated 4 aerobes and 3 anerobes indicating the significance of Deep tissue culturing in Diabetic foot ulcers.

The mean no. of organisms in the Superficial specimens was 1.78 & 5.18 in the Deep specimens. The standard deviation was 0.89 in the swab specimens while it was 1.57 in the deep, with a P value <0.001 which is very significant statistically.We also looked at the sensitivity pattern of the isolates of the superficial and deep specimens & compared the sensitivity patterns.

In the family Enterobacteriaceae, other than Ciprofloxacin, Levofloxacin & Trimethoprim sulfamethoxazole, the isolates were more sensitive to all antibiotics in the Deep specimens than in the swab specimens. While the non-fermenters, Pseudomonas & Acinetobacter showed all deep isolates more sensitive than the Superficial isolates to all the antibiotics

ESBLs and Amp C were detected by phenotypic methods according to CLSI 2010 guidelines.[46] Among Enterobacteriaceae 18isolates were found to be ESBL positive, with 5 Escherichia coli, 5 Klebsiella pneumoniae, 4 Enterobacter species, 2 Proteus species, 1 Citrobacter species & I Morganella morganii. We did not find resistance to Carbapenems in Enterobacteriaceae isolates. However we found few isolates of Acinetobacter showing Carbapenem resistance.[46]

9 isolates were Amp C producing with 2 Escherichia coli, 2 Enterobacter species,2 Providencia rettgeri, 1Citrobacter freundii, 1 Morganella morganii, 1 Klebsiella pneumoniae. All patients in our study were treated according to the sensitivity of the

isolates from deep tissue. [47] patients received a change in treatment after the sensitivity report was given.

Totally **six** patients in our study were admitted for amputation with very badly infected limbs. With immense cooperation from the surgery and the medicine departments, timely collection of the deep tissue samples and the meticulous culture of all the organisms that infected the foot were studied and the patient was treated according to the sensitivity report. The patients were discharged without amputation, saving their limbs.

Out of 50 badly infected diabetic foot infections, most cases altered the treatment after deep tissue report was given with appropriate antibiotics, with patients responding well to the treatment and the level of amputations were lowered or avoided and mostly discharged with well healed lesions.

However, it is possible that the superficial colonizing or contaminating organisms may be recovered from the deep tissues also while inappropriate collection. This can be avoided to a large extent by careful sampling after thorough cleaning of the superficial aspect, debridement and then taking a punch biopsy under strict aseptic precautions.

Discussion of few cases:

DF-14/10: Patient with cellulitis and ulcer was admitted for amputation and was on parenteral Augmentin and Metrogyl. Pus was aspirated and deep tissue was collected for culture. The aspirated pus showed no growth but the deep tissue isolated 4 aerobic organisms, (Enterococcus species, Enterobacter species, Proteus vulgaris, Klebsiella

pneumoniae) & 3 anaerobic organisms (peptococci, peptostreptococci, propionibacterium species). All aerobic organisms were multi drug resistant but sensitive to Chloramphenicol. Thus the patient was immediately started with oral Chloramphenicol and parenteral Metrogyl. Patient responded very well and was discharged without Amputation.

DF-21/10: Patient had a grade III ulcer , with parenteral Clindamycin and Metrogyl on admission. Organisms isolated in the superficial samples were only Enterobacter agglomerans, Klebsiella pneumoniae. After the deep tissue culture, the organisms isolated were Enterobacter species, Klebsiella pneumoniae, Acinetobacter species and Proteus mirabilis. According to their antibiotic sensitivity, The patient was treated with Oral Doxycycline and Levofloxacin. Patient improved considerably. However patient got discharged against medical advice & after about 2 weeks patient came back with a very badly infected limb and had to be amputated. We treated his infected amputated stump with deep tissue culture successfully (Picture 19 & 20)

DF-26: Superficial sample identified Providentia rettgeri, Enterococcus species, CONS but Deep tissue of this patient isolated MRSA and BH Streptococci(group G) with Providencia rettgeri, Enterococcus species and Escherichia coli withanerobes, Bacteroides fragilis, Peptococci, Peptostreptococci and Propionibactrium species. The patient was started with parenteral Amikacin, oral Linezolid and Metrogyl. The patient improved within one week and was discharged satisfactorily. (picture 17 & 18)

DF-27/10: Patient with grade IV ulcer with Diabetic Ketoacidosis and Septicemia was admitted. The consent was taken for amputation. Surgeons obliged to wait for the Deep

tissue culture report. After a thorough deep tissue study, the organisms isolated were Enterococcus species, Klebsiella pneumoniae and Candida albicans. He was started with parenteral Ciprofloxacin and Metrogyl. With good glycemic control & regular debridement, patient improved drastically and discharged without amputation (picture 21-24).

DF-39/11 : Providencia rettgeri, Staphylococcus aureus (MSSA), Enterococcus species were isolated from the deep tissue with Peptococci and Peptostreptococci. The patient was treated with parenteral Ceftriaxone and Gentamycin with Metrogyl and patient was discharged satisfactorily with a healed wound.

DF-41/11: BH Streptococci (group A), Proteus mirabilis, Peptostreptococci, Peptococci, Fusobacterium, Bacteroides fragilis were isolated from the deep tissue of this patient and was treated with parenteral Penicillin, Gentamicin and Metrogyl after deep tissue culture sensitivity reporting, Patient improved and was discharged without amputation.

DF-46/11: Patient admitted with grade II ulcer for about 2 months. Deep tissue had Candida albicans, with Escherichia coli and Enterococcus species. Candida albicans was not seen in the superficial sample. Patient improved with Oral fluconazole 50 mg OD for 7 days with parenteral Ceftriaxone and Gentamicin and was discharged without amputation.

Superficial swabs which are usually collected for microbiological diagnosis of diabetic foot infections usually shows only surface contaminants. This study though small in number has brought out additional organisms & anaerobes, isolated from deeper tissues in diabetic foot infections. Superficial swabs are not useful for isolation of anaerobes.

Treatment based on superficial swab isolates may not be effective since the actual pathogens are deeper in the tissues which are identified by processing deep tissue biopsy specimen/ aspirated pus.

Deep tissue cultures obtained by punch biopsy or aspiration provides the most reliable bacteriologic information in diabetic foot infections. However deep tissue isolates may be contaminated with colonizers during collection but still, deep tissue gives better knowledge on the infecting microorganisms and avoids antibiotics to be directed only against superficial contaminants.

Hence we recommend that there should be a uniform policy to collect deeper tissue for microbiological study of DFIs. Collection of superficial or surface swabs from the ulcers or wounds should be discouraged or totally avoided and in every hospital this should be communicated to the treating consultant and the clinical microbiologist.

Depending upon the microbiological data from deep tissue samples in DFIs an appropriate empiric therapy of antibiotic policy could be developed in each hospital or health care facility where DFIs are routinely treated.

In this study we found that empirically a combination of an Aminoglycoside, a Fluoroquinolone or Linezolid and Metrogyl or Clindamycin proved useful in the treatment of DFIs. Depending upon the organisms isolated from the deep tissues and their antibiotic sensitivity patterns, the therapy can be de-escalated or changed to the sensitivity of the etiological agents.

This in some cases may avoid un necessary amputations which has happened in 6 of our cases. Needless to say that this is a great benefit to the patient with DFIs. Furthermore early identification of the microorganisms and appropriate therapy promptly will reduce the further complications of DFIs.

We do not recommend the use of Carbapenems routinely, unless there is an overwhelming systemic infections such as septicemia or septic shock.

CONCLUSION

1. Collection of Superficial swab for the diagnosis of Diabetic foot infections for etiological diagnosis is not appropriate and should be avoided or stopped and has to be made aware to all Clinicians and Microbiologists.

2. A punch biopsy from deeper tissues after debridement should be collected aseptically for microbiological studies.

3. Preferably both aerobic and anaerobic cultures should be done to isolate and identify the infecting organisms.

4. Most diabetic foot infections are poly microbial in nature. Antibiotic sensitivity should be studied at least for all the aerobic isolates. Therapy should be directed against all bacteria isolated according to sensitivity patterns and if anaerobes are present, anti-anaerobic therapy should be included.

5. Good wound debridement, glycaemic control and appropriate antibiotic treatment can save the patient from complications of DFIs and also save the limbs from amputations.

Summary

This study was conducted to evaluate the micro-organisms of superficial swab and the deep tissue. Specimen collection with swab has been abandoned in many countries and switched over to the deep tissue sampling, but in most hospitals in India this is the main method of sample collection especially in diabetic foot infections. This study revealed that swab does not isolate all organisms infecting the foot and deep tissue helps in identifying the real pathogens including the anaerobes. A thorough deep tissue study could help the treating physician/surgeon in choosing the right antibiotic and at several times avoid amputations and save the limb.

REFERENCES

1.Khanolkar MP, Bain SC, Stephens JW. The Diabetic Foot. QJ Med 2008;101:685-695.

2.Bronze MS. Diabetic Foot Infections. Medscape reference. 2011 jul. Available from:http://emedicine.medscape.com/article/237378-overview

3.Lipsky BA, editor. Optimizing antimicrobial therapy in Diabetic Foot Infections: Proceedings of symposium;2009;Feb;Chennai.

4.Lipsky BA, Berendt AR, Derry G, Embil JM, Joseph WS, Karchmer AW, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004;39:885-910.

5.Swartz MN, Pasternack MS. Cellulitis and sub cutaneous tissue infections.In :Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases. 6th ed. Philadelphia USA:Elsevier,2005:1172-1193.

6.Ventura CS. The History of Diabetes Mellitus-A Maltese perspective, Malta 2002.Available from: http://staff.um.edu.mt/csav1/books/diabetes_hist.pdf

7.Tattersall RB. The History of Diabetes Mellitus. In:Holt R, Cockram C, Flyvberg A, Goldstein B, eds. Text book of Diabetes. 4th ed. Nottingham UK:Blackwell,2010:1-22.

8.Moses SG. "Diabetic foot syndrome" – A few Dictums. Int J DiabDev Countries 1994;14:64-65.

9.Siitonen OI, Niskanen LK, Laakso M, Siitonen JT, Pyorala K. Lower-extremity amputations in diabetic and non-diabetic patients. A population-based study in eastern Finland. Diabetes Care 1993; 16:16–20.

10.Trautner C, Haastert B, Giani G, Berger M. Incidence of lower limb amputations and diabetes. Diabetes Care 1996;19:1006–009.

11.Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower extremity ulcers in patients with diabetes from two settings.Diabetes Care 1999; 22:157–62.

12.Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. Jama 2005; 293:217–28.

13.Tentolouris N, Al-Sabbagh S, Walker MG, Boulton AJ, Jude EB. Mortality in diabetic and nondiabetic patients after amputations performed from 1990 to 1995: a 5-year follow-up study. Diabetes Care 2004; 27:1598–604.

14.Vileikyte L. Diabetic foot ulcers: a quality of life issue. Diabetes Metab Res Rev 2001; 17:246–49.

15.Diabetes facts-World Diabetes Foundation. 5th May 2011. Available from:http://www.worlddiabetesfoundation.org/composite-35.htm

16. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J PatholMicrobiol 2008;51:204-208.

17.Joshi SR. Management of Obese Indian Patient. Indian Journal of Obesity. 2005; I (1) : 11-20.

18. Viswanathan V, Pendsey S, Bal A. Diabetic foot in India. Medicine update 2005:220-222.

19. Viswanathan V, Thomas N, Tandon N, Asirvatham A, Rajasekar S, Ramachandran A, Senthilvasan K et al.Profile of Diabetic Foot Complications and its Associated Complications - A Multicentric Study from India.JAPI 2005;53:933-936

20.Vijay V, Snehalatha C, Ramachandran A. Socio-cultural practices that may affect the development of the diabetic foot. IDF Bulletin 1997;42:10–2.

21.Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M, et al. Bacteriology of diabetic foot lesions 2004;22:175-178.

22.Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, Abramson MA. Bacteriology of Moderate – to- severe Diabetic foot infections and In vitro activity of antimicrobial agents. J ClinMicrobiol 2007;45:2819-2828

23.Sotto A, Richard JL, Combescure N, Jourdan N, Schuldiner S, Bouziges N, Lavigne JP. Beneficial effects of implementing guidelines on microbiology and costs of infected diabetic foot ulcers. Diabetologia 2010;53:2249-2255.

24.Bowler PG, Davies BJ. The microbiology of infected and non-infected leg ulcers. Int. J. Dermatol 1999; 38:573–578.

25.Voneiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect. Dis.2002; 2:677–685.

26.Wall IB, Davies CE, Hill KE, Wilson MJ, Stephens P, Harding KG et al.Potential role of anaerobic cocci in impaired human wound healing. Wound Repair Regen.2002; 10:346–353.

27.Zeillemaker AM., Veldkamp KE, Van Kraaij MG, Hoekstra JB, Papendrecht AA, DiepersIootRJ. Piperacillin/tazobactam therapy for diabetic foot infection. Foot Ankle Int. 1998;19:169–172.

28.Nai AA, Ikem IC, Akinloye OO, Ikem RT, Kassim OO. Characterization of bacterial isolates from diabetic foot infections in Ile Ife, Southwestern Nigeria. The Foot 2006;16:158-164.

29.Lipsky BA. Osteomyelitis of the foot in diabetic patients. Clin Infect Dis 1997;25: 1318- 26.

30.Lipsky BA. Medical treatment of diabetic foot infections. Clin Infect Dis 2004;39 Suppl 2:S104-14.

31.Dang CN, Prasad YD, Boulton, AJ, Jude, EB. Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet Med 2003;20:159–161.

32.Game, F, Jeffcoate W. MRSA and osteomyelitis of the foot in diabetes. Diabet Med 2004;21 :16–19.

33.Anaerobe Laboratory. Outline of clinical methods in anaerobic bacteriology, 2nd revision. Virginia Polytechnic Institute and State University;Blacksburg, Va :1970.

34.Aldridge KE, Ashcraft D, Cambre K, Pierson CL, Jenkins SG, Rosenblatt JE. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of Bacteroides fragilis group, Prevotella, Fusobacterium, Porphyromonas, and Peptostreptococcus species. Antimicrob Agents Chemother 2001;45:1238-43.

35.Baron EJ, Citron DM. Anaerobic identification flowchart using minimal laboratory resources. Clin Infect Dis 1997;25 Suppl 2:S143-6.

36.Fierer, J, Daniel D, Davis C. The fetidfood; lower extremity infections in patients with diabetes mellitus. Rev. Infect. Dis1979;1:210-217.

37.Chen SC, Gottlieb T, Palmer JM, Morris G, Gilbert GL. Antimicrobial susceptibility of anaerobic bacteria in Australia. J Antimicrob Chemother1992;30:811-20.

38.Wybo I, Piérard D, Verschraegen I, Reynders M, Vandoorslaer K, Claeys G, et al. Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. J AntimicrobChemother 2007;59:132-9.

39.Falagas ME, Siakavellas E. Bacteroides, Prevotella, and Porphyromonas species: a review of antibiotic resistance and therapeutic options. Int J Antimicrob Agents 2000;15:1-9.

40.Catherine AS. Colayco M.D, Mendoza MT, Marissa MD, Alejandria M, Concepcion F. Microbiologic and Clinical Profile of Anaerobic Diabetic Foot Infection. Phil J Microbiol Infect Dis 2002; 31(4):151-160

41.Smith RG.Validation of Wagner's Classification: A Literature Review. Available from:http://www.o-wm.com/content/validation-wagners-classification-a-literature-review

42.Oyibo S, Jude EB, Tarawneh I, Nguyen H, Harkless LB, Boulton AJ. 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.

43.Wagner Classification of Diabetic Foot Ulcers. Available from: http://www.woundbegone.com/knowledgebase/?p=132

44.Schaper NC, Diabetic foot ulcer classification system for research purposes. DiabetMetab Res Rev. 2004;20(SUPPL I): S90-S95.

45.Lavery LA, Armstrong DG, Murdoch DP. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. Clin Infect Dis2007;44:562-565.

46.Clinical and Laboratory Standards Institute (2010). Performance Standards for Antimicrobial Susceptibility Testing-Nineteenth Informational Supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

47.Kelkar U, Kagal A, Bacteriology of diabetic ulcers:effect of sample collection method. Diab Foot 2004;(Autumn)

48.Senneville E, Melliez H, Beltrand E, Legout L, Valette M, Cazaubiel M et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: Concordance with ulcer swab cultures. Clin Infect Dis 2006;42:57-62.

49.Sotto A, Richard JL, Combescure N, Jourdan N, Schuldiner S, Bouziges N, Lavigne JP. Beneficial effects of implementing guidelines on microbiology and costs of infected diabetic foot ulcers. Diabetologia 2010;53:2249-2255.

50.The Anaerobic infections. In:Winn W, Allen S, Janda W, Koneman E, Procop G, ASchreckenberger P et al. Koneman'scolor Atlas & Textbook of Diagnostic microbiology.6th ed. Baltimore MD: Lippincott Williams & Wilkins,2006:877-941.

51.Allen SD, Emery CL, Siders JA. Anaerobic bacteriology. In: Truant AL, ed. Manual of Commercial methods in Clinical Microbiology. Washington, D.C., ASM Press, 2002:50-81.

52.Allen SD, Siders JA, Marler LM. Current issues and problems in dealing with anaerobes in the clinical laboratory. Clin Lab Med 1995;15:333-364.

53.Arnon SS. Infant botulism. In:Finegold SM, George WL,eds. Anaerobic infections in humans. New York, NY,Academic Press, 1989:601-609.

54.Capobianco CM, Stapleton JJ. Diabetic foot infections : a team oriented review of medical and surgical management. Diabetic foot and ankle 2010;1:5438-5844.

55.Ramakant P, Verma AK, Misra R, Prasad KN, Chand G, Msira A et al. Changing microbiological profile of pathogenic bacteria in diabetic foot infections : time for a rethink on which empirical therapy to choose? Diabetologia 2011;54:58-64.

56.Gadepalli R, Kapil A, Dhawan B, Ammini AC, Sreenivas V, Chaudhry R. A clinico - microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29:1727-1732.

57.Colayco CA, Mendonza MT, Alejandria MM, Ang CF. Microbiologic and clinical profile of anaerobic diabetic foot infections. J Microbiol Infect Dis 2002;31:151-160.

58.Bansal E, Garg A, Bhattia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J PatholMicrobiol 2008;51:204-208.

59.Sharma VK, Khadka PB, Joshi A, Sharma R. Common pathogens isolated in diabetic foot infection in Bir hospital. Kathmandu Univ Med J 2006;15:295-301.

60.Lily SY, Kwang LL, Yeow SC, Tan TY. Anaerobic culture of diabetic foot infections : Organisms and antimicrobial susceptibilities. Ann Acad Med Singapore2008;37:936-9.

61.Abdulrazak A, Bitar ZI, Al-Shamali AA, Mobasher LA. Bacteriological study of diabetic foot infections. J. Diabetes Complications2005; 19: 138– 141.

62. Dhanasekaran G, Sastry G, Viswanathan M. Microbial pattern of Soft tissue infections in diabetic patients in South India. Asian J. Diabet. 2003;5: 8–10.

63.Doern GV, Ferraro MJ, Gilligan PH, Janda M, von Graevenitz A eds. Bacteriology (sec IV). In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (Eds.): Manual of Clinical Microbiology. 8th ed. Washington: ASM Press. pp. 249-831.

64.Fosse S, Hartemann-Heurtier A, Jacqueminet S, Ha Van G, Grimaldi A, Fagot-Campagna A. Incidence and characteristics of lower limb amputations in people with diabetes Diabet. Med 2003;26: 391–396.

65.Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R.A Clinico-microbiological Study of Diabetic Foot Ulcers in an Indian Tertiary Care Hospital. Diabetes Care 2006; 29: 1727-1731.

66.Ge Y, MacDonald D, Hait H, Lipsky B, Zasloff M, Holroyd K Microbiological profile of infected diabetic foot ulcers. Diabet Med 2002; 19: 1032–1035.

67.Lipsky BA, Berendt AR, Gunner Deery H, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS (2004). Infectious Diseases Society of America. Diagnosis and Treatment of Diabetic Foot Infections. CID, 39: 885–910.

68.Raja NS. Microbiology in diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. J. Microbiol. Immunol. Infect 2007;40: 39-44.

69.Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial aetiology of diabetic foot infections in South India. Eur. J.Int. Med 2005;16: 567–570.

70.Schaper NC, Prompers LM, HuijbertsMS.Treatment of Diabetic Foot Ulcers.ImmunEndoc&Metab Agents in Med Chem 2007;7:95-104

Sl No.	Age yrs	Sex	Address	Ip/ Op No.	Lab No.	H/O Diabetes (yrs)	Anti Diabetic Treatment	Diab Control	H/O Ulcer	Antibiotic Administered
1	76	F	New Extension Kolar	568368	DF-1	9yrs	Regular	Poor	2 Weeks	No
2	75	M	Thondenahally Mundavadi Post Kolar	582599	DF-2	11yrs	Not Regular	Poor	3 Months	No
3	70	M	Hanchala Kolar	585017	DF-3	7yrs	Not Regular	Poor	1 Week	No
4	65	M	Mulbagal Kolar Dist.	585018	DF-4	5yrs	Regular	Poor	3 Months	T.Ciplox 5 Days
5	65	M	Gangadanellur ChittorTaluk	597956	DF-5	8yrs	Regular	Poor	3 Months	Inj.Ceff One Week
6	74	M	ChowdareddyPalya Chinthamani	604463	DF-6	15yrs	Not Regular	Poor	6 Months	No
7	55	M	DoomLight Circle Kolar	604162	DF-7	5yrs	Regular	Poor	3 Days	No
8	48	M	Malur	255235	DF-08	2yrs	Not Regular	Poor	1 Week	No
9	67	M	Sugutur Kolar	200235	DF-09	10yrs	Not Regular	Poor	4 Months	Bactoclav
10.	60	M	Hoskote	256683	DF-10	14yrs	Not Regular	Poor	1 Month	No
11	52	M	Bangarpet	257475	DF-11	1 week	Regular	Good	1 Week	Augmentin Metrogyl
12	45	M	Malur	256902	DF-12	1 week	Regular	Good	2 Months	No
13	45	F	Malur	258382	DF-13	8yrs	Regular	Good	2 Months	No
14	70	M	Srinivaspura	259383	DF-14	1 week	Started	Good	1 Week	Augmentin Metrogyl
15	52	M	Malur	259515	DF-15	1 week	Started	Good	10 Days	No
16	61	M	V Kote	260119	DF-16	7 yrs	Regular	Good	1 Day	No
17	62	M	Bangarpet	258373	DF-17	14yrs	Not Regular	Poor	2 Months	Monoceff
18	58	M	Mulbagal	261135	DF-18	2 weeks	Started	Good	2 Months	Augmentin Metrogyl From 10 Days
19	35	M	Vempalli Srinivaspura Kolar	624133	DF-19	2 weeks	Started	Good	2 Weeks	NO
20	70	M	Kempapura	261720	DF-20	1yr	Not Regular	Poor	8 Days	NO
21	65	M	Malur Kolar	262256	DF-21	22yrs	Regular	Poor	10 Days	Clindamycin Metrogyl
22	60	M	Hudukula Kolar	619534	DF-22	13yrs	Not Regular	Poor	2 Months	No
23	65	M	Sugutur, Kolar	600135	DF- 23	4	Not Regular	Poor	8 days	No
24	73	M	Chinthamani	602362	DF-24	15	Not Regular	Poor	6 months	No
25	65	M	Vemgal, Kolar	266699	DF-25	14	Not Regular	Poor	2 weeks	No
26	55	F	Thyavarahaly Kolar	266739	DF-26	5	Regular	Good	3 months	Levofloxacin

27	59	M	Gn Road Vijayapura	269693	DF-27	8	Not Regular	Poor	1 week	
28	50	M	PatalammaBadavane Malur	267225	DF-28	4	Not Regular	Poor	1 month	Inj.Ceff Inj.Metrogyl Inj.Amikacin
29	35	M	Channakal V Malur	265823	DF-35	1 mnth	Regular	Fair	15 days	Inj.Monoceff Inj.Ornidazole T.Ciplox
30	55	M	Swarna Nagar Kgf	270335	DF- 36	10 Yrs	Not Regular	Poor	20 days	Inj.Bactoclav Inj.Metrogyl
31	68	M	Krs Temple Street Mulbagal	269434	DF-37	1 Yr	Not Regular	6 Months	6 months	Inj.Ciplox Inj.Montaz Inj.Amikacin
32	55	M	Vemgal, Kolar	267871	DF-38	2 Yrs	Not Regular	Poor	2 months	Inj.Ceff Inj.Ornidazole Inj.Amikacin
33	48	M	Thyavarahaly Kolar	269942	DF-39	1	Regular	Good	1 month	Inj.Ceff Inj.Amikacin Inj. Metrogyl 1 Week
34	50	M	Doddipalli Chittoor	267038	DF-40	3 Yrs	Not Regular	Poor	1 month	Inj.Ceff
35	35	F	Pc Extension Kolar	652618	DF-41	5 Yrs	Not Regular	Good	2 weeks	Inj.Ceff
36	45	M	Hudukula Kolar	652698	DF-36	8	Not Regular	Poor	8 months	Inj.Bactoclav Inj.Metrogyl
37	52	M	Sugutur, Kolar	653602	DF- 37	3	Regular	Good	1 month	No
38.	67	M	Chinthamani	273112	DF-38	12	Not Regular	Poor	1 year	Inj.Ceff Inj.Metrogyl
39	35	M	Vemgal, Kolar	276001	DF-39	6	Not Regular	Poor	1 month	
40.	60	M	Kamandahally Kolar	2275799	DF-40	8	Not Regular	Poor	1 month	Inj.Ceff Inj.Metrogyl
41	50	F	DoddapetChintamani	277454	DF-41	13	Not Regular	Poor	2 months	--
42	65	F	Kamanur Village Mulbagal	277453	DF- 42	15	Not Regular	Poor	1 month	-
43.	50	M	Thayalur Mulbagal	277451	DF-43	3	Not Regular	Poor	2 days	Ceff Metrogyl
44	35	F	Kodagodi Bangalore	277622	DF-44	4	Not Regular	Poor	1 year	Ceff Metrogyl
45	67	M	Sugutur Kolar	273951	DF-45	12	Not Regular	Poor	1 year	-
46	51	M	Chinthamani	277841	DF-46	20	Not Regular	Poor	2 months	-
47	61	M	Kolar	278134	DF-47	10	Not Regular	Poor	20 days	InjCiplox Inj. Clindamycin
48	80	M	Kolar	279718	DF 48	20	Not Regular	Poor	1 month	Inj. Augmentin Inj.Ornida
49	53	M	Chinthamani	279465	DF-49	12	Not Regular	Poor	1 month	-
50	45	M	Mulbagal	281242	DF-51	10	Not Regular	Poor	10 days	

Sl no.	**Superficial samples**	**Deep tissue samples**	
	Aerobes	**Aerobes**	**Anaerobes**
DF-1	Enterococcus species CONS	Enterococcus sp. Staphylococcus aureus(MSSA)	Peptococci Peptostreptococci
DF-2	Enterococcus species Proteus vulgaris CONS	Enterococcus sp. Proteus vulgaris	Bacteroides sp. Fusobacterium sp. Peptococci Peptostreptococci
DF-3	Escherichia coli	Staphylococcus Aureus(Mssa) Escherichia Coli	Bacteroides Sp. P.Melaninigenica Propionibacterium Peptococci Peptostreptococci
DF-4	Staphylococcus aureus(Mssa)	Staphylococcus aureus (MSSA) Escherichia Coli	Bacteroides sp. Peptococci Peptostreptococci
DF-5	Staphylococcus aureus(MRSA) Proteus vulgaris Morganella morganii	Staphylococcus aureus(MRSA) Morganella morganii Enterobacter species Proteus mirabilis	Peptococci Peptostreptococci
DF-6	Enterococcus species CONS Escherichia coli	Proteus mirabilis Escherichia coli Enterobacter species Citrobacter diversus	Fusobacterium sp. Peptococci Peptostreptococci
DF-7	Enterococcus species	Enterococcus sp. MRSA Escherichia coli	Bacteroides sp. Fusobacterium sp. Peptococci Peptostreptococci
DF-8	MSSA Diphtheroids	MSSA Enterococcus faecalis Proteus vulgaris	Peptococci Peptostreptococci Bacteroides species
DF-9	Citrobacter diversus Morganella morganii CONS	Citrobacter diversus Morganella morganii Enterococcus faecalis Klebsiella pneumoniae	Peptococci Peptostreptococci
DF-10	Escherichia coli Proteus vulgaris Enterococcus faecalis	Enterococcus faecalis Proteus vulgaris Klebsiella pneumoniae MSSA	Bacteroides species Peptococci Peptostreptococci
DF-11	Pseudomonas aeruginosa CONS providencia rettgeri	Pseudomonas aeruginosa Providentia rettgeri Enterococcus faecalis	Peptococci Peptostreptococci

DF-12	Pseudomonas aeruginosa	Pseudomonas aeruginosa Morganella morganii Enterococcus faecalis	Peptococci Peptostreptococci
DF-13	MSSA Enterobacter species CONS	MRSA Klebsiella pneumoniae Enterococcus faecalis Acinetobacter species	Peptococci Peptostreptococci
DF-14	No growth	Enterococcus faecalis Enterobacter species Proteus vulgaris Klebsiella pneumoniae	Peptococci Peptostreptococci Propionibacterium species
DF-15	Pseudomonas aeruginosa Proteus vulgaris Klebsiella pneumoniae	Proteus vulgaris Klebsiella pneumoniae Enterococcus faecalis	Peptococci Peptostreptococci Propionibacterium species Bacteroides species
DF-16	Escherichia coli	MSSA Escherichia coli	Peptococci Peptostreptococci
DF-17	Klebsiella pneumoniae Morganella morganii MRSA	Klebsiella pneumoniae Morganella morganii Enterococcus faecalis MRSA	Propionibacterium species Peptococci Peptostreptococci
DF-18	Pseudomonas aeruginosa	Pseudomonas aeruginosa	No growth
DF-19	Proteus mirabilis Klebsiella pneumoniae Enterobacter species	Proteus mirabilis Klebsiella pneumoniae Citrobacter freundii MSSA	Peptococci Peptostreptococci
DF-20	Enterococcus faecalis CONS	MRSA Enterococcus faecalis	Peptococci Peptostreptococci
DF-21	Enterobacter agglomerans Klebsiella pneumoniae	Enterobacter species Klebsiella pneumoniae Acinetobacter species Proteus mirabilis	Bacteroides sp. Peptococci Peptostreptococci
DF-22	Pseudomonas aeruginosa	Klebsiella pneumoniae Enterobacter species	Peptococci Peptostreptococci Propionibacterium species
DF-23	Citrobacter diversus β- haemolytic streptococci (group a)	Citrobacter diversus Streptococci (group a) MSSA Escherichia coli	Bacteroides sp. Fusobacterium sp. Peptococci Peptostreptococci
DF-24	Morganella morganii β- haemolytic streptococci (group a)	Morganella morganii Escherichia coli Streptococci(group a)	Peptococci Peptostreptococci

Df-25	Providentia rettgeri Escherichia coli	Providentia rettgeri Klebsiella pneumoniae	Clostridium novyii Peptococci Peptostreptococci Propionibacterium sp Prevotella melaninogenica
Df-26	Providentia rettgeri Enterococcus faecalis CONS	Providentia rettgeri MRSA Streptococci (group g) Escherichia coli Enterococcus faecalis	Bacteroides fragilis Peptococci Peptostreptococci Propionibactrium species
Df-27	Candida albicans Klebsiella pneumoniae	Enterococcus faecalis Klebsiella pneumoniae Candida albicans	Peptococci Peptostreptococci
Df-28	Citrobacter diversus Acinetobacter species	Acinetobacter species Enterobacter species	Peptococci Peptostreptococci Bacteroides .fragilis
DF-29	Pseudomonas aeruginosa Enterobacter agglomerans	Pseudomonas aeruginosa Staphylococcus aureusMRSA) B- haemolytic streptococci (group a)	Peptococci Peptostreptococci
DF-30	Escherichia coli	Escherichia coli Enterococcus faecalis	Peptococci Peptostreptococci Bacteroides .fragilis
DF-31	staphylococcus aureus(MSSA)	MSSA Escherichia coli	Peptococci Peptostreptococci
DF-32	Pseudomonas aeruginosa Klebsiella pneumoniae Enterobacter species	Pseudomonas aeruginosa Klebsiella pneumoniae Enterobacter species Enterococcus faecalis	Peptococci Peptostreptococci
DF-33	Pseudomonas aeruginosa	Pseudomonas aeruginosa	not found
DF-34	Enterobacter agglomerans Escherichia coli	Enterobacter agglomerans Staphylococcus aureus(MRSA)	Peptococci Peptostreptococci Bacteroides fragilis
DF-35	b-hemolytic Streptocci(group a)	b-hemolytic Streptococci (group a) Morganella morganii	Peptococci Peptostreptococci
DF-36	β-hemolytic Streptococci (group A)	B- hemolytic Streptococci (group A) Acinetobacter species Pseudomonas aeruginosa	Peptococci Peptostreptococci
DF-37	Staphylococcus aureus(MSSA)	Staphylococcus aureus(MSSA)	Peptococci Peptostreptococci

DF-38	Acinetobacter species Diphtheroids	Acinetobacter species Proteus mirabilis	Peptococci Peptostreptococci
DF-39	Enterococcus faecalis CONS Providencia rettgeri	Providentia rettgeri Staphylococcus aureus (MSSA) Enterococcus faecalis	Peptococci Peptostreptococci
DF-40	Pseudomonas aeruginosa	Pseudomonas aeruginosa	not found
DF-41	Pseudomonas aeruginosa proteus mirabilis	B-haemolytic Streptococci (group A) Proteus mirabilis	Peptostreptococci Peptococci Fusobacterium Bacteroides fragilis
DF-42	Diphtheroids B-haemolytic Streptococci (group A)	B-haemolytic Streptococci (group A) Proteus mirabilis Enterococcus species MSSA	Peptococci Peptostreptococci Propionibacterium
DF-43	Escherichia coli Diphtheroids	Escherichia coli Enterococcus .species	Peptococci Peptostreptococci
DF-44	Escherichia coli	Enterococcus species Bhaemolytic Streptococci (group A) Escherichia coli	Peptococci Peptostreptococci
DF-45	MSSA Proteus mirabilis Diphtheroids	MSSA B haemolytic Streptococci (group A) Proteus mirabilis	Peptococci Peptostreptococci Propionibacterium
DF-46	Escherichia coli Enterococcus species	Escherichia coli Enterococcus species Candida albicans	Peptococci Peptostreptococci
DF-47	Diphtheroids	Escherichia coli Enterococcus faecalis	Peptococci Peptostreptococci
DF-48	Klebsiella pneumoniae CONS	Klebsiella pneumoniae Morganella morganii Enterococcus faecalis	Peptococci Peptostreptococci
DF-49	Escherichia coli	Escherichia coli MRSA MSSA	Peptococci Peptostreptococci
DF-50	Enterococcus species CONS	B haemolytic Streptococci (group G) Enterococcus faecalis MSSA	Peptococci Peptostreptococci

CONSENT

I, Mr. / Miss ……. Attending R.L.J.H & R.C voluntarily give permission to test the samples for different tests for the diagnosis of Diabetic foot infection. I am made aware that the results of test will be communicated to the treating physician as and when the results are available.

Date:

Participant
Name:
Sig:

Consent in Case of Minor

I, Mr. / Mrs…….Guardian/ Parent of the child, Voluntarily give permission to test the samples for different tests for the diagnosis of diabetic foot infections. I am made aware that the results of the tests will be communicated to the treating physician as and when the results are available.

Date:

Guardian / Parent
Name:
Signature: