STUDY ON BACTERIOLOGICAL PROFILE OF WOUND INFECTIONS IN TERTIARY CARE HOSPITAL

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University

In partial fulfillment of the regulations

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH – IV

GOVT. KILPAUK MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA.

APRIL 2011

CERTIFICATE

This is to certify that this dissertation entitled "**STUDY ON BACTERIOLOGICAL PROFILE OF WOUND INFECTIONS IN TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr.B.RAVICHANDRAN,** Post graduate in Microbiology, under my overall supervision and guidance in the department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

Dr.V.KANAKASABAI , M.D.,
Dean,
Govt. Kilpauk Medical College,
Chennai-600 010.

Dr.THYAGARAJAN RAVINDER, M.D.,
Professor & Head,
Department of Microbiology
Govt. Kilpauk Medical College,
Chennai-600 010

DECLARATION

I solemnly declare that this dissertation **" STUDY ON BACTERIOLOGICAL PROFILE OF WOUND INFECTIONS IN TERTIARY CARE HOSPITAL"** is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr.THYAGARAJAN RAVINDER, M.D.,** Professor and Head, Department of Microbiology, Govt. Kilpauk Medical College, Chennai-10.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in April 2011.

Place : Chennai.
Date :

Dr.B.RAVICHANDRAN

AKNOWLEDGEMENT

I humbly submit this work to the Almighty who has given the health and ability to pass through all the difficulties in the compilation and proclamation of this blue print.

I wish to express my sincere thanks to our Dean ***Dr.V.KANAKASABAI , M.D.,*** *for permitting me to use the resources of this institution for my study.*

I feel indebted to Prof. ***Dr.THYAGARAJAN RAVINDER, M.D.,*** *Professor & Head, Department of Microbiology, Govt. Kilpauk Medical College, for his constant encouragement, innovative ideas, and timely suggestion during my work.*

I owe special thanks to my professors ***Dr.RADHIKA KATRAGADDA, M.D.,DA., Dr.K.V.LEELA, M.D.,DGO.,*** *Department of Microbiology, Govt. Kilpauk Medical College, for their constant support, invaluable suggestions, erudite guidance in my study and for being a source of inspiration in my endeavours.*

I express my thanks and gratitude to our former Professor and Head, ***Dr.NIYANTHRINI SRIDHAR, M.D.,*** *for her guidance and support.*

I express my sincere thanks to our Assistant Professors ***Dr.S.MEENAKSHI, M.D.,DNB., Dr.J.RAJA, M.D., Dr.SUGANTHI, M.D.,DCH.,*** *for their valuable guidance and technical support for my study.*

I would like to thank ***Dr.PRIYA, M.D.,*** *Department of Community Medicine, Govt. Kilpauk Medical College for her statistical guidance and data analysis in my study.*

I would like to thank all my colleagues and technical staff of Department of Microbiology, Govt. Kilpauk Medical College, Chennai for their help.

I would like to thank the Institutional Ethical Committee for approving my study.

I also extend my thanks to all the patients who participated in my study.

Finally I am indebted to my parents for their constant support and encouragement.

CONTENTS

.

Introduction

INTRODUCTION

Skin, the largest organ in the human body, plays a crucial role in the sustenance of life through the regulation of water and electrolyte balance, thermoregulation, and by acting as a barrier to external noxious agents including microorganisms, however , when the epithelial integrity of skin is disrupted, a wound results.[1]

A wound is breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity provides a moist, warm and nutritive environment that is conducive to microbial colonization and proliferation.[2]

In developing countries like India, large number of people die daily of preventable and curable diseases such as wound infections.

Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality.[3,4]

The importance of wound infections, in both economic and human terms, should not be underestimated [5]. In a study on an average, patients with an infected wound stay about 6-10 days more than if the wounds heal without infections[6].

The wound infection depends on a complex interaction between host factors like immunity, nutritional status and age , wound related factors like

magnitude of trauma, dead space, devitalization and presence of hematoma and microbial factors like toxins, invasion and resistance to antibiotics[7].

Most wound infections can be classified into two major categories, skin and soft tissue infections, although they often overlap as a consequence of disease progression[8].

Exogenous wound infection include those associated with traumatic injury or decubitus pressure ulcer, animal or human bites, burns or foreign bodies in skin or mucous membrane.

Endogenous wounds and abscess may be associated with appendicitis, cholecystitis, cellulitis, dental infection, septic arthritis, osteomyelitis, empyema, sinusitis. Most of these processes are nosocomial contracted after invasive procedures, surgical manipulation, placement of prosthesis. Others derived from hematogenous spread from primary site of infection[9].

The potential wound pathogens are Gram positive cocci (Staphylococcus aureus, Streptococcus species, Coagulase negative Staphylococcus , Enterococcus species), Gram negative bacilli(Escherichia coli, Pseudomonas aeruginosa , Klebsiella pneumoniae, Proteus species, Enterobacter species), anaerobes(Bacteroides, Clostridial species)[10] .Wound infections are mostly due to nosocomial pathogens that differ from country to country and from hospital to another within the same region[11], which remains the major source of postoperative morbidity[12].

Wound infection by resistant bacteria worsens the condition[13]. Rapid spread of resistant microbes affected the effectiveness of antimicrobials creating worldwide problem[11]. The condition is serious in developing countries owing to irrational prescription of antimicrobial agents[14]. The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians, and drug companies looking for effective drugs. Measures to control problem include development of new antimicrobial, better infection control program and more appropriate use of existing antimicrobial agents[15,16,17].Many researchers made different recommendations on the susceptibility of microorganisms to drugs[18].

This study aims to find out common bacterial isolate and their antibiotic resistance pattern, the incidence of ESBL producers and MRSA in wound infections.

Review of Literature

REVIEW OF LITERATURE

Background:

Hippocrates (Greek physician and surgeon, 460-377 BC) known as father of medicine, used vinegar to irrigate open wounds and wrapped dressings around wounds to prevent further injury. Galen (Roman gladiatorial surgeon,130AD) was first to recognize that pus from wounds inflicted by the gladiators heralded healing (pus bonum et laudabile). Unfortunately this observation was misinterpreted and the concept of pus preempting wound healing preserved well into the eighteenth century. The link between pus formation and healing was emphasised so strongly that foreign material was introduced into wounds to promote pus formation. The concept of wound healing remained a mystery, as highlighted by the famous saying by Ambrose Pare (French military surgeon, 1510-1590 AD) “I dressed the wound god healed it”.

The scale of wound infections was most evident in times of war. During the American civil war, erysipelas and tetanus accounted for 17,000 deaths, according to an anonymous source in 1883.

Because compound fractures at that time almost invariably were associated with infection, amputation was the only option, despite a 25-90% risk amputation stump infection.

Koch (Professor of Hygiene and Microbiology, Berlin, 1843-1910) first recognized the cause of infective foci as secondary to microbial growth in his nineteenth century postulates. Semmelweis (Austrian Obstetrician, 1818-1865) demonstrated a 5 fold decrease in puerperal sepsis by hand washing between performing postmortem examinations and entering the delivery room. Joseph Lister (Professor of Surgery, London, 1827-1912) and Louis Pasteur (French Bacteriologist, 1882-1895) revolutinised the entire concept of wound infection. Lister recognized that antisepsis could prevent infection. In 1867, Lister placed carbolic acid into open fractures to sterilize the wound and to prevent sepsis and hence the need for amputation. In 1871, Lister began to use carbolic spray in the operating room to reduce contamination. However, the concept of wound suppuration persevered even among eminent surgeons, such as John Hunter(1728-1793.)

World War I resulted in new types of wounds from high velocity bullet and shrapnel injuries coupled with contamination by the mud from the trenches. Antoine Depage (Belgian military Surgeon,(1865-1925) reintroduced wound debridement and delayed wound closure and relied on microbiological assessment of wound brushings as guidance for the timing of

secondary wound closure. Alexander Fleming (Microbiologist, London, 1881-1955) performed many of his bacteriological studies during World War I and is credited with discovery of penicillin.

As late as nineteenth century, aseptic surgery was not routine practice. Sterilization of instruments began in the 1880 as did the wearing of gowns, masks and gloves. Halsted (Professor of Surgery, John Hopkins university, United States, 1852-1922) introduced rubber gloves to his scrub nurse because she was developing skin irritation from the chemicals used to disinfect instruments. The routine use of gloves was introduced by Halsteds student J.Bloodgood.

Penicillin was first used clinically in 1940 by Howard Florey. With the use of antibiotics, a new era in the management of wound infections commenced.

WOUND MICROBIOLOGY :

Microbial Colonization:

Exposed subcutaneous tissue provides a favourable substratum for a wide variety of microorganism to contaminate and colonize, and if the involved tissue is devitalized (Eg. Ischemic, hypoxic or necrotic) and the host immune

response is compromised, the conditions become optimal for microbial growth.

Wound contaminants are likely to originate from three main sources ;

1. The environment (exogenous) microorganism in the air or those introduced by traumatic injury,
2. The surrounding skin (involving members of the normal skin flora such as Staphylococcus epidermidis, Micrococci, Skin Diptheroids and Propionibacteria).
3. Endogenous sources involving mucous membranes (primarily the Gastrointestinal, Oropharyngeal and Genitourinary mucosa)[23] the normal microflora of the gut, the oral cavity, and the vagina are both diverse and abundant and these sources (particularly the oral and gastrointestinal mucosa) supply the vast majority of microorganisms that colonize wounds.

Whereas a minor, healing wound may allow sufficient time for only relatively small number of skin contaminants to take residence, the continued exposure of devitalized tissue associated with a slowly healing chronic wound is likely to facilitate the colonization and establishment of a wide variety of endogenous microorganisms. Dental plague, the gingival crevice,

and the contents of the colon contain approximately 10^{10} microorganism per g of tissue, of which up to 90% of the oral microflora[24] and upto 99.9% of the colonic microflora are anaerobes[25].

In view of this situation it is reasonable to predict that wounds with a sufficiently hypoxic and reduced environment are susceptible to predict that wound with a sufficiently hypoxic and reduced environment are susceptible to colonization by a wide variety of endogenous anaerobic bacteria. However to date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa and beta haemolytic Streptococci are primary causes of delayed healing and infection in both acute and chronic wound. This was so because the isolation of anaerobic bacteria was minimal or omitted, whereas when wounds are investigated by appropriate microbiological techniques anaerobes are found to form a significant proportion of microbial population in both acute and chronic wounds.

Factors Predisposing to Microbial Proliferation :

Surgical wounds will heal rapidly if blood perfusion is maximized, thus delivering O_2, nutrients and cells of the immune systems to the site of injury and providing minimal opportunity for micro organism to colonize and proliferate[26] . In contrast, chronic, non healing wounds are frequently hypoxic as a consequence of poor blood perfusion (ischemia), and host and microbial

cell metabolism contributes further to a lowering of the local PO_2. Thus, cell death and tissue necrosis caused by tissue hypoxia or anoxia are likely to create ideal growth condition for members of wound microflora, including fastidious anaerobes that will proliferate as residual O_2 is consumed by facultative bacteria.

As well as being essential for cell growth and wound healing, O_2 is a critical component of the respiratory burst activity in polymorphonuclear leukocytes resulting in the intracellular production of highly potent antimicrobial metabolites. A Significant reduction in the killing capacity of polymorphonuclear leukocytes at a PO_2 of < 30 mmHg has been reported[27] and in this respect, poorly perused wound tissue is considered to be far more susceptible to infection than are wounds involving well perfused tissue.[28]

Wound infection :

Infection occurs when virulence factors expressed by one or more micro- organisms in a wound compete the host natural immune systems and subsequent invasion and dissemination of microorganism in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulites around a wound[29].

The progression of a wound to an infect state is likely to involve a multitude of microbial and host factors, including the type, site and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the microbial load combined level of virulence expressed by the types of micro organism involved. Most acute and chronic wound infections involve mixed populations of both aerobic and anaerobic micro organisms.

An acute wound usually occurs in a normal, healthy person and is either closed primarily or allowed to close by secondary intention. Most injuries to whole organs or tissues can be considered acute wounds.

Surgical Wound infections:

Definition : Clinically a surgical site is considered to be infected when there is purulent discharge from the incision site [30,31]. According to Centre for disease control (CDC) definition surgical site infection (SSI) is diagnosed on the basis of one of the following.[32]

a) Purulent discharge from a incision site a drain

b) Positive results obtained from culture of fluid obtained from a surgical site closed primarily.

c) Surgeons or attending physician's diagnosis of infection.

d) Surgical site which requires reopening.

Bacteria account for majority of SSI Staphylococcus aureus, Staphylococcus epidermidis and enteric Gram negative bacteria are common in clean surgeries. When surgery involves the gastrointestinal, respiratory or genitourinary tract the pathogens are polymicrobial involving aerobic and anaerobic organisms.

D.C. Berridge et al and Bengt Gastrin et al [33,34] their studies on Orthopaedic surgeries found Staphylococcus aureus and Staphylococcus epidermidis to be the common isolates. Enterobacteriaceae, Enterococci, Streptococcal species, Bacteriodes and Pseudomonas were the other isolates.

Studies involving a large number of generalized wound types have reported overall infection rates of 3.4% in 5129 operations,[35] 4.7% in 62939 operations [36]and 9.4% in 1,770 operations. [37] In the last two studies, the infection rates ranged from 1.5% and 5.9% following clean surgery to 40% and 52.9% following contaminated surgery.

Minimizing the incidence of postoperative wound infection relies on adequate sepsis and antisepsis and preservation of the local host defenses.[38] Asepsis involves the utilization of effective infection control procedures (eg. air filtration, skin barrier garments, disinfection) to minimize exogenous microbial contamination during surgery. Antisepsis involves the use of skin antiseptics on the operative site and also in the case of dirty surgical procedures, administration of prophylactic antibiotics at a time point just

prior to surgery that will ensure adequate tissue levels of antibiotic during surgery.

As part of the surgical procedure, the endogenous and exogenous microbial contamination must be minimized by ensuring good aseptic, skilled surgical techniques and minimizing the duration of surgery, while also optimizing the local wound conditions.[39]. This primarily involves removing any devitalized tissue to re establish blood flow to the wound area thereby maintaining adequate perfusion to enable the delivery of immune cells, oxygen and nutrients and reducing the microbial load.

Acute soft tissue infections:

Acute soft tissue infections include cutaneous abscesses, traumatic wounds and necrotizing infection. In a cataloging of the bacteriology of a large number of cutaneous abscesses (with unspecified individual predisposing causes), Staphylococcus aureus was the single most common aerobic facultative isolate followed in frequency by streptococci, both groupable (A,B,C,D) and non groupable.[40] Among anaerobic isolates Bacteroides species (most commonly Bacteroides fragilis group) were most frequent followed by Peptostreptococcus species and Clostridium species. These abscesses are commonly polymicrobial (mixed aerobic and anaerobic). As might be predicted Staphylococcus is the principle isolate in infections (both abscesses and wounds) of the extremities and trunk, whereas anaerobes

are more numerous than aerobic facultative species in such infections In the genital, perirectal, inguinal, and head and neck areas.

In two studies microbiological investigations have shown that Staphylococcus aureus is the single causative bacterium in approximately 25 to 30% of cutaneous abscesses [41,42]. Staphylococcus aureus has also been recognized as being the most frequent isolate in superficial infections seen in hospital accident and emergency departments. However other studies have demonstrated that approximately 30 to 50% of cutaneous abscesses [41,40] ,50% of traumatic injuries of varied etiology[43,44] and 47% of necrotizing soft tissue infection[45] have a polymicrobial aerobic and anaerobic microflora. Necrotizing soft tissue infection, they involve the skin, (Eg. Clostridial and Non Clostridial anaerobic cellulitis) subcutaneous tissue to the muscle fascia (necrotizing fasciitis) and muscle tissue (Streptococcal myositis and Clostridial myonecrosis).

Cellulitis:

Cellulitis is an acute, spreading infection of the skin that extends deeper and involves the subcutaneous tissues. Group A Streptococcal or Staphylococcal aureus is the most common etiologic agent. Previous trauma (laceration, puncture wound) or an underlying skin lesion (furuncle, ulcer) predisposes to the development of cellulitis. Occasionally, Cellulitis results from blood- borne spread of infection to the skin and subcutaneous tissues;

rarely, it is caused by direct spread from subjacent infections (subcutaneous abscesses. fistulas from osteomyelitis).

Cellulitis is a serious disease because of the propensity of infection to spread (via)lymphatics and blood stream. Cellulitis of the lower extremities in older patients may be complicated by thrombophlebitis. A polymorphonuclear leukocytosis is usually present regardless of the bacterial etiology. Data from studies employing culture of needle aspirates from areas of cellulitis have provided the best information on the most likely pathogens.[46,47]

A pathogen was identified in 30% of 284 patients gram positive bacteria (mainly Staphylococcus aureus, group A Streptococci, group B Streptococci, viridans Streptococci, and Enterococcus faecalis) represented 79%, the reminder were Gram negative bacteria (Enterobacteriacea, Hemophilus influenza, Pastuerella multocida, Pseudomonas aeruginosa and Acinetobacter species).

A broader spectrum of pathogens has been isolated from deep wounds or debrided tissue in diabetic patients with limb threatening infections including cellulitis). Theses comprised Gram positive aerobes in 56% of patients (Staphylococcus aureus, Enterococcus species and various Streptococcal species)Gram negative aerobes in 22% (Enterobacteriaceae, Acinetobacter, Pseudomonas aeruginosa) and anaerobes in 22% (Bacteriodes,

Peptococcus). In cellulitis complicating decubitus ulcers, this broad range of microorganism also be considered as potential pathogens. If this complication develops in a hospitalized patient, resistant nosocomial pathogens should be considered when deciding on empirical antibiotic coverage.

Chronic wounds :

Chronic wounds remain one of the most costly unsolved problem in health care today. Leg ulcers, pressure ulcers, ischemic ulcers and diabetic foot ulcers are examples of commonly encountered chronic wound infections.

Four basic condition exist in open wounds resulting from the level of bioburden present (bacterial contamination normal but short lived state, colonization-normal state, critical colonization -abnormal state and infection - abnormal state). The two abnormal states have the potential to disrupt the orderly healing sequence, which result in the development of a chronic wound.

Many wounds healing by secondary intention become indolent. Extending periods of patient discomfort and inconvenience and increasing health care costs and staff overload. Although wound cease to heal for many reasons, perhaps the most common emanates from the effects of wound bioburden, due to invasive infection, the quantity of colonizing microbes,

the mixture of species in the wound base, or effect of their toxins, in addition, the impact of the wounds anatomical position, shape or presentation, the patients level of health and control of underlying pathologies, presence of infection and potentiating factors such as foreign bodies, hematoma, and necrotic tissue, all require consideration.

Open wound pathogens are commonly considered to be aerobic, (Essentially Staphylococci and Streptococci species) But anaerobic species (Peptostreptococcus, Prevotella, Porphyromonas and Bacteroides) are also now thought to have a role to play because the frequency of their isolation increases in clinically infected chronic wounds. They may act synergistically to invade the tissue even if they themselves do not penetrate far into the deep wound compartment.[48] Recent in vitro research [49]shows how anaerobic species cause healing delay by inhibiting fibroblast and keratinocyte proliferation, keratinocyte wound repopulation and endothelial tubule formation.

A third group of organisms, Gram negative bacteria (eg. Pseudomonas aeruginosa, Escherichia coli, Klebsiella, Proteus, Acinetobacter and Enterobacter species) tend to appear in the open wound at approximately 4 week from intiation. This group generally does not penetrate, but adds to the large members of organisms in the wound bioburden. Gram negative bacteria possess antiphagocytic and adherence mechanisms, endotoxins and

some exotoxins making them difficult to remove and kill and allowing the toxins to prolong the inflammatory response into a chronic disordered process.

Pseudomona's exotoxin pyocyanin can cause wound extension without cellulitis. At a certain quantity , these organisms may start quorum sensing or communicating chemically and turn on expression of virulence factors and the production of biofilm causing a great deal of fuss and bother but no classic cellulitic infection in the open wound. Hence chronic infected wounds are polymicrobial and of mixed aerobe/anaerobe populations making it impossible to designate the pathogens, Although competition through cohabitation on intact skin appears to decrease the virulence of an individual species. The polymicrobial natural of the open wound is likely to provide opportunities for synergism, producing infection or delayed healing.

Another consideration is the effect of specific species on the wound. Beta hemolytic Streptococci, notably (Streptococcus pyogenes), are pathogenic at numbers that are significantly lower than many other species. Other species (Eg .Streptococcus aureus, Proteus and Escherichia coli) may have a positive effect by provoking a inflammatory response, accelerating wound repair by stimulating blood flow [50.51]. Increased angiogenesis produced by a low bioburden has a positive effect, but uncontrolled angiogenesis is linked to excessive scar formation.

The acceleration stimulus also may be paralleled by an increased strength in the wound because of increased collagen production. On the other hand, the outcome of high bioburden is often decreased strength.

Trengrove et al[52] support the notion that the presence of multiple species (four or more) delays healing. In general fewer species and numbers are better for normal healing progress.

A diagnosis of critical colonization is made from two main signs; cessation/delay in healing (despite receiving what would normally be considered effective therapy) and the absence of cellulitis. In addition, corroborative signs include a wet rather than moist wound, abnormal smell, change in exudates color, dull dark red or overly bright red discoloration of granulation, a edematous wound base that does not have a granular appearance.

Diabetic foot ulcer infections :

Diabetic patients have always suffer from foot ulceration. This complication has become more prevalent since advances in the general medical care of diabetes, particularly the discovery of insulin, have prolonged the life expectancy of patients with this disease.

Despite progress in the treatment of ulcers, prevention and achieving healing of established ulcers remains a considerable challenge. With

enthusiasm and the application of basic principles, however much can be achieved in treating patient with this common complication of diabetes. It is first important to appreciate that the etiology of diabetic foot disease is truly multifactorial. Within any individual patient, one factor may predominate over all or some of the others, but generally foot disease arises from more than one cause.

Factors to consider include neuropathy, macrovascular and microvascuar disease, infection, connective tissue abnormalities and hematological disturbances. Identification of the dominant causative factors in each case is essential in planning treatment and the concept of the neuropathic foot, the neuroischaemic foot and the ischemic foot is very useful.

S.Fredenburg stated that altered immune response, peripheral vascular disease and neuropathy are key factors in the production of infection.[53] Joseph W.S. also stated that three main factors are responsible for the diabetic foot infections. (Neuro, angio and immunopathy) [54], Wheat., L.J. et al stated that successful treatment of diabetic foot infection requires accurate assessment of the extent and etiology of infections and often involves broad antibiotic coverage and surgery.[55]

The infection is virtually always polymicrobial with Gram positive and negative aerobes and anaerobes.[55,56,57,58], (Staphylococcus

aureus, Bacteroides, Proteus, Enterococcus, Clostridia and Escherichia Coli being present. Lipksy, B.A. et al described that aerobic Gram positive cocci are the major pathogens in the diabetic foot infections. Aerobic Gram negative bacilli or anaerobes are present in chronic or previously treated infections.[59]

Staphylococcus aureus was the commonest bacterial species isolated while anaerobic bacteria comprised only 10% of the isolates in a study done by Jones E.W. [60]Anaerobes are occasionally isolated in the osteomyelitis of the foot in diabetic foot infections. [59] Armstrong DG et al reviewed that anaerobic species were isolated in only 5% of all cultures.[61]

Antibiotic treatment can be valuable when infection is local or superficial. The choice of drug should take account of the polymicrobial nature of these lesions. There is some evidence that prolonged antibiotic treatment for small ulcers result in a more favourable outcome, although there is some debate on this issue. Once there is tissue destruction secondary to infection surgical debridement is required, although broad spectrum antibiotics still have an important role to play.

Bamberger, D,M.et al reviewed that diabetic foot infection in absence of extensive necrosis or gangrene usually responds to antimicrobial therapy without the need for an ablative surgical procedure.[62] Peterson L.R. et al

suggested that ciprofloxacin offers promise for the improved outcome of patient with the serious infected diabetic foot infections.[63]

Conservative treatment including culture guided parenteral and oral antibiotics is successful without amputation in a large proportion of diabetic patients admitted for foot ulcers.[56, 64] However, with optimal treatment involving debridement of devitalized tissue, the use of appropriate dressings and pressure relief wound infection can be minimized. Boultonj et al [65]reported an infection rate of 2.5% in diabetic wounds treated with a moisture retentive hydrocolloid dressing, compared with a 6% infection rate under a traditional gauze dressing. Laing [66] also observed a similar infection rate (2%) in diabetic foot ulcers treated wit a hydro colloid dressing, despite the number of species increasing during treatment.

As bactericidal activity of neutrophils is impaired in diabetic, G-CSF which increase the release of neutrophils from the bone morrow and improves neutrophil function is assessed as adjuvant therapy for the treatment of severe diabetic foot infections.[67]

Other adjunctive therapy using hyperbaric oxygen and topical growth factors can be helpful in treating diabetic foot infections.[68]Self foot care behavior , as well as foot care given by health care providers reduced the prevalence of lower extremity clinical disease in patients with diabetes.

WOUND – SAMPLING METHODS :

Wound tissue sampling :

The acquisition of deep tissue during biopsy following initial debridement and cleansing of superficial debris is recognised as being the most useful method for determining the microbial load and the presence of invasive pathogens [69]. Another technique involving dermabrasion has recently been described that enables the acquisition of deeper tissue without being as invasive as the biopsy method.[70]

Wound fluid sampling :

When a copious volume of wound fluid exists, sampling by needle aspiration can be employed. This is the most useful procedure for sampling purulent fluid from intact cutaneous abscesses. In cavity wounds such as some pressure sores, irrigation with sterile saline and gentle massaging may be performed to provide fluid for aspiration.

Wound swabbing:

Most frequently involves the use of a cotton tipped swab to sample superficial wound fluid and tissue debris, and this enables a semiquantitative and qualitative analysis of the wound microflora. Johnson et al [71] demonstrated superior isolation of anaerobic bacteria from infected diabetic

foot ulcers by a swab technique than by a needle aspiration technique. Studies by Bowler and Davies.[48] have demonstrated the efficacy of the swab sample in isolating anaerobes from a various acute and chronic wounds.

Specimen Transport :

Following the acquisition of wound fluid or tissue for microbiological analysis, prompt delivery of the specimen to the laboratory is considered to be of utmost importance particularly if anaerobic bacteria are being investigated. Aspirates of purulent fluid and tissue samples are considered to be preferred to swabs[72] because they will maintain the condition required to sustain microbial viability (a moist and reduced environment) if processed promptly.

However pre reduced commercially available transport media offer advantages if specimen culture is delayed beyond 1-2 hours after isolation. For specimens that cannot be transferred to the laboratory within 12 hours, storage at room temperature if considered to be appropriate for the maintenance of aerobic and anaerobic microorganisms; elevated temperatures may cause differential growth or death of some microorganisms, and lower temperatures will cause increased O_2 diffusion. [73]

Analysis of wound specimen :

Information regarding the type of wound (Eg. surgical, traumatic, leg ulcer or pressure ulcer) position of the wound, clinical signs of infection, presence of necrosis, associated malodor, and antimicrobial therapy will greatly assist the microbiologist in predicting the microorganisms that are most likely to be involved and therefore the types of culture media and complementary analyses that should be used. Also, the provision of information regarding current antibiotic treatment may assist the microbiologist in determining which microorganisms are most likely to persist in a wound and therefore guide appropriate culturing procedures. Since microbial culture and antibiotic sensitivity result cannot be generated in less than 48h (and may, on occasion, take considerably longer), a number of rapid investigations must be considered at the outset.

Gram Stain:

Despite being used for over a century Gram's stain is still the most important stain in microbiology [74] and is widely used as a rapid technique for guiding antibiotic therapy in life threatening infections such as bacterial meningitis, in wound management, Gram staining of a known volume of tissue biopsy specimen homogenate has been used to rapidly estimate the microbial load of a wound and thus facilitate successful closure of surgical wounds.[75] However in diabetic foot infection and burn wounds, both of which involve complex microbial ecosystems a poor correlation between

Gram stain and culture results from deep tissue biopsy specimens has been reported.

Meislin et al [42] reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure Straphylococcus aureus. Similarly, this procedure may also facilitate identification of the etiological agent of wound infection following clean surgery, where there is a higher probability of one microorganism being involved (Eg. Clusters of Gram positive cocci) in most other wound types that are characterized by complex aerobic-anaerobic micro flora, the Gram stain has little value, although the combined presence of leucocytes and bacteria is likely to be a good indicator of infection. With the exception of Gram positive spore forming anaerobes such as Clostridium perfringens, differentiation between aerobic and anaerobic bacteria is difficult and is further complicated by the fact that many Gram positive anaerobes become Gram variable on exposure to oxygen.[76]

Culture of wound specimen and Antibiogram:

Routine analysis of wound specimen normally involves the use of selective and non selective agar media to culture aerobic bacteria and yeasts and if a specimen is purulent and or malodorous, anaerobic bacteria also.

Although anaerobic bacteria often constitute a significant proportion of the total micro flora in wounds, their culture and isolation is prolonged and more resource demanding than investigation of aerobic bacteria, and consequently, anaerobic microbiology is often excluded from a routine analysis.

Following incubation under aerobic or anaerobic conditions for 24 to 48 hours, qualitative and semi quantitative assessments of the cultures are normally made. With the exception of Clostridium species anaerobes (if investigated) are likely to be reported as being mixed with aerobic microflora. Antibiograms are most frequently performed for the aerobic pathogens, particularly if they are cultured in abundance and with minimal cohabiting microflora. If aerobes are absent, but the wound is reported as being clinically infected, anaerobes should be suspected and investigated more thoroughly.

EXTENDED SPECTRUM BETA LACTAMASES:

In recent years there has been an increased incidence and prevalence of ESBL (Amber's class A penicillinases) that hydrolyze and cause resistance to oxyamino cephalosporins (extended spectrum cephalosporins) and aztreonam.[77,78] ESBLs are now found in a significant percentage of Escherichia coli and Klebsiella pneumoniae strains. They have also been found in Pseudomonas aeruginosa and other Enterobacteriaceae strains like

Enterobacter, Citrobacter, Proteus, Morganello morganii, Serratia marsescens, Shigella dysenteriae.[79]

Production of these enzymes is either chromosomally mediated or plasmid mediated, Pointed amino acid substitution of the classical plasmid mediated beta lactamases like TEM-1 TEM-2 and SHV-1 increase the spectrum of activity from earlier generation beta lactams to 3rd generation cephalosporins and monobactams. However, they retain their stability against cephamycins and carbapenems and are inhibited to an extent by beta lactamase inhibitors (clavulanic acid, sulbactam and tazo bactam). Today over 575 different ESBLs have been described.[80] Being plasmid mediated these enzymes spread fast among various bacteria and are important by infection control, clinical and therapeutic implication.

DETECTION METHODS: [81]

Double disk synergy test:

A disk diffusion test in which synergy between third generation cephalosporin (3GC) and clavulanate is detected by placing a disk of amoxicillin/clavulanate 20µg/10µg) and a disk of third generation cephalosporin (3GC) (30µg), 15mm apart (centre to centre) on an inoculated agar plate. A clear extension of the edge of the 3 GC inhibition zone toward

the disk containing clavulanate is interpreted as synergy, indicating the presence of the ESBL.

(CLSI) RECOMMENDED METHODS FOR ESBL DETECTION : [81]

1.Screening for ESBL producers

i) Disk diffusion method

The CLSI has proposed disk diffusion methods for screening for ESBL using disk diffusion methods for antibiotic susceptibility testing and screen for ESBL production by noting specific on diameters, which indicate a high level of suspicion for ESBL production cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone was used.

A zone inhibition diameter lower than the following values should be investigated with confirmatory tests, ceftazidime (<22mm), cefotaxime and aztreonam (<27mm) and cerftriaxone(<25mm). In the case of cefpodoxime, the cut off for Proteus mirabilis was (<22mm), whereas in the remaining 3 species E.coli, Klebsiella pneumoniae, Klebsiella oxytoca was (< 17mm) Criteria for screening for ESBL production in other Enterobacteriaceae have not been established by the CLSI.

Broth dilution method :

This method can also be used for screening for ESBL producers. It is recommended that Escherichia coli, Klebsiella pneumoniae and Klebsiella

oxytoca strains with Minimum inhibitory concentration (MIC<2 µg/m1) against cefotaxime, ceftazidime, cefriaxone or aztreonam and MIC <8µg/m1 for cefpodoxime should be investigated using specific phenotypic confirmatory tests for ESBL production. For Proteus mirabilis isolates confirmatory tests should be performed if strains demonstrate MIC >2µg/m1 for cefotaxime, ceftazidime or cefpodoxime.

PHENOTYPIC CONFIRMATORY TESTS FOR ESBL PRODUCTION:

(a) Cephalosporin/Clavulanate combination disks:

The CLSI advocates the use of cefotaxime 30µg or ceftazidime disks (30 µg) with and without clavulanate (10 µg) for phenotypic confirmation of the presence of ESBL . The disk tests are to be performed with confluent growth on Mueller Hinton agar. A difference of 5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin / clavunate disk is taken to be phenotypic confirmation of ESBL production.

b) Broth microdilution:

Phenotypic confirmatory testing can also be performed by broth microdilution assays using ceftazidime (0.25 to 128 µg/ml), ceftazidime plus

clavulanic acid (0.25 to 128 µg/ml), cefotaxime (0.25 to 64 µg/ml), and cefotaxime plus clavulanic acid (0.25/4 to 64/4 µg/ml). A twofold serial dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid was compared to its MIC when tested alone.

Implications of positive phenotypic confirmatory tests:

According to CLSI guidelines, isolates which have positive phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephamycins, cefoxitin and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin.

Reporting of microbiological results:

The abrupt onset and rapid progression of acute wound infection such as necrotizing fascitis usually requires therapeutic intervention (in terms of surgical debridement and empiric antibiotic therapy) long before the microbiology laboratory can generate result, and consequently the role of the laboratory in this situation is limited. [82] In contrast, the laboratory has a key role to play in providing information about the wounds that are slowly deteriorating or failing to heal.

From a microbiological perspective, the main pathogen or group of microorganisms that the microbiology laboratory should routinely detect and report (with antibiograms being provided when appropriate) are as follows,

Staphylococcus aureus, Pseudomonas aeruginosa, beta haemolytic Streptococci, Coliform bacteria, pigmented Gram negative anaerobes (Prevotella and Porphyromonas species), non pigmented Gram negative anaerobes (primarily Bacteroides,. Prevotellam and Fusobacterium species), Peptostreptococcus species and Clostridium species.

In order that the microbiology laboratory can provide the wound care practitioners with clinically relevant information, it is essential that the microbiology results (Eg. Gram stain, culture and antibiogram) be interpreted in association with clinical information provided by the practitioner (Eg. wound type, location, condition, signs of infection and sampling method).

Control of microbial populations in wounds:

The reporting by the microbiology laboratory of specific microorganisms isolated from a wound and the associated antibiogram may be interpreted by the practitioner as a diagnosis of wound infection that requires antimicrobial treatment. However, with clinical signs of infection and careful consideration, a wound should not be treated with systemic antibiotics, and it is for this reason that all clinical observation and microbiological findings should be taken into consideration before that medical microbiologist provides an expert opinion.

Although systemic antibiotic therapy is essential for advancing cutaneous infections and those that involve deeper tissues, wound that exhibit only localized signs of infection or are failing to heal but not to have clinical signs of infection (heavy colonization) may initially be treated with topical agents. Topical antimicrobial agents include both antiseptics and antibiotics and the wide choice available creates a further problem to wound care practitioner. Other treatment options such as hyperbaric oxygen therapy, which facilitates the host immune response and may also have a direct antimicrobial effect against some anaerobic bacteria (Eg. Clostridium perfringens) antimicrobial peptides, and botanical extracts may also have roles to play in wound management and are worthy of consideration.

Infected and non infected, non healing wounds can also benefits considerably form surgical debridement, since devitalized tissue both obstructs the healing process and often forms the focus for microbial proliferation. As a consequence, surgical debridement will significantly reduce the microbial load as well as exposing healthy tissue required for wound healing.

Aim and Objectives

AIM AND OBJECTIVES

1. To isolate and identify the organism causing wound infections.

2. To detect the antimicrobial susceptibility pattern of the bacterial isolates.

3. To find out the incidence of Methicillin Resistant Staphylococcus aureus.

4. To find out the incidence of Extended Spectrum Beta Lactamase producers among Enterobacteriaceae isolates.

4. To select appropriate antibiotic for effective treatment.

Materials and Methods

MATERIAL AND METHODS

Study population:

A total of 289 patients with wound infection attending as outpatient and inpatient in Kilpauk Medical College and Hospital ,Chennai were included in the study.

Study period:

March 2009 to February 2010

MATERIALS AND MEHTODOLOGY :

Clinical samples like pus, tissue material and discharge from the incised lesions or ulcers were analyzed for bacteriological profile and antimicrobial susceptibility pattern. Staphylococcus aureus strains were analyzed for MRSA and Enterobacteriaceae isolates obtained were analyzed for ESBL production.

The methodology included,

1. Collection of specimen
2. Specimen processing
3. Identification of pathogens
4. Antimicrobial susceptibility testing
5. Double disk synergy test for ESBL
6. Minimum inhibitory concentration

7. Phenotypic confirmatory test for MRSA

Collection of specimens:

1. **Pus:**

The area over the abscess was wiped with sterile saline or 70%alcohol and with a sterile syringe and needle, pus was aspirated and collected in a sterile test tube.

2. **Swab:**

The wound was wiped with sterile saline and the swab was rolled along the leading edge of the wound and placed in a sterile test tube. Two swab specimens were collected, one for smear examination and one for culture.

3. **Tissue bits:**

For chronic wounds, wound area was wiped with sterile saline and tissue bits were collected using sterile punch biopsy forceps in a sterile test tube containing a small amount of sterile saline to keep the specimen moist.

SPECIMEN PROCESSING:

Once the specimen reached the laboratory, smears were prepared by smearing the swab or purulent material on a clean glass slide .Tissue specimens were ground or minced using sterile scissors and forceps before processing. Smears were routinely subjected to Gram staining and examined.

The specimens were inoculated onto Blood agar plate, Macconkey agar plate and incubated aerobically at 37° c for 18-24 hours. Organisms were identified by colony morphology, Gram staining, motility and biochemical reactions.

Information from the primary plates in conjunction with the atmospheric requirements .Grams stain and colonial morphology of a pure isolate provides presumptive identification of anaerobic organisms.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

Routine disk susceptibility testing of the aerobic isolates were performed by Kirby Bauer method in Mueller Hinton agar medium obtained from Himedia. 25 ml of prepared medium was poured in to a petridish of 90mm diameter to obtain a thickness of 4mm.

Preparation of 0.5 Mcfarland's turbidity standard for inoculum preparation:

0.05 ml of 1% Barium chloride solution was added to 9.95 ml of 1% Sulphuric acid in a test tube with constant stirring to maintain a uniform suspension. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of same size as those used in growing or diluting the bacterial inoculum. The tube was tightly sealed and stored in refrigerator.

Before each use it was shaken vigorously until all the deposit was raised into uniform suspension.

Preparation of inoculum and inoculation:[83]

Morphologically similar colonies from an agar medium was touched with a wire loop and the growth transferred to a test tube containing 1.5ml of Nutrient broth. The tube was incubated at 35°c until it is matched in density with 0.5 Mcfarland's standard which corresponds to 150 million organism per ml.

Within 15 minutes of preparation of the suspension, a sterile cotton wool swab was dipped into the suspension and surplus removed by rotation of the swab against the side of the tube above the fluid level. The medium was inoculated by even streaking of the swab over the entire dried surface of the MHA plate 3 times, rotating the plate approximately 60 degrees each time. Finally the rim of the agar was swabbed. The lid of the dish was left apart for 3 to 5 minutes but not longer than 15 minutes, for the surface of agar to dry before placing the antibiotic disks.

Antibiotic disks:

The antimicrobial susceptibility testing for Staphylococcus aureus and Coagulase negative Staphylococcus included Penicillin 10U , Erythromycin 15 µg, Cotrimoxazole 25 µg, Oxacillin 1 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, and Amikacin 30 µg disks and Vancomycin 30 µg disk used only for Oxacillin resistant strains.

For Beta hemolytic Streptococci, the antimicrobial susceptibility testing included Penicillin 10U , Erythromycin 15 µg, Cotrimoxazole 25 µg and Cephalexin 30 µg disks.

For Enterococci the antimicrobial susceptibility testing included Penicillin 10U , Erythromycin 15 µg, and Amikacin 30 µg disks.

For Gram negative bacilli the antimicrobial susceptibility testing included Ampicillin 10µg , Cotrimoxazole 25 µg, Ciprofloxacin 5 µg, Cefotaxime 30 µg, Ceftazidime 30 µg, Gentamicin 10 µg and Amikacin 30 µg disks and Imipenem disk for ESBL producers.

Antibiotic disks were applied with forceps and pressed gently to ensure even contact with the medium. The plates were inverted and incubated at 35°c to 37°c for 16 to 18 hours. Plates were incubated, not stacked more than three high.[84]

Reading zones of inhibition:

The diameters of the zones of complete inhibition were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black nonreflecting background and illuminated with reflected light.

Interpretation:

The size of the zones of inhibition were interpreted by referring to the NCCLS table -2; volume 20; No. 1; 2000(zone diameter interpretive

standards) and reported as susceptible, intermediate or resistant to the agents that have been tested.

ESBL DETECTION:

Enterobacteriaceae isolates with zone of inhibition diameter <27mm for cefotaxime and <22 mm for ceftazidime were further tested for ESBL production by following methods.

DOUBLE DISK DIFFUSION SYNERGY TEST:

In the DDST, synergy was determined between a disk of Augmentin (20µg Amoxycillin and 10µg Clavulanic acid)and a 30 µg disk of 3rd generation Cephalosporin(Ceftazidime) placed at a distance of 15mm apart (center to center) on a lawn culture of the resistant isolate under test on MHA plate. A clear extension of the edge of the 3rd generation cephalosporin inhibition zone toward the disk containing Clavulanate was interpreted as synergy indicating the presence of an ESBL. This extension occurs due to the Clavulanate in the Augmentin disk which inactivates the ESBL produced by the test organism.

AGAR DILUTION METHOD:

Mueller Hinton agar was prepared in flasks and autoclaved. It was then allowed to cool in a 50°c water bath. Serial dilution of 3rd generation

Cephalosporins (Ceftazidime and Cefotaxime) was prepared in sterile distilled water to give a final concentration ranging from 2µg to 2048µg/ml. After adding the drugs to medium at 50° c, it was mixed well and poured into sterile Petri dishes. (the media was used immediately otherwise potency of drugs will be affected).

A control plate containing the test medium without the antibiotic was prepared for each series of test. Plates of various concentrations were divided into nine quadrants. 0.003ml of inoculums that matched 0.5Mcfarland standard was put into the appropriate quadrant and incubated at 37°c for 16-20 hours. Nine to twelve organisms can be tested using a single plate.

MIC noted as the lowest concentration at which no visible growth occurred. Isolates were tested for various concentration of 3rd generation Cephalosporins combined with 2µg/ml of Clavulanic acid(0.25µg to 2048µg/ml of agar) and the MIC was determined.

PHENOTYPIC CONFIRMATORY TEST:

Antibiotic sensitivity testing was done on MHA with 0.5Mcfarland standard of the organism. The drugs used were Cefotaxime and Ceftazidime each 30µg alone and in combination with Clavulanic acid 10µg.Organisms with 5mm increase in zone of inhibition with third generation cephalosporin and Clavulanic acid were confirmed as ESBLs . Quality control strains of non ESBL producing organism (Escherichia coli ATCC 25922) and an ESBL

producing organism (Klebsiella pneumonia ATCC 700603) were used as control.

DETECTION OF MRSA STRAINS:

SCREENING FOR MRSA: Oxacillin disk (1µg)

Disk diffusion test is performed with lµg of Oxacillin disk which was placed on MHA plate . The zone of inhibition is determined after 24hrs of incubation at 37˚C. The zone size is interpreted according to CLSI guideline.

Susceptible	>13 mm
Intermediate	11-12mm
Resistant	< 10 mm

Cefoxitin disk (30µg) diffusion test:

The test was performed with 30 µg of Cefoxitin disk placed on Muller Hinton agar plate without NaCl supplementation. The zone of inhibition is determined after 24 hrs of incubation at 37°C. The zone size is interpreted according to CLSI guidelines.

Susceptible	>19mm
Resistant	< 20mm

Quality control used for MRSA detection:

ATCC Staphylococcus aureus 43300 (Positive control)

ATCC Staphylococcus aureus 25923 (Negative control)

STATISTICAL ANALYSIS:

A statistical analysis was carried out using statistical package for social sciences (SPSS) and Epi-info software by a statistician. The proportional data of the cross sectional study was tested using Pearson's Chi-square analysis test and Binomial proportion test.

Results

TABLE 1

AGE AND SEX DISTRIBUTION OF CASES (N=289)

AGE IN YEARS	MALE	FEMALE	TOTAL
0-10	24	16	40
11-20	17	27	44
21-30	32	57	89
31-40	35	24	59
41-50	9	5	14
51-60	14	13	27
61-70	8	4	12
71-80	4	-	4
TOTAL	143(49.48%)	146(50.52%)	289

TABLE 2

DISTRIBUTION OF ORGANISMS

ORGANISMS	NO OF ISOLATES	PERCENTAGE
Staphylococcus aureus	89	54.26
Klebsiella pneumoniae	40	24.39
Pseudomonas aeruginosa	22	13.41
Escherichia coli	5	3.04
Enterococci	5	3.04
Coagulase negative staphylococcus	2	1.21
Acinetobacter	1	0.6

TABLE 3

DISTRIBUTION OF ORGANISMS IN BURN WOUND

ORGANISMS	BURN WOUND	PERCENTAGE
Staphylococcus aureus	63	56.75
Klebsiella pneumoniae	29	26.12
Pseudomonas aeruginosa	13	11.71
Escherichia coli	1	0.90
Enterococci	2	1.80
Coagulase negative Staphylococcus	2	1.80
Acinetobacter	1	0.90

TABLE 4

SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS

ANTIBIOTICS	STAPHYLOCOCCUS AUREUS N=89 (PERCENTAGE)
Amoxycillin	45(50.56)
Gentamicin	57(64.04)
Ciprofloxacin	50(56.17)
Erythromycin	36(40.44)
Cefotaxime	58(65.16)
Cephalexin	36(40.44)
Piperacillin/tazobactum	61(68.53)
Levofloxacin	81(91.01)
Amikacin	82(92.13)
Vancomycin	89(100%)

TABLE 5

SENSITIVITY PATTERN OF KLEBSIELLA PNEUMONIAE

ANTIBIOTICS	KLEBSIELLA PNEUMONIA N=40 (PERCENTAGE)
Amikacin	36(90)
Ciprofloxacin	34(85)
Cephatoxime	10(25)
Cephalexin	5(12.5)
Piperacillin/tazobactum	20(50)
Imipenem	24(60)
Gentamicin	18(45)

TABLE 6

SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA

ANTIBIOTICS	PSEUDOMONAS AERUGINOSA N=22 (PERCENTAGE)
Amikacin	18(81.81)
Ciprofloxacin	13(59.09)
Cephatoxime	8(36.36)
Cephalexin	6(27.27)
Piperacillin/tazobactum	12(54.54)
Imipenem	22(100)
Gentamicin	11(50)

TABLE 7

DETECTION OF MRSA BY OXACILLIN SCREEN AGAR TEST.

ZONE(mm)	NO.OF ISOLATES	PERCENTAGE (%)
>14 (MSSA)	55	61.79
<10 (MRSA)	34	38.21

TABLE 8

CONFIRMATION OF MRSA BY CEFOXITIN DISK TEST.

ZONE(mm)	NO.OF ISOLATES	PERCENTAGE (%)
>20 (MSSA)	53	59.55
<19 (MRSA)	36	40.44

TABLE 9

RESISTANCE PATTERN OF MRSA ISOLATES TO ANTIBIOTICS.

ANTIBIOTICS	MRSA ISOLATES (N=36) PERCENTAGE (%)
Amoxicillin	84.4
Gentamicin	47.3
Ciprofloxacin	41.7
Erythromycin	48.4
Cefotaxime	66.7
Cephalexin	75
Piperac illin/tazobactum	72.3
Levofloxacin	2.8
Amikacin	5.6
Vancomycin	0

TABLE 10

NO OF ENTEROBACTERIACEAE RESISTANT TO 3RD GENERATION CEPHALOSPORINS.

NUMBER OF ISOLATES	RESISTANT TO 3rd GENERATION CEPHALOSPORINS
45	19

TABLE 11

ESBL DETECTION BY VARIOUS METHODS.

METHODS	DDST	MIC	PCT
Positive isolates n=19	19(100%)	19(100%)	19(100%)

TABLE 12

DISTRIBUTION OF ESBL PRODUCING ENTEROBACTERIACEAE

ORGANISMS	TOTAL	ESBL	PERCENTAGE
Klebsiella pneumoniae	40	17	42.50
Escherichia coli	5	2	40
Total	45	19	41.25

TABLE 13

DISTRIBUTION OF ESBL PRODUCING ENTEROBACTERIACEAE FROM VARIOUS WOUNDS.

ORGANISM	BURNS		SSI		TRAUMATIC WOUND		ABSCESS		TOTAL	
	Total	ESBL	Total	ESBL	Total	ESBL	Total	ESBL	Total	ESBL
Klebsiella pneumoniae	29	12	7	3	2	0	2	2	40	17
Escherichia coli	1	1	2	1	-	-	2	-	5	2
Total	30	13	9	4	2	-	4	2	45	19

TABLE 14

RESISTANCE PATTERN OF ESBL PRODUCERS TO OTHER ANTIBIOTICS.

ANTIBIOTICS	ESBL PRODUCERS(N=19)%
Amikacin	10.5
Ciprofloxacin	58
Cefotaxime	89.5
Cephalexin	95
Piperacillin/tazobactum	79
Imipenem	58
Gentamicin	58

TABLE 15

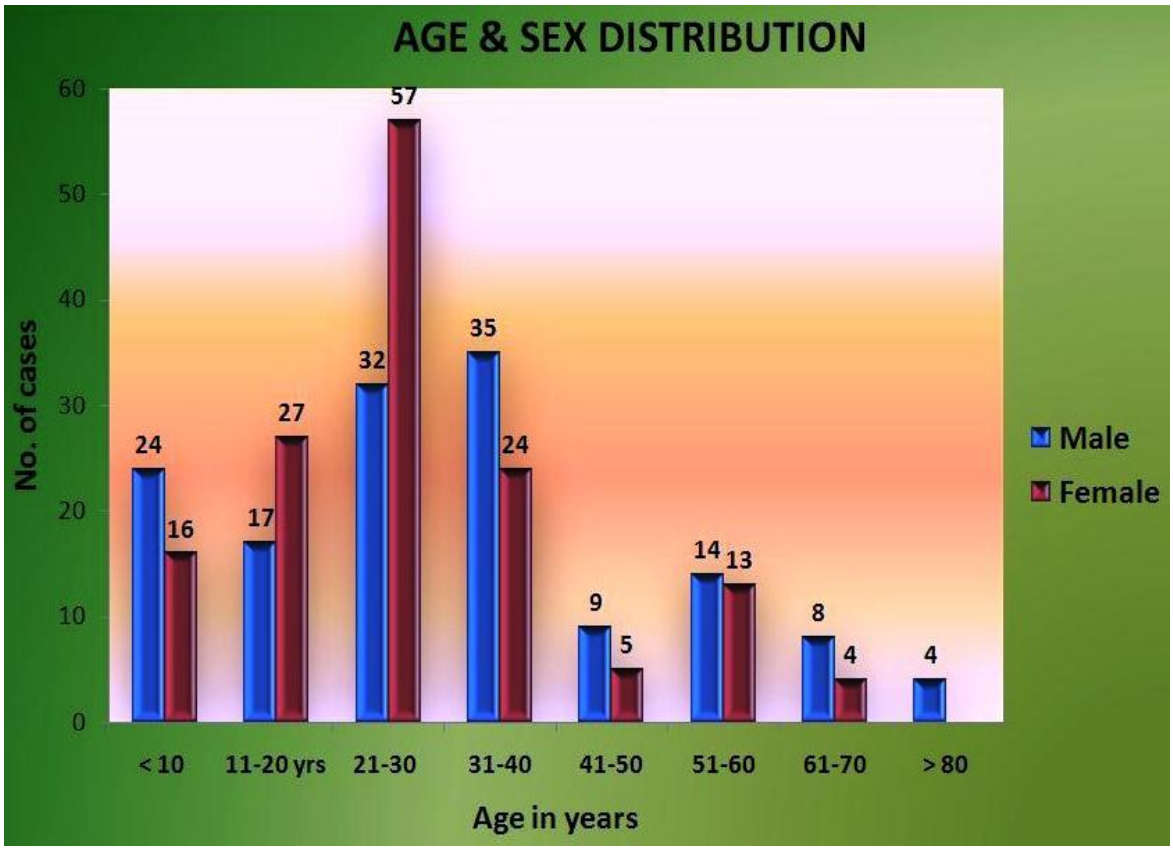
MIC OF ENTEROBACTERIACEAE TO CEFOTAXIME AND CEFOTAXIME WITH CLAVULANIC ACID.

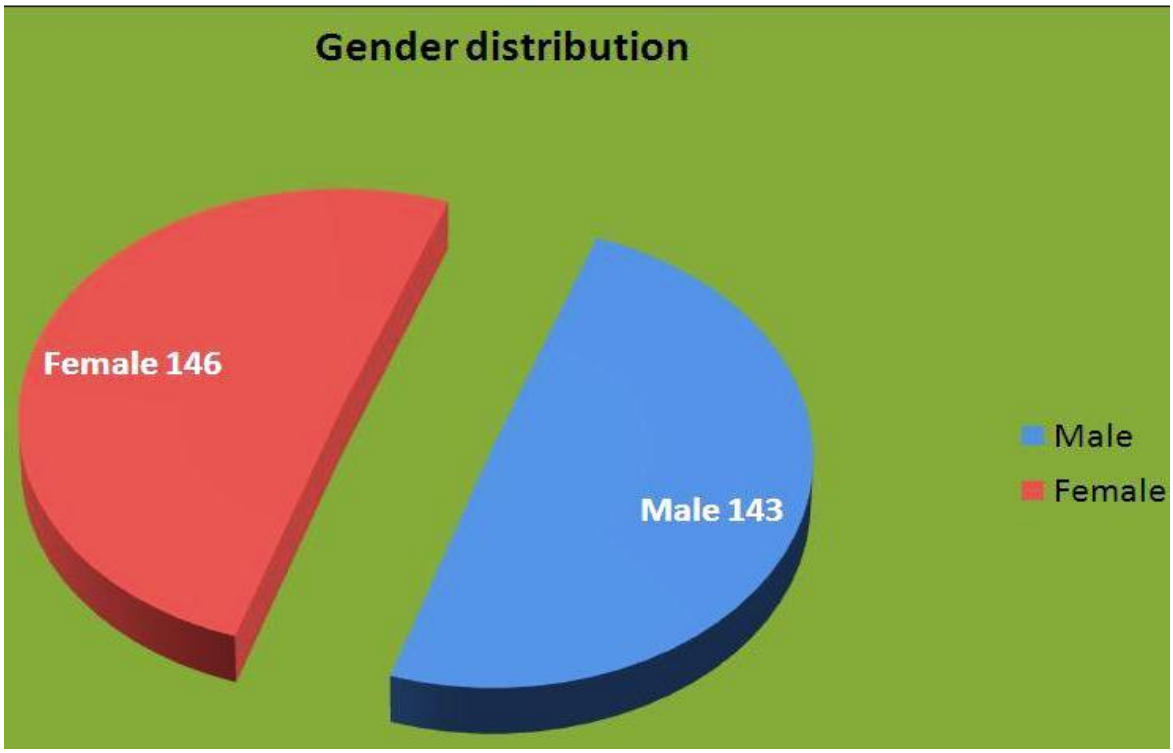
CEFOTAXIME µG/ML	NO OF ISOLATES INHIBITED	CEFOTAXIME & CLAVULANIC ACID(2µG/ML)	NO OF ISOLATES INHIBITED
1	-	0.125	1
2	-	0.25	3
4	-	0.5	5
8	-	1	4
16	-	2	7
32	3	4	4
64	3	8	1
128	5	16	1
256	4	32	-
512	6	64	-
1024	8	128	2
2048	9	256	-

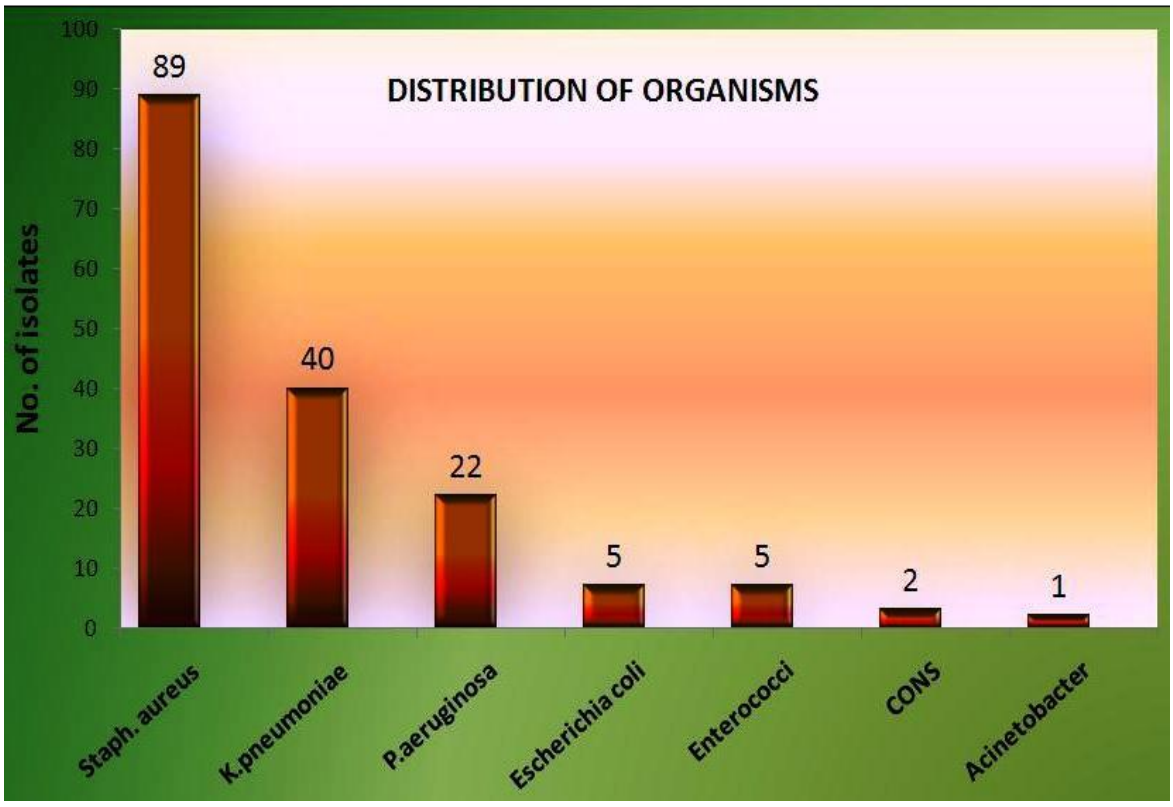
TABLE 16

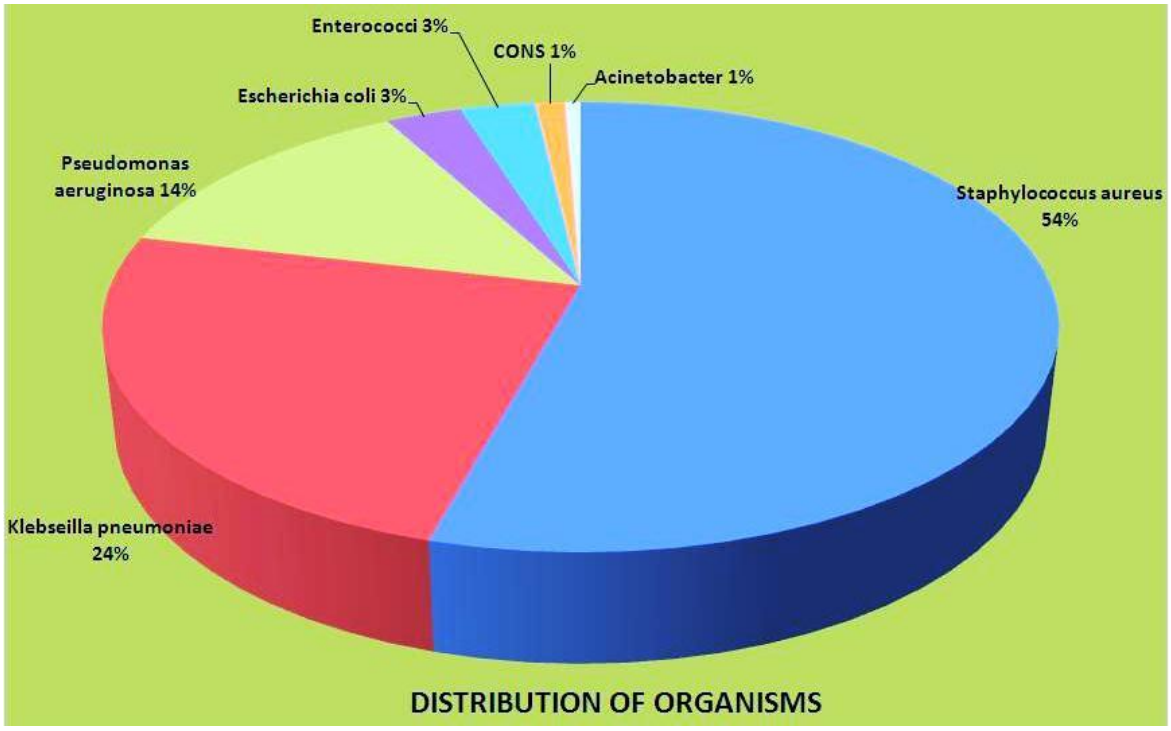
MIC OF ENTEROBACTERIACEAE TO CEFTAZIDIME AND CEFTAZIDIME WITH CLAVULANIC ACID.

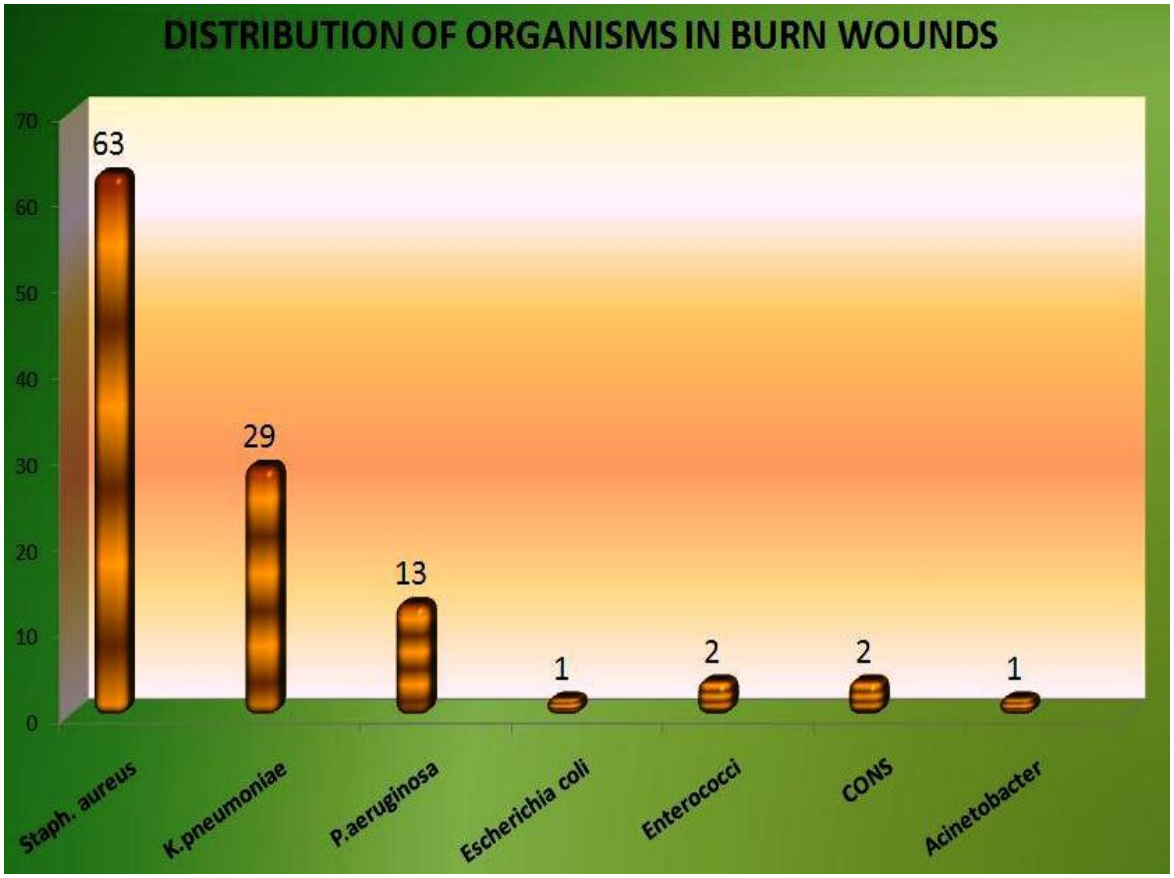
CEFTAZIDIME µG/ML	NO OF ISOLATES INHIBITED	CEFTAZIDIME & CLAVULANIC ACID(2µG/ML)	NO OF ISOLATES INHIBITED
1	-	0.125	2
2	-	0.25	3
4	-	0.5	5
8	-	1	8
16	-	2	7
32	5	4	3
64	6	8	1
128	9	16	-
256	9	32	-
512	8	64	-
1024	5	128	-
2048	4	256	-

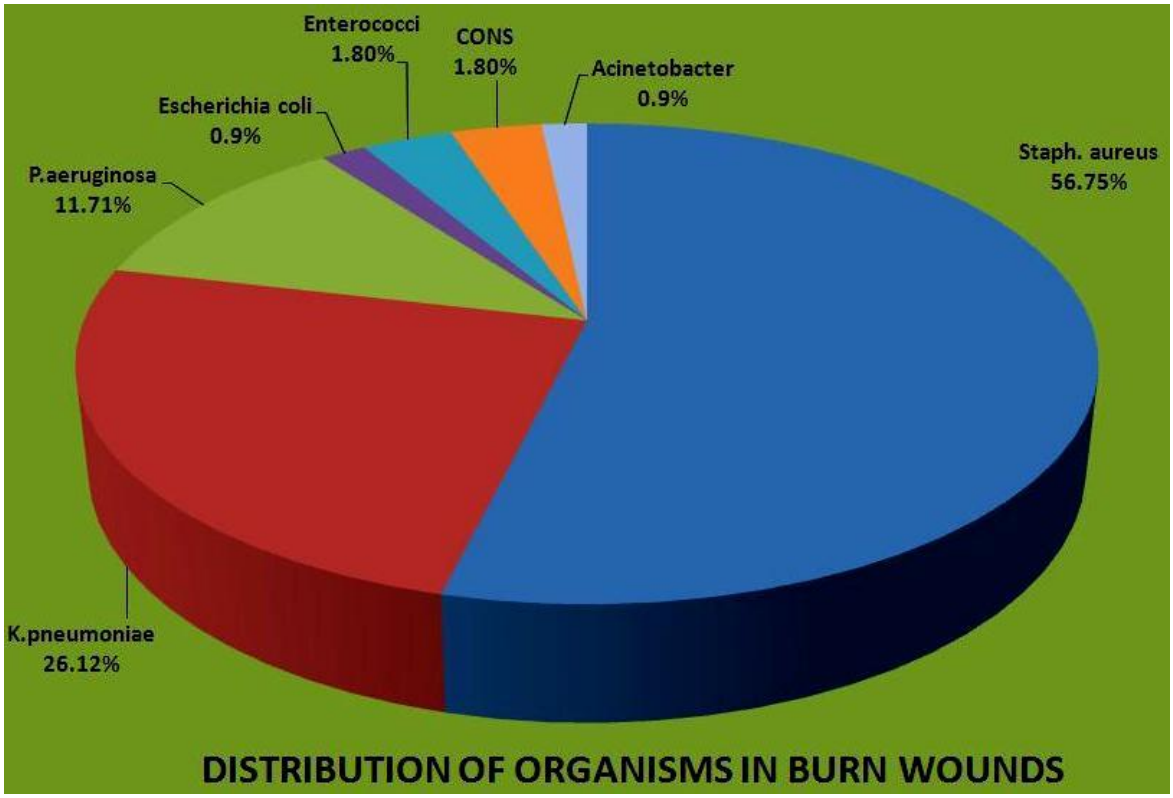

AGE & SEX DISTRIBUTION
No. of cases
60
50
40
30
20
10
0
24
16
17
27
32
57
35
24
9
5
14
13
8
4
4
< 10
11-20 yrs
21-30
31-40
41-50
51-60
61-70
> 80
Age in years
Male
Female

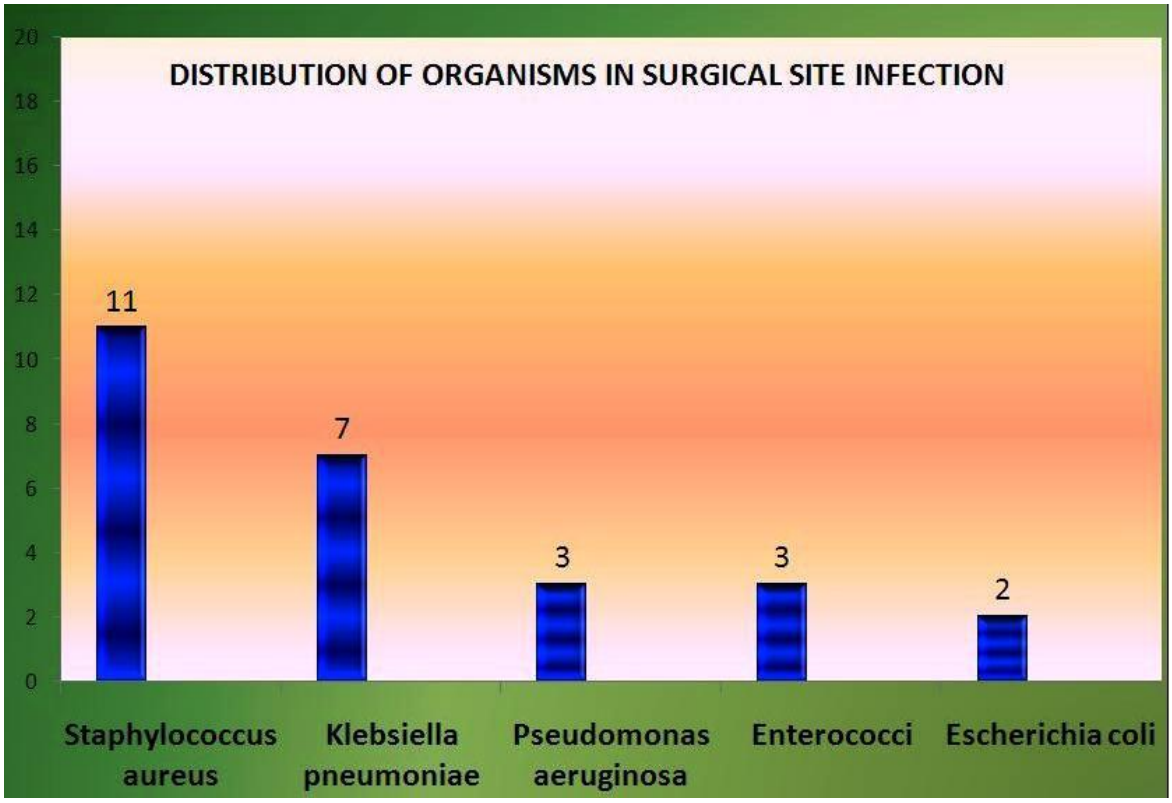

Gender distribution
Female 146
Male 143
Male
Female

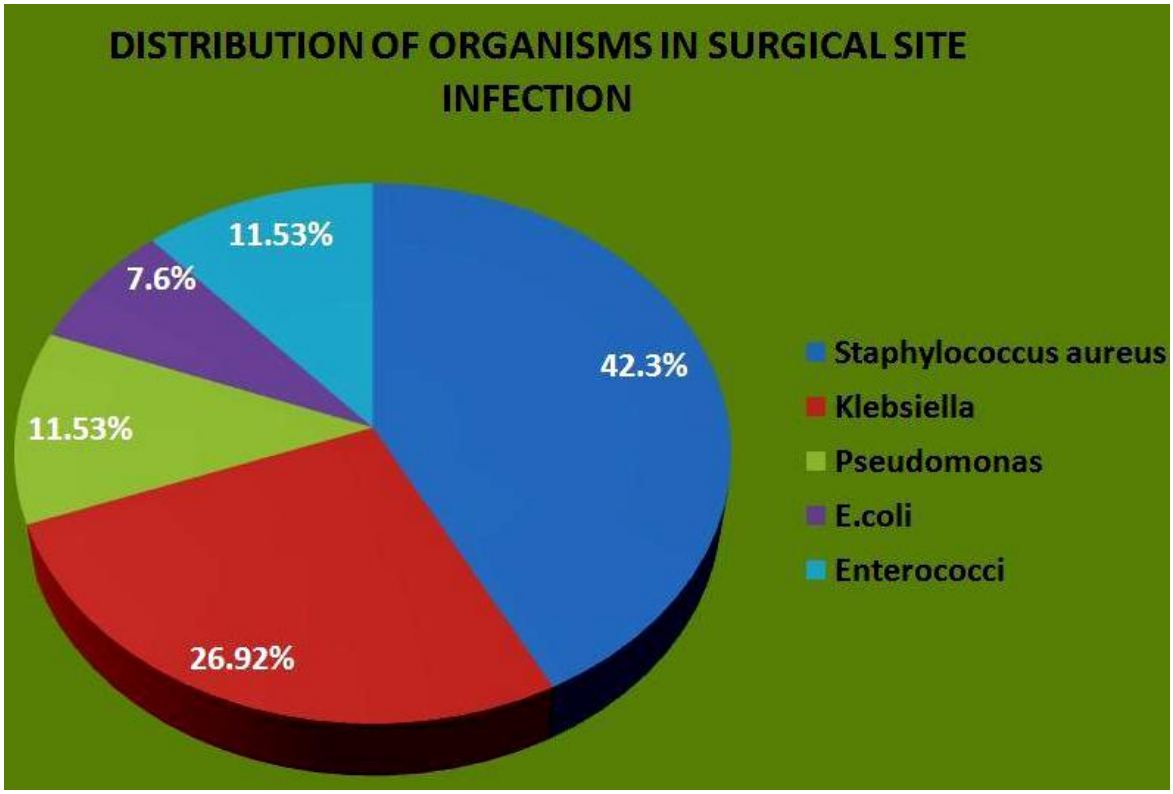

DISTRIBUTION OF ORGANISMS
No. of isolates
100
90
80
70
60
50
40
30
20
10
0
89
40
22
5
5
2
1
Staph. aureus
K.pneumoniae
P.aeruginosa
Escherichia coli
Enterococci
CONS
Acinetobacter

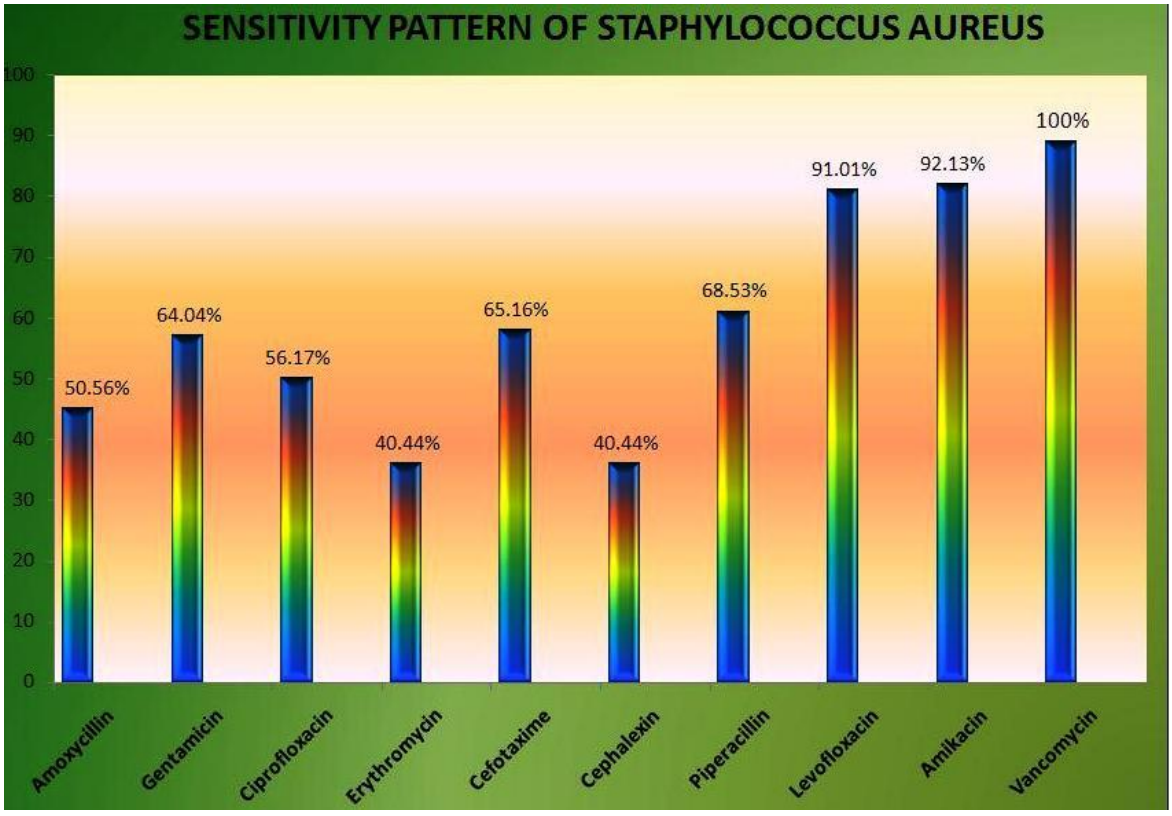

Enterococci 3%
CONS 1%
Acinetobacter 1%
Escherichia coli 3%
Pseudomonas aeruginosa 14%
Staphylococcus aureus 54%
Klebseilla pneumoniae 24%
DISTRIBUTION OF ORGANISMS

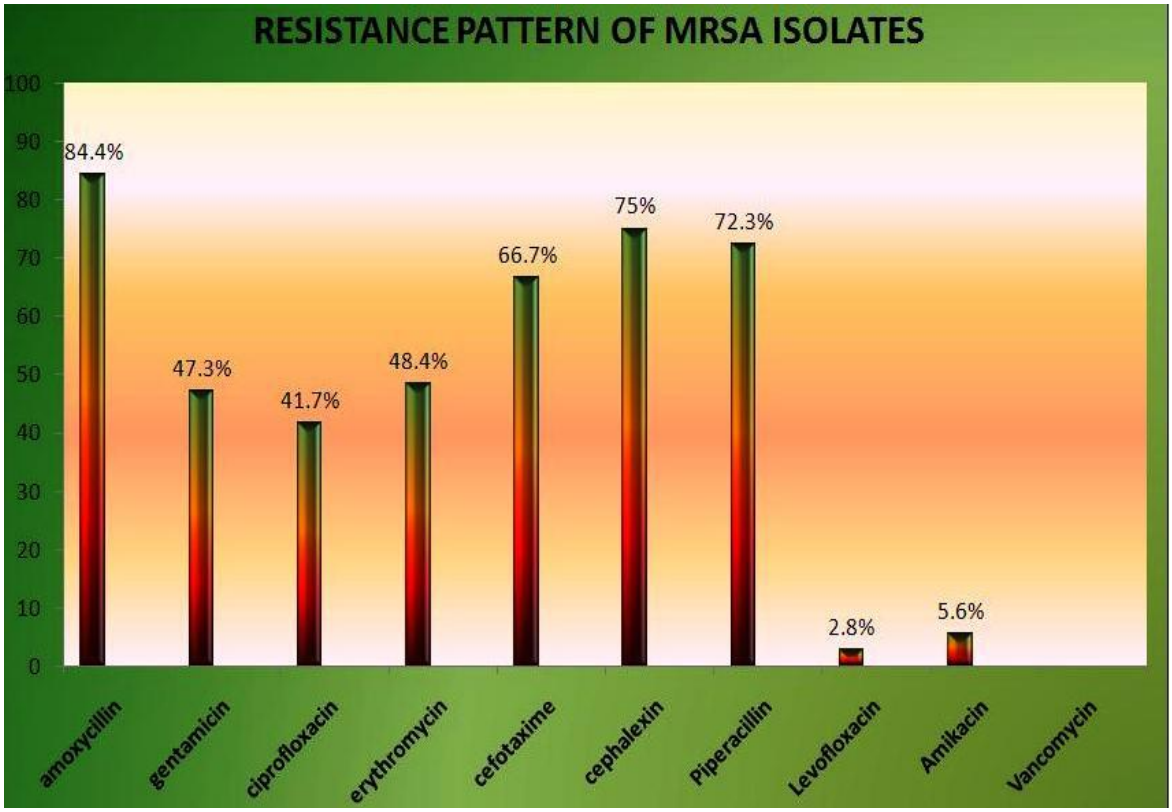

DISTRIBUTION OF ORGANISMS IN BURN WOUNDS
70
60
50
40
30
20
10
0
63
29
13
1
2
2
1
Staph. aureus
K.pneumoniae
P.aeruginosa
Escherichia coli
Enterococci
CONS
Acinetobacter

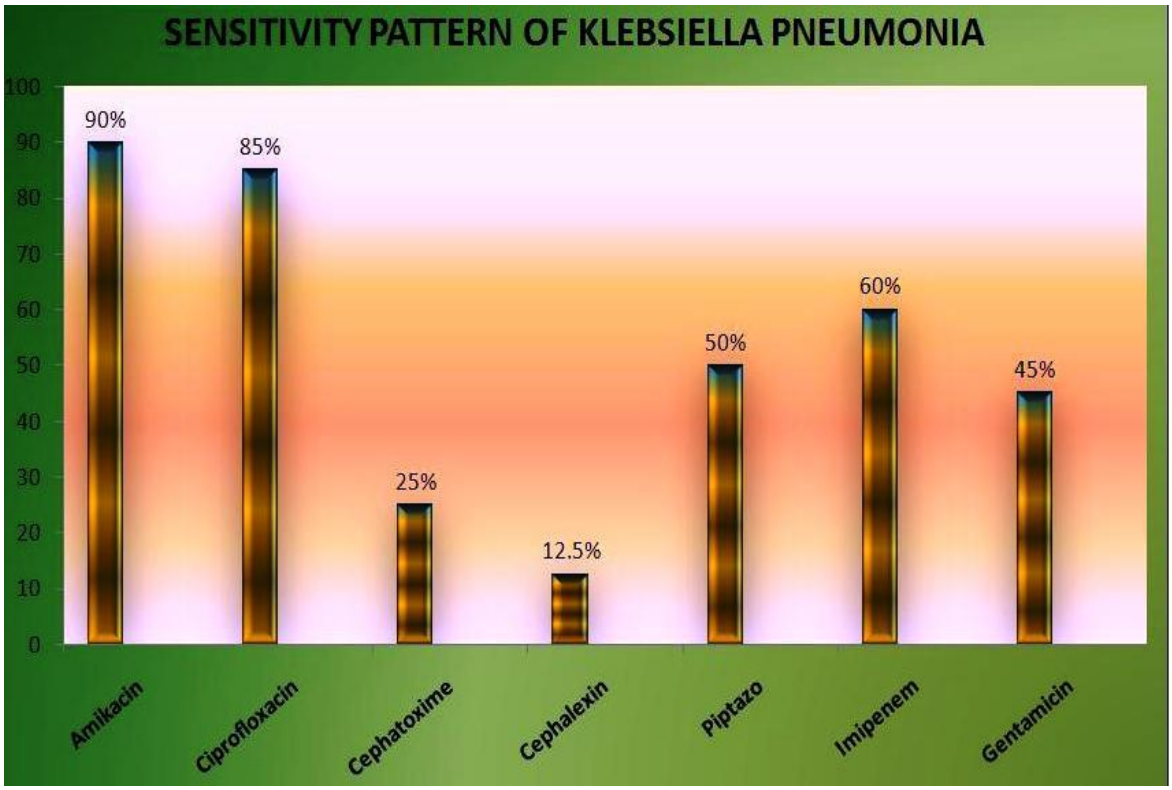

Enterococci
1.80%
CONS
1.80%
Acinetobacter
0.9%
Escherichia coli
0.9%
P.aeruginosa
11.71%
Staph. aureus
56.75%
K.pneumoniae
26.12%
DISTRIBUTION OF ORGANISMS IN BURN WOUNDS

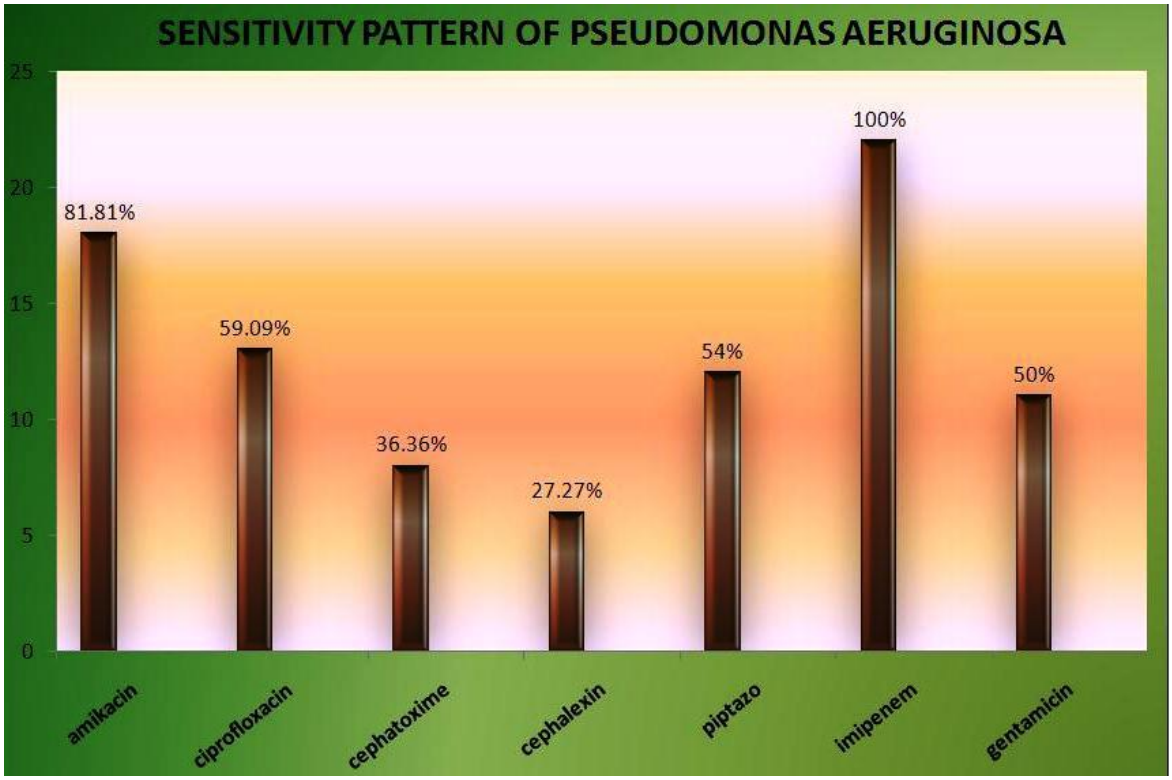

DISTRIBUTION OF ORGANISMS IN SURGICAL SITE INFECTION
20
18
16
14
12
10
8
6
4
2
0
11
7
3
3
2
Staphylococcus aureus
Klebsiella pneumoniae
Pseudomonas aeruginosa
Enterococci
Escherichia coli

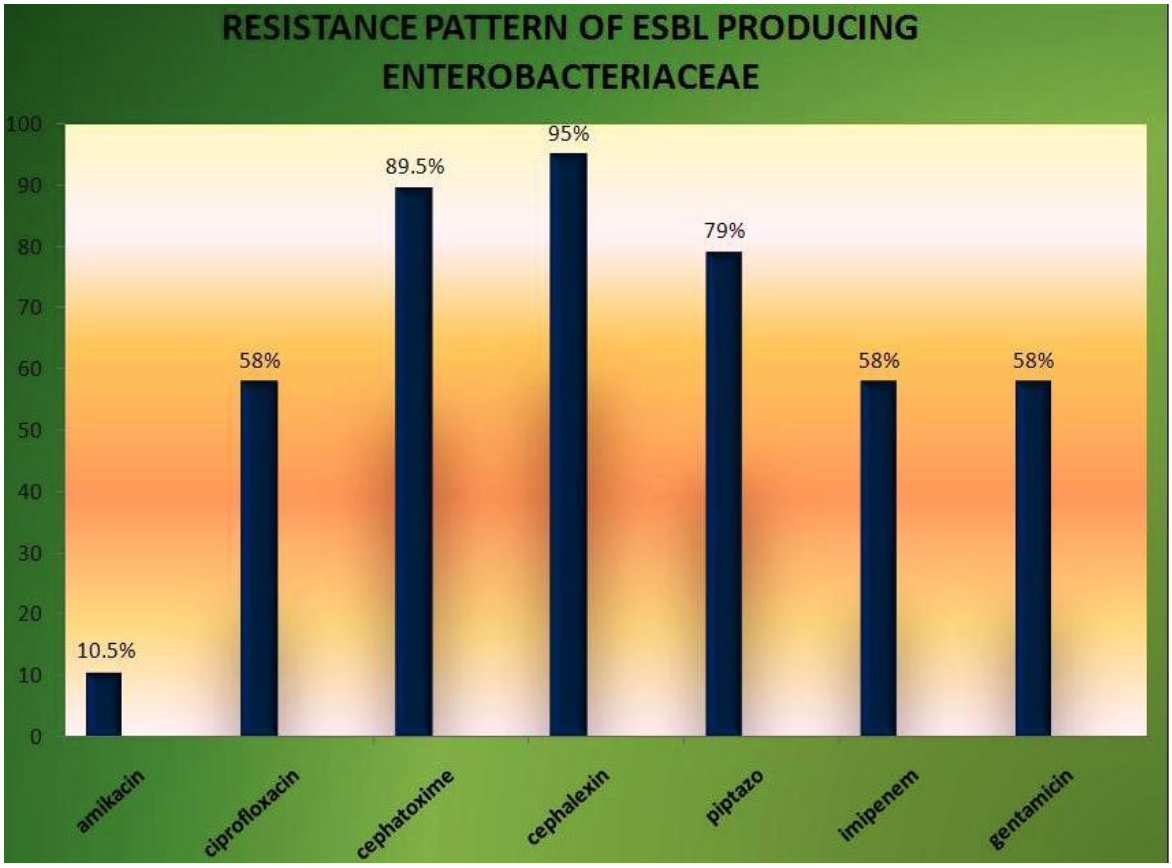

DISTRIBUTION OF ORGANISMS IN SURGICAL SITE INFECTION
11.53%
7.6%
42.3%
11.53%
26.92%
Staphylococcus aureus
Klebsiella
Pseudomonas
E.coli
Enterococci


SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS
100
90
80
70
60
50
40
30
20
10
0
50.56%
64.04%
56.17%
40.44%
65.16%
40.44%
68.53%
91.01%
92.13%
100%
Amoxycillin
Gentamicin
Ciprofloxacin
Erythromycin
Cefotaxime
Cephalexin
Piperacillin
Levofloxacin
Amikacin
Vancomycin


RESISTANCE PATTERN OF MRSA ISOLATES
100
90
80
70
60
50
40
30
20
10
0
84.4%
47.3%
41.7%
48.4%
66.7%
75%
72.3%
2.8%
5.6%
amoxycillin
gentamicin
ciprofloxacin
erythromycin
cefotaxime
cephalexin
Piperacillin
Levofloxacin
Amikacin
Vancomycin


SENSITIVITY PATTERN OF KLEBSIELLA PNEUMONIA
100
90
80
70
60
50
40
30
20
10
0
90%
85%
25%
12.5%
50%
60%
45%
Amikacin
Ciprofloxacin
Cephatoxime
Cephalexin
Piptazo
Imipenem
Gentamicin


SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA
25
20
15
10
5
0
81.81%
59.09%
36.36%
27.27%
54%
100%
50%
amikacin
ciprofloxacin
cephatoxime
cephalexin
piptazo
imipenem
gentamicin


RESISTANCE PATTERN OF ESBL PRODUCING ENTEROBACTERIACEAE
100
90
80
70
60
50
40
30
20
10
0
10.5%
58%
89.5%
95%
79%
58%
58%
amikacin
ciprofloxacin
cephatoxime
cephalexin
piptazo
imipenem
gentamicin

RESULTS

Specimens obtained from patients with wound infection attending Surgical, Orthopaedic, Burns ward, OG, IMCU, Plastic surgery departments as OP and IP were studied from March 2009 to Feb 2010 to identify the bacteriological profile of wound infection, antimicrobial susceptibility pattern of the organisms isolated ,incidence of MRSA and ESBL producing Enterobacteriaceae among them. Study included patients of both sexes and up to 80 years of age .Specimen included were pus, wound swab.

Wound swabs from 289 patients were analyzed in the study. Male patients constituted 143(49.48%), Female patients constituted 146(50.51%), age ranged from 3 months to 80 years. Bacterial isolates were found in 164 (56.74%). The isolation rate was significantly higher in females (51.21%), compared to males (48.78%). The predominant isolates were Gram positive bacteria 96(58.53%). The most frequently isolated organisms were Staphylococcus aureus 89(54.26), followed by Klebsiella pneumoniae (24.39%), Pseudomonas aeruginosa 22(13.41%), Escherichia coli 5(3.04%), Enterococci 5(3.04%), Coagulase negative Staphylococcus 2(1.21%), Acinetobacter 1(0.60%).

The results were analyzed as follows:

Total number of cases taken for study were 289, which included total of 143 males (49.48%) and 146 females(50.51%). Maximum cases were recorded in the age group between 21-30. In all age groups except 11-20 and 21-30 the sex distribution was predominantly male.(Table 1)

Out of 289 samples collected, 164 showed culture positivity.The isolated organisms were Staphylococcus aureus 89(54.26%), followed by Klebsiella pneumoniae 40 (24.39%), Pseudomonas aeruginosa 22(13.41%), Escherichia coli 5 (3.04%), Enterococci 5 (3.04%) Coagulase negative staphylococcus 2 (1.21%), Acinetobacter 1 (0.60%).(Table 2)

Out of 111 burn wound isolates, 63 (56.75%) were Staphylococcus aureus, 29(26.12%) were Klebsiella pneumoniae, 13(11.71%) were Pseudomonas aeruginosa ,1(0.9%) were Escherichia coli, 1(0.9%) were Acinetobacter, 2(1.8%) were Enterococci, 2(1.8%) were Coagulase negative Staphylococcus.(Table 3).

The organisms isolated from 26 specimen of surgical site infections were Staphylococcus aureus 11(42.30%), Klebsiella pneumoniae 7 (26.92%), Pseudomonas aeuroginosa 3(11.53%), Enterococci 3(11.53%), Escherichia coli 2 (7.6%).

Out of 22 cutaneous abscess isolates, 13(59.09%) were Staphylococcus aureus, 2(9.09%) were Klebsiella pneumoniae, 5(22.72%) were Pseudomonas aeruginosa, 2(9.09%) were Escherichia coli.

Out of 5 traumatic wound , 2(40%) were Staphylococcus aureus, 2(40%) were Klebsiella pneumoniae, 1(20%) were Pseudomonas aeruginosa.

Antibiotic sensitivity pattern :

Out of 89 Staphylococus aureus 45(50.56%) were sensitive to amoxycillin, 57(64.04%) were sensitive to gentamicin, 50(56.17%) sensitive to ciprofloxacin, 36(40.44%) were sensitive to erythromycin, 58(65.16%) were sensitive to cefotaxime, 36(40.44%) were sensitive to cephalexin, 61(68.53%) were sensitive to piperacillin/tazobactum, 81(91.01%) were sensitive to levofloxacin, 82(92.13%) were sensitive to amikacin, and 100% sensitive to vancomycin.(Table 4).

Cogaulase negative Staphylococcus were 100% sensitive to amoxycillin, gentamicin, erythromycin, cefotaxime, cephalexin, piperacillin/tazobactum, levofloxacin, amikacin vancomycin, and 50% sensitive to ciprofloxacin.

Out of 5 Enterococci, 4(80%) were sensitive to ciprofloxacin, 3(60%) were sensitive to amikacin, 2(40%) were sensitive to gentamicin, erythromycin, cefotaxime, cephalexin, levofloxacin and 100% sensitive to vancomycin.

Out of 40 Klebsiella pneumoniae 36(90%) were sensitive to amikacin, 34(85%) were sensitive to ciprofloxacin, 10(25%) were sensitive to cefotaxime, 5(12.5%) were sensitive to cephalexin, 20(50%) were sensitive

to Piperacillin/tazobactum , 24(60%) were sensitive to imipenem, 18(45%) were sensitive to gentamicin.(Table 5)

Out of 22 Pseudomonas aeruginosa, 18(81.81%) were sensitive to amikacin, 13(59.09%) were sensitive to ciprofloxacin, 8(36.36%) were sensitive to cefotaxime, 6(27.27%) were sensitive to cephalexin, 12(54.54%) were sensitive to Piperacillin/tazobactum, 22(100%) were sensitive to imipenem, 11(50%) were sensitive to gentamicin.(Table 6)

Out of 5 Escherichia coli , 4(80%) were sensitive to amikacin, imipenem, 3(60%) were sensitive to ciprofloxacin,2(40%) were sensitive to cephalexin, 1(20%) were sensitive to cefotaxime, piperacillin/tazobactum gentamicin.

Acinetobacter is 100% sensitive to imipenem and piperacillin/tazobactum.

SCREENING FOR MRSA:

Oxacillin disk (1 µg)

All the 89 isolates of Staphylococcus aureus were screened for Methicillin resistance using oxacillin disk (1µg) and out of them 34(38.21%) were found to have inhibition zone less than 10mm.(Table 7).

Using Cefoxitin disk (30µg)

All the 89 isolates of Staphylococcus aureus were then further confirmed for Methicillin resistance using cefoxitin disk (30µg).(Table 8).

The MRSA isolates were resistant to Amoxycillin(84.4%), Gentamicin(47.3%),Ciprofloxacin(41.7%),Erythromycin(48.4%),Cefotaxime (66.7%),Cephalexin(75%), Piperacillin/tazobactum(72.3%), Amikacin(5.6%) Levofloxacin (2.8%) but not resistant to vancomycin.(Table 9).

SCREENING FOR ESBL:

Out of 45 Enterobacteriaceae isolates 19(42.22%) found to be ESBL producers by screening method ,were subjected to further tests and by all methods were confirmed as ESBL producers.(Table 10 and Table 11)

Out of 40 Klebsiella pneumoniae isolates, 17(42.50%) were ESBL producers. Out of 5 Escherichia coli isolates, 2(40%) were ESBL producers.(Table 12).

In burn wound out of 30 Enterobacteriaceae isolates, 13(43.33%) were ESBL producers. Out of 1 Escherichia coli isolate, 1(100%) were positive, out of 29 Klebsiella pneumoniae isolates, 12(41.37%) were ESBL producers.(Table 13).

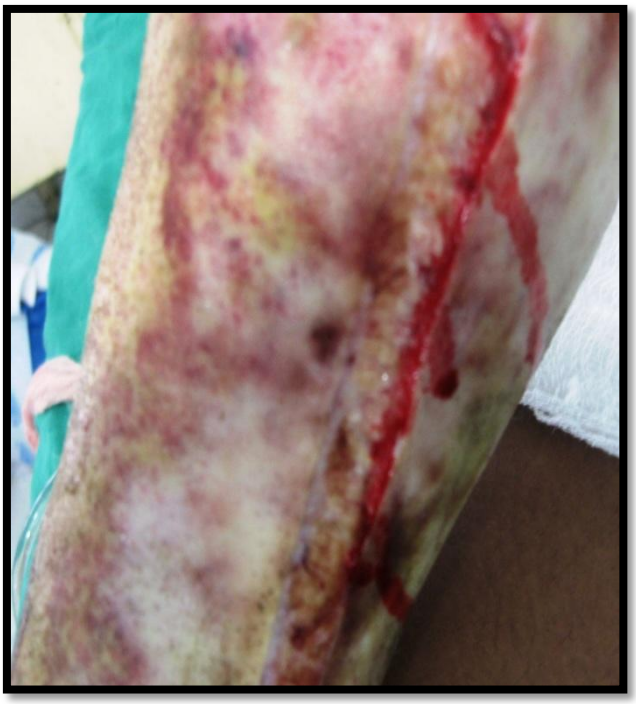
In Surgical site infection out of 9 Enterobacteriaceae isolates, 4(44.44%) were ESBL producers. Out of 7 Klebsiella pneumoniae 3(42.85%) were ESBL producers, out of 2 Escherichia coli isolates 1(50%) were ESBL producers.(Table 13).

In cutaneous abscess out of 4 Enterobacteriaceae isolates, 2 (50%) were ESBL producers. Out of 2 Klebsiella pneumoniae isolates, 2(100%) were ESBL producers.(Table 13)

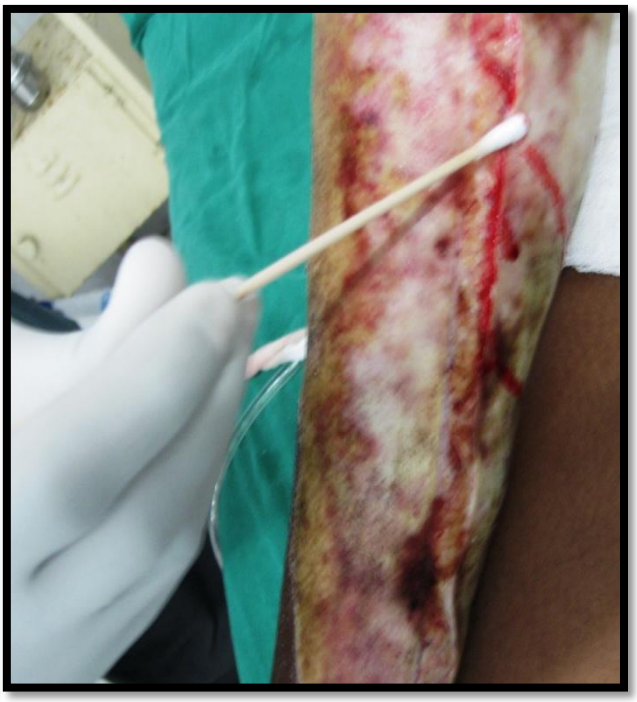
The ESBL producers were resistant to amikacin(10.5%) gentamicin(58%),ciprofloxacin(58%),cefotaxime(89.5%), cephalexin (95%, Piperacillin/tazobactum(79%), imipenem(58%).(Table 14).

MIC for the isolates was between 32µg/ml to 2048µg/ml of agar for cefotaxime and ceftazidime which was reduced to 0.125 to 128 µg/ml of agar respectively in the presence of 2 µg of clavulanic acid/ml of agar. (Table 15&16).

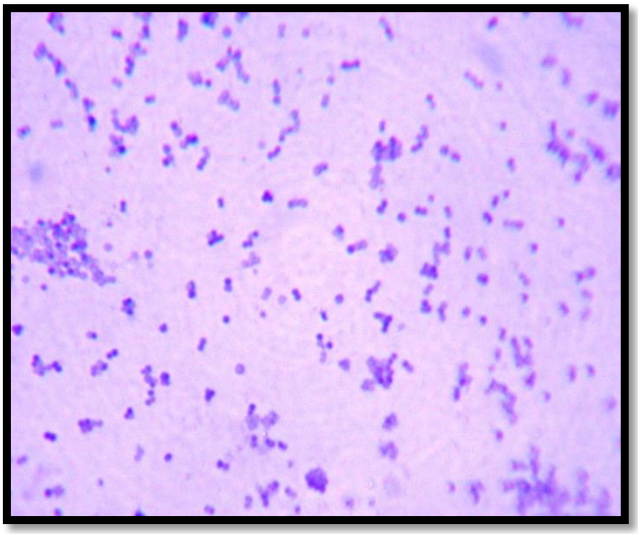
A CASE OF BURN WOUND



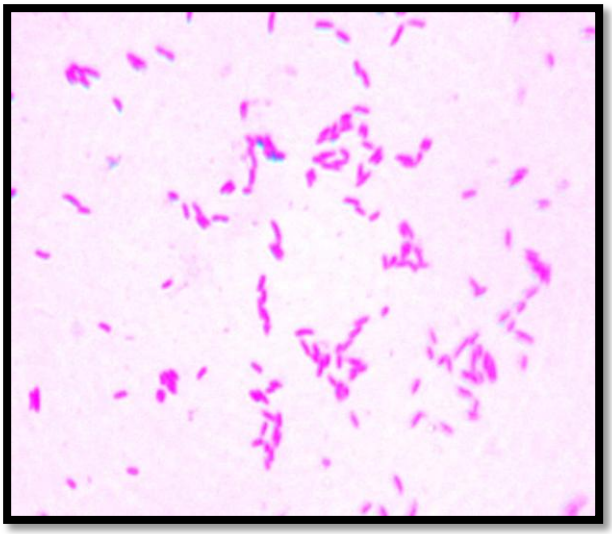
SAMPLE TAKEN BY SWAB



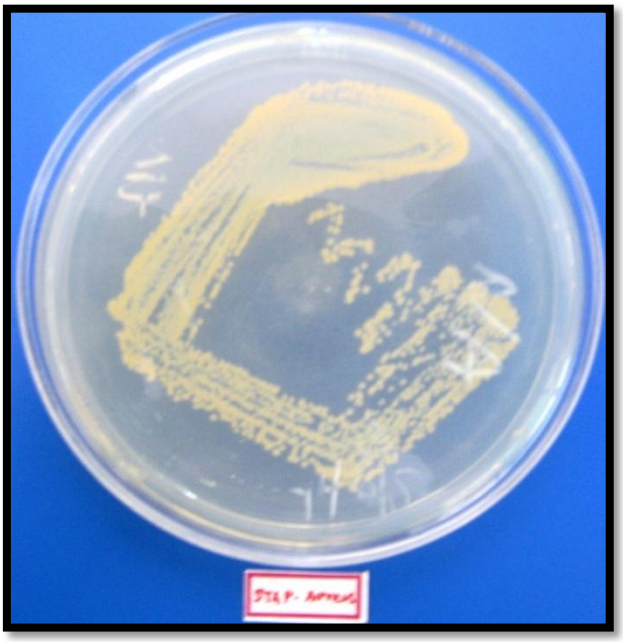
GRAM POSITIVE COCCI IN CLUSTERS



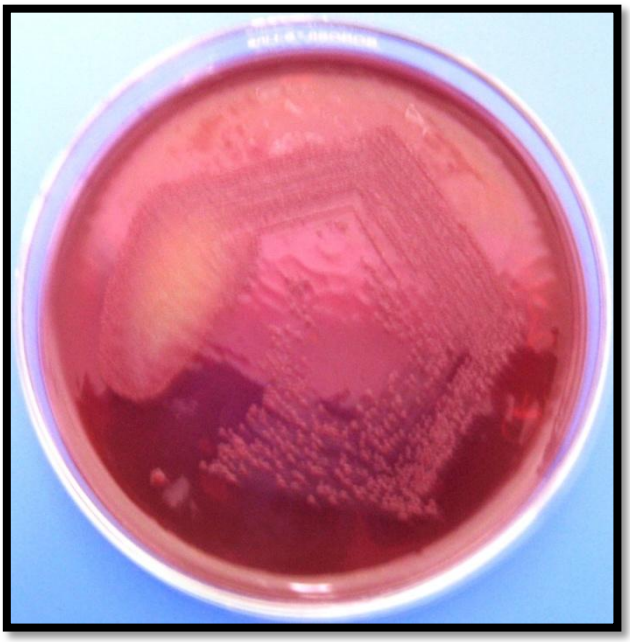
GRAM NEGATIVE BACILLI



STAPH.AUREUS IN NUTRIENT AGAR AGAR

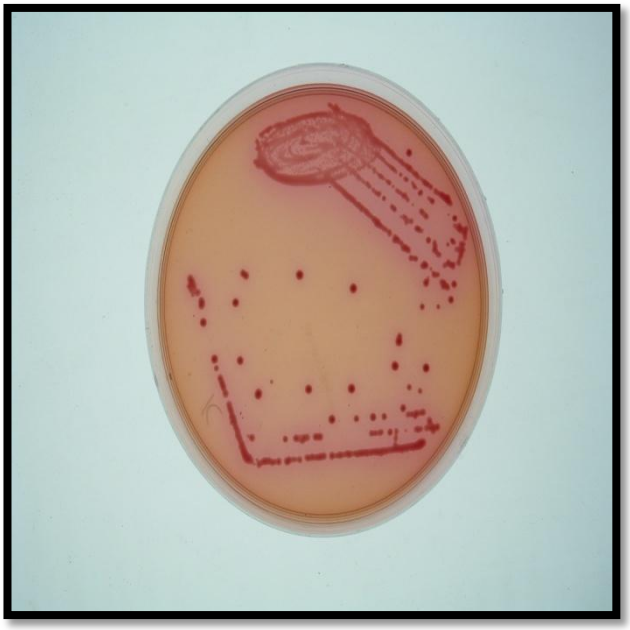


E.COLI IN MACCONKEY



PSEUDOMONAS IN NUTRIENT AGAR AGAR

KLEBSIELLA IN MACCONKEY

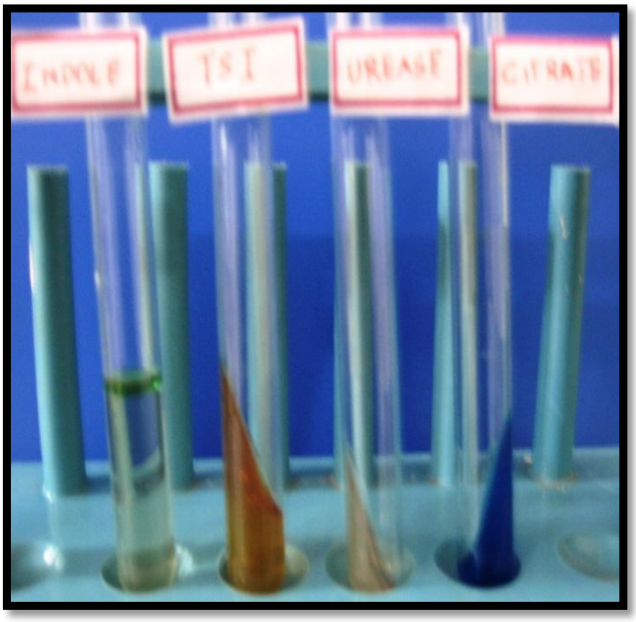


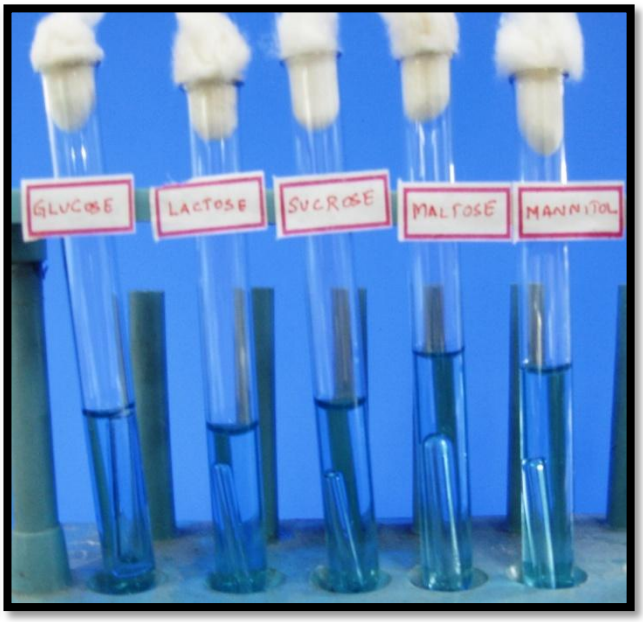
COAGULASE TEST

BIOCHEMICAL REACTION STAPH.AUREUS

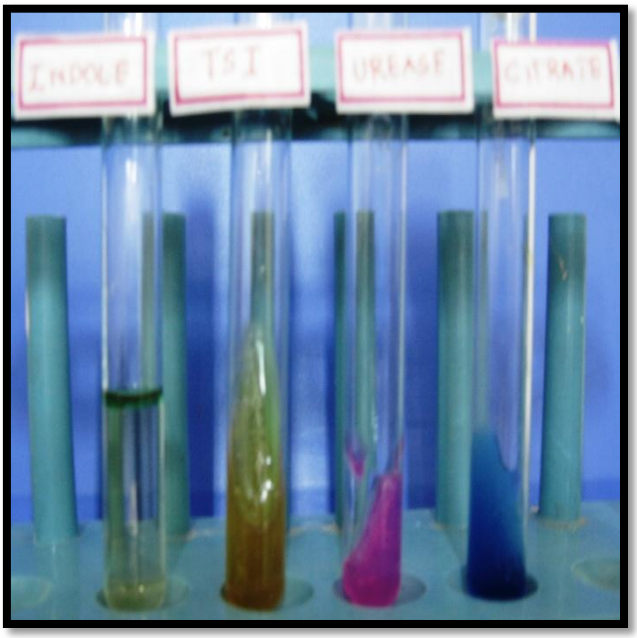
BIOCHEMICAL REACTIONS OF E.COLI

BIOCHEMICAL REACTIONS OF PSEUDOMONAS

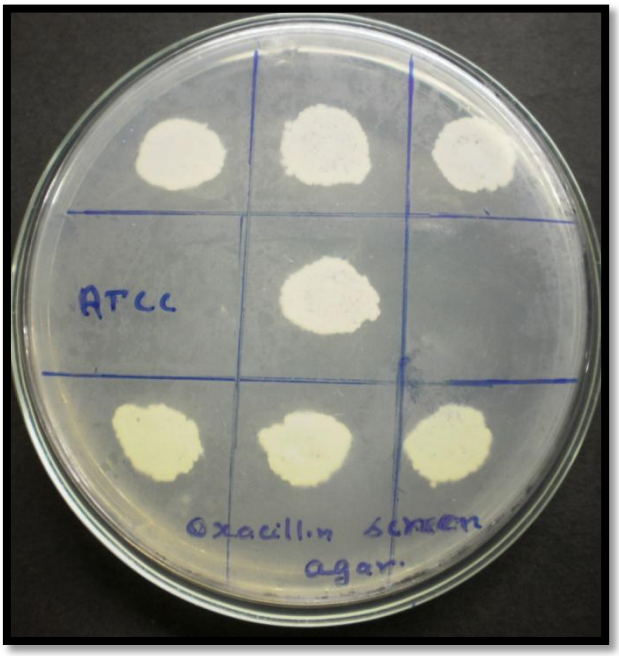




BIOCHEMICAL REACTIONS OF KLEBSIELLA

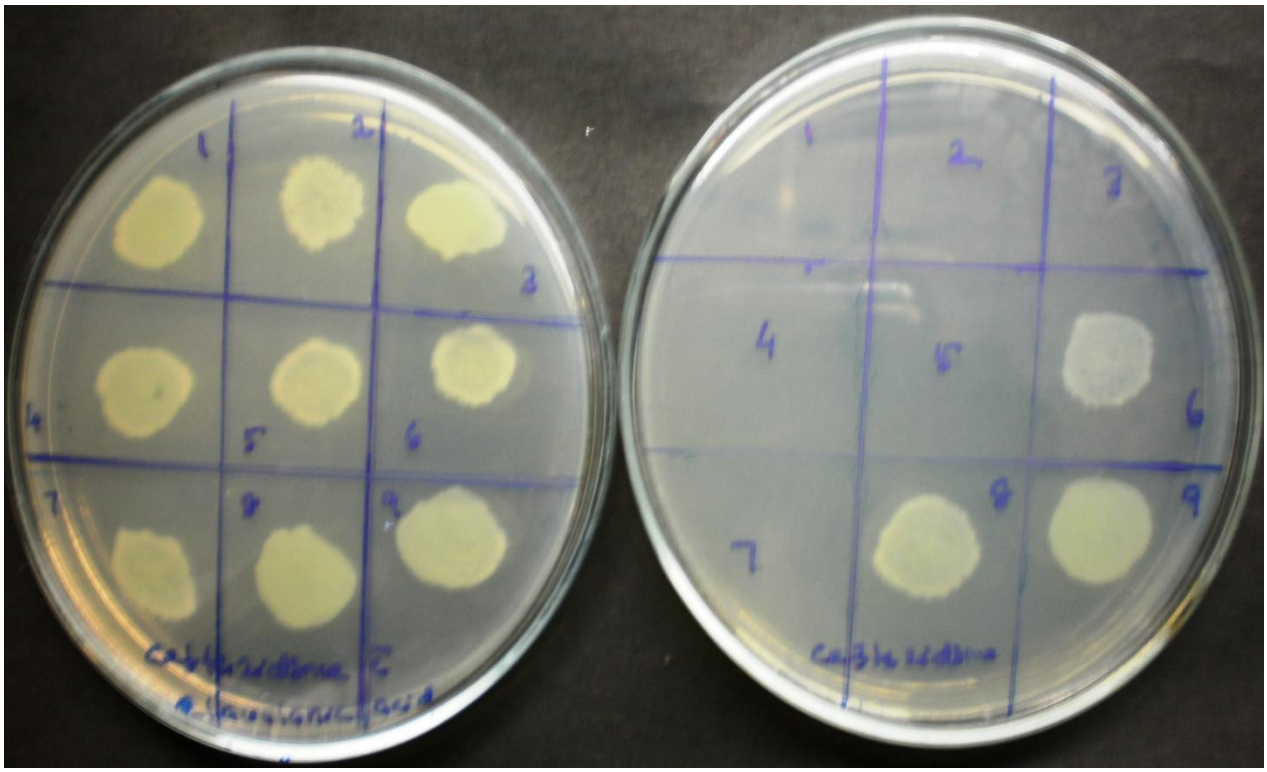


OXACILLIN SCREEN AGAR



DOUBLE DISK SYNERGY TEST

ESBL DETECTION



Discussion

DISCUSSION

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to greater incidence of infections caused by ESBL producing strains and MRSA.

The clinical microbiological laboratory has the task of screening and confirming isolates for ESBL production and MRSA, therefore pus culture and antibiotic susceptibility testing plays an important role in the treatment of wound infection and also prevents from developing further complications.

289 cases of both male and female from up to 80 years of age with various wound infection were included in this study which gives a ratio of 0.97 :1 (143/146).

In developing countries like ours, inspite of strict aseptic precautions, vigorous antibiotic prophylaxis and meticulous surgical techniques wound infection is still a challenge to the surgeon however skilled he may be.

In the present study out of 289 specimens, 164 isolates were identified.(56.74%)

In the present study the organisms isolated from 26 specimen of surgical site infections were Staphylococcus aureus 11 (42.30%) Klebsiella pneumoniae 7 (26.92%), Pseudomonas aeruginosa 3 (11.53%), Enterococci 3 (11.53%), Escherichia.coli 2 (7.6%).

Similar results were observed in the study by Jonathan isibor et al [85,] the predominant bacterial isolate in SSI was Staphylococcus aureus- 35%, followed by Pseudomonas aeruginosa 7.4%, Escherichia coli 13%,.

In study by Eveline geubbels et al[86], the predominant isolate was Staphylococcus aureus (35%).

A study by Jyoti sonawane[87] et al the predominant isolate in SSI was Staphylococcus aureus which was (29.26%) followed by Escherichia coli (18.70%), Pseudomonas aeruginosa (15.3%).

In Shittu et al and Brook et al [88, 89] studies ,Staphylococcus aureus was the predominant organism isolated from surgical site infections ,22.22% and 26.54% respectively . Data from the national nosocomial infections survelliance system[90].reveals that most common incisional SSI pathogens are Staphylococcus aureus, Enterococcus, Enterobacteriaceae, Pseudomonas aeruginosa .

In SP Lilani[91] et al study the predominant isolate in SSI was Staphylococcus aureus followed by Pseudomonas aureginosa. In study by Mangram, Pearson [92] et al and study by Prabhakar et al [93]the predominant isolate in SSI was Staphylococcus aureus.

In a study by Arti kapil[94] the predominant isolate in SSI was staphylococcus aureus which was 40% followed by Pseudomonas aeruginosa 12%, Escherichia coli -11%, Klebsiella pneumoniae 8%.

In a study by Hayath kowner[95], the predominant isolate in SSI was staphylococcus aureus which was 23.37% followed by pseudomonas aeruginosa 23.37% , Escherichia coli 4.8%, Klebsiella pneumoniae 8%.

In a study by Prabhat ranjan [96]the predominant isolate in SSI was Pseudomonas aeruginosa 29.6%, Escherichia coli 20.3%, Klebsiella pneumoniae 16.6%, Staphylococcus aureus 14.3% which is contrast to our study.

In the present study the prevalence of Klebseilla pneumoniae in surgical site infections were 26.92%.

Similar results were observed in a study by Rezwana haque et al[97] in which the prevalence of Klebsiella pneumoniae were 26.76%.

The results in contrast to our study were observed .In a study by Prabhat ranjan[96] et al the prevalence of Klebsiella pneumoniae were 16.6%.In a study by Jyoti sonawarne et al[87] the prevelance of Klebsiella pneumonia were 14.07% .In a study by Arti kapil et al [94]the prevalence of klebsiella pneumonia were 8%. This shows that there is increase in the prevalence of Klebsiella pneumoniae in the present.

The aerobic isolates of burn wound in the present study included Staphylococcus aureus 63(56.75%), kKebsiella pneumonia 29(26.12%), Pseudomonas aeruginosa 13(11.71%), Enterococci 2(1.8%), Coagulase negative Staphylococcus 2(1.8%), Escherichia coli 1(0.9%) and Acinetobacter (0.9%). In concordance with our study, in the study by Misra

et al[98] the commonest pathogen isolated was Staphylococcus aureus (60%). In the study by Revathi et al [99]the predominant isolates was Staphylococcus aureus . %. In a study by S.Vidhani et al [100]the predominant isolate was Staphylococcus aureus 41.8%.

A study by NP Singh et al[101] the predominant isolate in burns wound was pseudomonas aeruginosa 31% followed by staphylococcus aureus 22% and klebseilla pneumonia 19% which is contrast to our study. A study by Manjula et al[102] shows contrast observation in which Pseudomonas aeruginosa was the commonest pathogen isolated from burns wound(51.5%) and Staphylococcus aureus was 11.15%. In another study by Ekrami and Kalantar et al[103] Pseudomonas aeruginosa was the commonest pathogen .In a study by Shankar srinivasan et al[104] the predominant isolate in burns wound was Klebseilla pneumonia 33.91%.. In contrast in study by Herjinder kaur et al[105] the predominant isolate was Pseudomonas aeruginosa 19%. In a study by Agnihotri et al[106] the predominant isolate was Pseudomonas aeruginosa 59%, followed by Staphylococcus aureus 17.9%, Klebsiella pneumonia 3.9%.

The predominant aerobic isolate obtained from 22 specimen of cutaneous abscess was Staphylococcus aureus 13(59.09%). The other isolates obtained were Pseudomonas aeruginosa 5(22.72%), Klebsiella pneumoniae 2(9.09%), Escherichia coli 2(9.095). This correlates with the study of Brook and Frazier[107] where Staphylococcus aureus (30.76%) was the commonest

isolate .In another study by Brook and Finegold also Staphylococcus aureus [108] (42.58%)was the commonest isolate .In another study by Fantahun biadglegne et al[109]the predominant isolate was Staphylococcus aureus 69.7%.

The aerobic isolates of traumatic wound in the present study included Staphylococcus aureus 2(40%), Klebseilla pneumoniae 2(40%), Pseudomonas aeruginosa 1(20%). s 29.14%. In Akinjogunla et al [134] study the predominant isolate were Staphylococcus aureus 37.8%, Pseudomonas aeruginosa 27% which correlates with our study. In Shittu et al [88] study the predominant isolates were Staphylococcus aureus 25%.In contrast , in Brook and Frazier[107] study the predominant isolates were Escherichia coli 22.55%, Staphylococcus aureus 16.57% Klebsiella pneumonia 4.34% .

The antibiotic sensitivity pattern of Staphylococcus aureus in our study shows , 45(50.56%) sensitive to amoxycillin, 57(64.04%) were sensitive to gentamicin, 50(56.17%) sensitive to ciprofloxacin, 36(40.44%) were sensitive to erythromycin, 58(65.16%) were sensitive to cefotaxime, 36(40.44%) were sensitive to cephalexin, 61(68.53%) were sensitive to piperacillin/tazobactum, 81(91.01%) were sensitive to levofloxacin, 82(92.13%) were sensitive to amikacin.

Similar results were observed in the study by Sarita yadav et al [110] sensitivity pattern of Staphylococcus aureus was 48.31% to erythromycin, 53.93% to cephalexin. In a study by Misra et al [98]sensitivity pattern of Staphylococcus aureus was 59% to cefotaxime, 56% to gentamicin, 75% to

ciprofloxacin. In a study by shilpa arora et al[111] sensitivity pattern of Staphylococcus aureus was 78% to amikacin, 46% to gentamicin, 47.2% to ciprofloxacin, 43.2 % to cephalexin.

In contrast to our study ,in the study by Fantahun biadglegne et al [109]the sensitivity of staphylococcus aureus to erythromycin and gentamicin showed 70% and 75.7% respectively. In a study by Sanjay dhar et al [112]sensitivity pattern of Staphylococcus aureus was 20% to amikacin, 26.66% to ciprofloxacin. ,. In a study by Jonathan osariemen isibor et al [85]sensitivity pattern of Staphylococcus aureus was 15.8% to ciprofloxacin, 63.2 % to gentamicin, 15.8% to cephalexin, 63.2 % to erythromycin.

The antibiotic sensitivity pattern of Klebsiella pneumoniae in our study shows , 36(90%) were sensitive to amikacin, 34(85%) were sensitive to ciprofloxacin, 10(25%) were sensitive to cephotoxime, 5(12.5%) were sensitive to cephalexin, 20(50%) were sensitive to piperacillin/tazobactum , 24(60%) were sensitive to imipenem, 18(45%) were sensitive to gentamicin.similar results observed in the study by Shankar srinivasan et al[104] sensitivity pattern of Klebseilla pneumonia was 66.9% to amikacin. In a study by sanjay dhar [112] sensitivity pattern of Klebseilla pneumonia was 100% to amikacin, 33.33% to ciprofloxacin.

In contrast to our study , in the study by Jonathan osariemen isibor et al [85]sensitivity pattern of Klebsiella pneumonia was 50% to ciprofloxacin , 50% to cephalexin, 100% to gentamicin. In a study by Jyoti sonawane et al[87]

sensitivity pattern of Klebsiella pneumonia was 37.62% to ciprofloxacin, 47.37% to gentamicin, 100% to imipenem.

The antibiotic sensitivity pattern of Pseudomonas aeruginosa in our study shows , 18(81.81%) were sensitive to amikacin, 13(59.09%) were sensitive to ciprofloxacin, 8(36.36%) were sensitive to cephotaxime, 6(27.27%) were sensitive to cephalexin, 12(54.54%) were sensitive to piperacillin/tazobactum, 22(100%) were sensitive to imipenem, 11(50%) were sensitive to gentamicin. Similar results were observed in the study by Shankar srinivasan et al[104] sensitivity pattern was 62.3% to amikacin. . In a study by Sanjay dhar et al [112] sensitivity pattern of Pseudomonas aeruginosa was 65% to amikacin, 30% to ciprofloxacin. In a study by Jyoti sonawane et al[87] sensitivity pattern of Pseudomonas aeruginosa was 54.22% to amikacin, 96.38% to imipenem. In a study by Jonathan osariemen isibor et al [85]sensitivity pattern of Pseudomonas aeruginosa was 35.7% to ciprofloxacin, 71.4% to gentamicin, 28.5% to cephalexin.

In a study by Fantahun biadglegne et al [109]sensitivity of Pseudomonas aeruginosa to gentamicin was 67%. In a study by Prabhat ranjan et al[96]sensitivity pattern of Pseudomonas aeruginosa was 76.9% to imipenem, 53% to amikacin, 36% to ciprofloxacin, 29.1% to gentamicin. In the study by shampa anupurba et al[113], the prevalence of pseudomonas aeruginosa in wound infection was 32% and were sensitive to ciprofloxacin 58%.

In contrast to our study , in the study by Misra et al [98]sensitivity pattern of Pseudomonas aeruginosa was 67% to cefotaxime, 12 % to getamicin, 60% to amikacin, 47% to ciprofloxacin.in a study by sp lilani et al[91] Pseudomonas aeruginosa was 100% resistance to gentamicin. In a study by Manjula Mehta et al [102]sensitivity pattern of Pseudomonas aeruginosa was 15% to amikacin, 11% to gentamicin, 21% to ciprofloxacin.

The antibiotic sensitivity pattern of Escherichia coli in our study shows, 4(80%) were sensitive to amikacin, imipenem, 3(60%) were sensitive to ciprofloxacin,2(40%) were sensitive to cephalexin, 1(20%) were sensitive to cephotaxime, pipercillin/tazobactum ,gentamicin. In a study by Shankar srinivasan et al[104] sensitivity pattern of Escherichia coli was 42.7% to amikacin. . In a study by Sanjay dhar et al [112] sensitivity pattern of Escherichia coli was 50% to amikacin, 30% to ciprofloxacin. In a study by Jonathan osariemen isibor et al[85] sensitivity pattern of Escherichia coli was 85.7% to ciprofloxacin, 57.1% to gentamicin, 85.75% to cephalexin.

In a study by Misra et al [98]sensitivity pattern of Escherichia coli was 65% to cefotaxime, 89% to amikacin, 35% to gentamicin, 19% to ciprofloxacin. In a study by Fantahun biadglegne et al [109]sensitivity of Escherichia coli to gentamicin was 75%.. In a study by Jyoti sonawane et al[87] sensitivity pattern of Escherichia coli was 13.86% to ciprofloxacin, 49.5% to gentamicin, 99.01% to imipenem.

MRSA is a major nosocomial pathogen causing significant morbidity and mortality[114].The important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers is the predominant mode for patient to patient transmission.[115]

The percentage of MRSA isolated in our study was 40.44% similar results were observed . In the study by Arti kapil et al[94] the MRSA was 30%. In the study by Shilpa arora et al[111] which was 46%. In the study Vidhani et al 100the MRSA was found to be 51.6%. In the study by Sarita yadav et al [110] methicillin resistance was documented in 60.6% of Staphylococcus aureus isolates which was alarmingly high prevalence of MRSA.

In contrast in the study Jyoti sonawane et al[87] prevalence of MRSA was 27.85%. In a study by Hayath kowner[95], MRSA isolated was 21.7%.a study by Srinivasan et al [135] 33.33% of staphylococcus isolates was identified as MRSA.

In the study on spectrum of antimicrobial resistance among MRSA, ciprofloxacin resistance was as high as 90% and Qureshi et al [116] had reported the same as 98.9%. In contrast in our study 44% of the strains are resistant to ciprofloxacin. Pulimood [117]had observed only 8% resistance of MRSA to gentamicin as against 44% in our study. Qureshi [116]had reported gentamicin resistance of 97.8% which is high compared to our study.

In our study we obtained high percentage of multidrug resistant MRSA .Majumder et al[118] from assam had reported 23.2% of the MRSA isolated found to be multidrug resistant. Anupurba et al [119]from uttar Pradesh had reported a higher percentage of multidrug resistant MRSA.

Vidhani et al [100]from delhi reported even a higher percentage of multidrug resistant MRSA.. In the study by Misra et al[98] MRSA isolates were resistance to erthyromycin (69%), 66.6%to gentamicin, 40% to ciprofloxacin, 69.2% to cefotaxime. In the study by Rajaduraipandi et al[120] the MRSA isolates were resistant to gentamicin 62%, cephalexin 60.8%, 60% to erythromycin.

This variation might be because of several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital.

The percentage of MDR strains among MRSA was found to be in various reports from other parts of the country, the burden of such strains has ranged from 23.2% to 32% to 63.6%.[118, 119,120] I

In the study by vidhani et al[100] the antibiotic sensitivity pattern of MRSA isolates were 11.5% to amoxicillin, 21.5% to cefotaxime, 17.7% to erythromycin, 26.6% to amikacin, 67% to ciprofloxacin.

In the study by hare Krishna tiwari et al[121] the MRSA isolates was 38.44% and they were resistant to gentamicin 56.14%, amikacin 42.98%, 75.56% to ciprofloxacin, 72.81% to erythromycin.

The most effective way to prevent MRSA infections is by doing continuous surveillance of antibiotic resistance profiles of local *S*taphylococcus aureus isolates to formulate antibiotic policies and effective infection control practices.

Extended spectrum beta lactam antimicrobial drugs are commonly included in empirical antibiotic regimen for treatment of Gram negative sepsis but the emergence of ESBL producing bacteria poses a serious threat to the continued use of this family of antibiotics [122.]

Therefore , infections caused by ESBL isolates need to be addressed with a general consensus in order to overcome the challenge of infection management worldwide .

There have been sporadic reports of ESBLs from major hospitals in India and some of them recorded the incidence to be as high as 60-68% (Mathur et al)[124] the unusually high incidence of ESBLs should be a cause of concern to the regulators of the hospital antibiotic policy. Over reliance on third generation cephalosporins to treat Gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics.

In our study the ESBL producing Enterobacteriaceae were 19 (42.22%) .In the study by C Rodrigues et al [125] the prevalence of ESBL was 53%.The occurrence of ESBL producing Klebsiella pneumoniae accounts for 42.5% in our study and E.coli was 40% which is correlating with the study of Ashwin et al [126]43.75% and 58.06% respectively. ESBLs among Klebsiella pneumoniae of this study also correlates with Leblebicioglu study[127] (50%),and in Ozgunes study [128]it was 47%.

But in contrast , a study by Shukla et al[129] showed 36.1% of ESBL producing Klebsiella pneumoniae. In a study by C Shanmuganathan et al[130] , there was an outbreak of ESBL producing Klebsiella pneumoniae. In contrast to our study the study by MS kumar et al[131] the ESBL producing Klebsiella pneumoniae was 14% and ESBL producing Escherichia coli was 63.7%. 85% of Klebsiella pneumoniae exhibited an MIC of 256 µg/ml to cefotaxime. 95% of Escherichia coli exhibited an MIC of 256µg/ml to cefotaxime.

In the study by Shukla et al [129]the prevelance of ESBL producing Klebsiella pneumonia was 30.18%.The MIC of 3rd generation cephalosporins test antibiotics against ESBL producers ranged between 2 to 128µg/ml.

In a study Rezwana haque et al [97]the percentage of ESBL producing bacteria was 46.67% and highest rate was found in Klebsiella pneumonia (57.89%) followed by Escherichia coli (47.83%), which correlates with our

study ESBL was 42.22% and Klebsiella pneumoniae (42.50%) and Escherichia coli (40%.)

In the postoperative wound infections the occurrence of ESBL producers in the study were33.33% which correlates with the Ashwins et al [126] study (22.4%).

In our study , 89.5% of ESBL positive bacterial strains were sensitive only to amikacin, while they showed significantly increasing resistance to all other antibiotics used, which implies that ESBL producing organisms are multidrug resistance. The prevalence of these multidrug resistant ESBL strains is also reported to be on the rise.[123]

The resistance pattern of ESBL producers in our study were 79% to piperacillin/tazobactum and 58% to gentamicin , ciprofloxacin, imipenem but 89.5% sensitive to amikacin which correlates with study by Baby padmini et al[133] where sensitivity of ESBL producers to amikacin was 82.6%. In the study by Dechen c tsering et al[132] the prevalence of ESBL was 34%. The ESBL producers were resistant to 51.9% to piperacillin/tazobactum, and ciprofloxacin, 54.3% to gentamicin.

The resistance pattern of ESBL producers in a study by Rezwana haque et al [97]was 100% to ampicillin, 81.82% to ciprofloxacin, 45.45% to gentamicin, but 100% sensitive to imipenem.

In a study by Jyoti sonawane et al [87] the ESBL isolates were frequently resistant to other antibiotics but showed nearly 100% sensitive to piperacillin/tazobactum and imipenem.

Minimum inhibitory concentrations (MIC) of Cefotaxime and Ceftazidime and of Cefotaxime and Ceftazidime with 2 µg/ml of Clavulanic acid by agar dilution method for ESBL producers. (N=19)

MIC of cefotaxime and ceftazidime for ESBL producing enterobacteriaceae isolates was between 32-2048 mg/ml of agar. MIC of cefotaxime for ESBL producing isolate was between 0.125 -128 mg/ml of agar in the presence of clavulanic acid at a concentration of 2mg/ml of agar showing 8 fold reductions in MIC. MIC of ceftazidime for ESBL producing isolate was between 0.125 -128 mg/ml of agar in the presence of clavulanic acid at a concentration of 2mg/ml of agar showing 8 fold reductions in MIC.

Monitoring and judicious usage of extended spectrum cephalosporins , periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBLs.

Summary

SUMMARY

- Two hundred and eighty nine patients with wound infection between March 2009 and February 2010 formed the study group. Specimens obtained from patients were cultured and bacterial pathogens were identified by Gram staining and various biochemical reaction.
- The predominant isolates of wound infection were Staphylococcus aureus 89(54.26%) followed by Klebsiella pneumonia 40(24.39%), Pseudomonas aeruginosa 22(13.41%).
- All 89 isolates of Staphylococcus aureus were screened for methicillin resistance using oxacillin disc (1µg) and confirmed using cefoxitin disk(30µg)The percentage of MRSA was found to be 40.44%. and were susceptible to vancomycin(100%), levofloxacin (97.2%), amikacin (94.4%).
- Antibiotic susceptibility testing was done with appropriate antibiotics including third generation cephalosporins and the Enterobacteriaceae isolates were screened for ESBL production by disk diffusion method(as per CLSI guidelines) and double disk synergy test. ESBL producers were confirmed by phenotypic confirmatory test and minimum inhibitory concentration determination by agar dilution method.

- Out of 45 Enterobacteriaceae isolates 19(42.22%) were found to be ESBL producers and were susceptible to amikacin(89.5%).
- MIC for the isolates was between 32µg/ml to 2048µg/ml for cefotaxime and ceftazidime which was reduced to 0.125 to 128 µg/ml respectively in the presence of 2 µg of clavulanic acid/ml of agar.

Conclusion

CONCLUSION

- The predominant isolate was found to be Gram positive bacteria 96 (58.53%) than Gram negative bacteria 68 (41.46%).However Staphylococcus aureus 89 (54.26%) was seen as the most common bacterial pathogen followed by Klebsiella pneumoniae 40 (24.39%) and Pseudomonas aeruginosa 22 (13.41%)

- Among the Staphylococcus aureus 36 (40.44%) were found to be MRSA and were susceptible to vancomycin (100%), levofloxacin (97.2%), amikacin (94.4%).
- Detection of MRSA were found to be high with cefoxitin disk than oxacillin screen agar .
- Among Enterobacteriaceae isolates 19 (42.2%) were found to be ESBL producers and were susceptible to amikacin (89.5%).
- By employing standard microbiological techniques meticulously the causative agents can be isolated and antimicrobial sensitivity can be assessed for proper management of wound infection.
- Essential infection control practices including hand washing by hospital personnel and provides better control of antibiotic resistant strains.

Annexures

ETHICAL COMMITTEE

GOVT. KILPAUK MEDICAL COLLEGE, KILPAUK, CHENNAI- 10.

Venue: PANAGAL HALL, KMC
Dt: 26.11.2009

CHAIRPERSON
Prof. Dr.V.KANAGASABAI, MD.,
The Dean
Govt. Kilpauk Medical College, Chennai-10

Sub: Ethical Committee project work - approved.
Ref: Lr.No.3944/P&D/09 Dt. 17.11.09.

With above reference, the Institutional Ethical committee meeting for the following students was conducted at our Institution on 26.11.09.

1.	Dr.Uma S. Pandian IIIr Yr (Micro)	Microbiological profile of Diabetic foot ulcers.
2.	Dr.V.Vasanthapriyan.M.R	Fungal isolates in human corneal ulcer
3.	Dr.B.Ravichandran	Study of Bacteriological profile in postoperative would infections.

We are glad to inform you that at the Ethical Committee meeting the documents were discussed and the above short term projects are Ethically approved.

CHAIRPERSON
Prof. Dr.V.KANAGASABAI, MD.,
The Dean
Govt. Kilpauk Medical College,
Chennai-10.

To
The Individuals

PROFORMA

NAME :

AGE/SEX :

OP/IP NO:

WARD:

DATE OF ADMISSION:

OCCUPATION:

PRESENT COMPLAINTS:

PAST HISTORY : DIABETES MELLITUS:
HYPERTENSION:
CORONARY HEART DISEASE:

CLINICAL FINDINGS:

DIAGNOSIS:

INVESTIGATIONS:

TREATMENT AND MANAGEMENT:

APPENDIX

1.STAINS AND REAGENT:

GRAMS STAIN:

Reagents

1. primary stain- crystal violet 10g

Absolute alcohol 100ml

Distilled water 1 litre

2. Iodine solution- iodine 10g

Potassium iodide 20g

Distilled water 1 litre

3. Acetone

4 .Counter stain- dilute carbol fuchsin

Procedure

1. Flood the fixed smear with crystal violet for one minute

2. Rinse gently with distilled water

3. Flood the slide with grams iodine for one minute

4. Rinse gently with distilled water

5. Decolourise with acetone for only 2-3 seconds.

6. Rinse the slide with distilled water to remove excess decoluriser

7. Flood the slide with dilute carbol fuchsin for 30 seconds.

8. Rinse the slide with distilled water, air dry and examine under oil immersion objective of the microscope.

2.MEDIA USED:

1. **PEPTONE WATER:**

Peptone	10g
Sodium chloride	5 g
Distilled water	1 Litre

Dissolve the ingredients in warm water, adjust the ph to 7.4 to 7.5 and filter. Distribute as required and autoclave at 121° c for 15 minutes.

2. **NUTRIENT AGAR:**

Ingredients	Grams per Litre
Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	15

Final ph at 25°c , 7.4±0.2

Suspend 28 grams in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure 121°c for 15 minutes and pour into sterile Petridishes.

3.**BLOOD AGAR:**

Sterile sheep blood	5ml
Nutrient agar	100 ml

Autoclave the Nutrient agar at 121°c for 15 minutes. Cool to 45-50°c and add blood with sterile precautions and distribute in Petri dishes.

4.**MACCONKEY AGAR:**

Ingredients	Grams per Litre
Peptic digest of animal tissue	17
Proteose peptone	3
Lactose	10
Bile salts	1.5
Sodium chloride	5
Neutral red	0.03
Agar	15

Final ph at 25°c 7.1±0.2

Suspend 51.53 grams in 1000ml of distilled water . Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure 121c for 15 mts and pour into sterile petridishes. Mix well before pouring.

5**. MUELLER HINTON AGAR:**

Ingredients	Grams per Litre
Beef infusion	300
Casein acid hydrolysate	17.50

Starch	1.5
Agar	17.00

Final ph at 25°c 7.3±0.2 .Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure 121°c for 15 minutes and pour into sterile Petridishes. Mix well before pouring. Pour 0-25 ml of it into petridishes of 9cm diameter to give a thickness of 4mm.

6. **MANNITOL SALT AGAR:**

Peptone	10g
Meat extract	50g
Mannitol	10g
Sodium chloride	75g
Distilled water	1 litre

Phenol red

Sterilize by autoclaving at 15 lbs pressure 121c for 15 mts and pour into sterile petridishes.

7**. INDOLE TEST MEDIUM:**

Peptone	20g
Sodium chloride	5g
Distilled water	1 litre

pH was adjusted to 7.4 Sterilize by autoclaving at 15 lbs pressure 121c for 15 mts

8. **SIMMONS CITRATE MEDIUM:**

Sodium chloride	5g
Magnesium sulphate	0.2g
Ammonium dihydrogen phosphate	1g
Potassium dihydrogen phosphate	1g
Sodium citrate	5g
Bromothymol blue(0.2%)	40ml
Distilled water	1 litre

Sterilize by autoclaving at 15 lbs pressure 121c for 15 mts in a flask and distribute as slopes in sterile test tubes.

9. **CHRISTENSENS UREASE MEDIUM:**

Peptone	1g
Sodium chloride	5g
Di potassium hydrogen phosphate	2g
Phenol red(1 in 500 aqueous solution)	6ml
Agar	20ml
Distilled water	1 litre

Glucose 10% solution

Urea 20% solution

Sterilize the glucose and urea solution by filteration. Prepare the basal medium without glucose or urea. Adjust the pH to 6.8-6.9 and Sterilize by

autoclaving at 15 lbs pressure 121c for 15 mts in a flask. Cool to about 50°c . Add glucose and urea solution and por as deep slopes in tubes.

10. **TRIPLE SUGAR IRON AGAR:**

Beef extract	3g
Yeast extract	3g
Peptone	15g
Proteose peptone	5g
Lactose	10g
Glucose	1g
Sucrose	10g
Ferrous sulphate	0.2g
Sodium chloride	5g
Agar	12g
Phenol red	0.024g
Distilled water	1 litre

Heat to dissolve the solids; add the indicator solution; mix and tube. Sterilize by autoclaving at 15 lbs pressure 121c for 15 mts and cool to form slopes with deep(3cms) butts

BIBLIOGRAPHY

1. Enoch, s. and price, p.2004. Cellular and biochemical differences in the Pathophysiology of healing between acute wounds, chronic wounds, and wounds in the aged.

2.Bowler PG, Duerden BI, Armstrong DG.2001. Wound microbiology and associated approaches to wound management. Clin. Microbiology review 14;244-269

3.Gottrup, F.Melling,a and Hollander. D. 2005. An overview of surgical site infections; aetiology, incidence and risk factors. EWMA journal; 5(2) 11-15.

4.Wilson. a. p.r. Gibbons, c. Reeves, b.c, Hodgson, b. Liu, m. and Plummer, d.2004. Surgical wound infections as a performance indicator; agreement of common definitions of wound infections in 4773 patients. BMJ ;329;720-722

5.Collier, m. 2004. Recognition and management of wound infections. Wounds.

6. Plowman, r.2005. The socioeconomic burden of hospital acquired infection. Euro.surveill; 5(4);49-50

7.Kernodle ds. Kaiser ab. Postoperative infections and antimicrobial prophylaxis. In; Mandell gl. Bennett je, Dolin r eds. Principle and practice of infectious diseases. 4th edition newyork, Churchill livingstone 1995; 2742-56

8.Hackner sm 1994. Common infection of the skin; characteristics, causes and cures.post graduate medicine. 96;43-529.

9. Konemans color atlas and Text book of diagnostic microbiology, 6th edirion 2006. Lippincott Williams and wilkins..

10.. Cooper R, Kingsley A, White R. *Wound Infection and Microbiology*. *:* Medical Communications (UK) Ltd for Johnson & Johnson Medical, 2003.

11.Mulu a, Moges f , Tessema b, Kassu a. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at university of Gondar teaching hospital, north west Ethiopia. Ethiop med j. 2006; 44 (2); 125-131

12.Nichols rl. Surgical wound infection. Am.J. Med. 1991; 16;91(3b) -6413.

13.Onyang d, Machoni f, Waindi en. Multi drug resistance of salmonella enteric serovars typhi and typhimurium isolated from clinical samples at two rural hospitals in western Kenya. J. infect developing countries.2008;2 106-111

14.Shears p. Antimicrobial resistance in tropics. Tropical doctor. 2003; 30(2); 114- 116

15. World health organization. Resistance to antimicrobial agent. WHO bulletin.1996;74(3);335-336

16.Turnidge j. What can be done about resistance to microbes? Br Med J.1998;317;645-647

17.Hart ca, Kairuki s. Antimicrobial resistance in developing countries.Br Med J 1998;317; 640-650

18.Rind r, Khan ts. Antibiogram sensitivity of bacterial organisms identified from surgical and non surgical wounds of animal. Pak J Bio.Sci.2000; 3(10); 1719-1723

19.Cohen IK. A Brief History of Wound Healing. Yardley, Pa: Oxford Clinical Communications Inc; 1998.

20.Lister J On a new method of treating compounfractures. Lancet. 1867;1:326-329,387-389,507-509.

21.Qvist G. Hunterian Oration, 1979. Some controversial aspects of John Hunter's life and work. *Ann R Coll Surg Engl*. Jul 1979;61(4):309-11.

22. Helling TS, Daon E. In Flanders fields: the Great War, Antoine Depage, and the resurgence of débridement. Ann Surg. Aug 1998;228(2):173-81

23. Duerden b i. virulence factors in anaerobes. Clin inf dis . 1994;18;s253-s259

24.Barlett jg gorbach s l. Anaerobic infections of head and neck. Otolaryngo clin North Am. 1976; 9; 655-678

25. Hentges d. Anaerobes as normal flora. In; Finegold sm, George w l. editors. Anaerobic infections in humans. San diego, calif; academic press, inc; 1989. Pp.37-53

26.Hunt t k Hopt h w. Wound healing and wound infection – what surgeons and anaesthesiologists can do. Surg Clin North Am. 1997; 77; 587-606

27. Hohn d c, Mackay r d, Halliday b. hunt t k. Effect of O2 tension on microbicidal function of leukocytes in wounds and in vitro. Surg forum. 1976;27;18-20

28. Niinikoski, j;Gottrup, f; hunt, t k. The role of oxygen in wound repair. In; Janssen h, Rooman r, Robertson j j s. editors. Wound healing . petersfield, united kingdom. Wrightson biomedical publishing ltd; 1991. Pp.165-173

29. Peel alg. Definition of infection. In; Taylor ew. Editor.Infection in surgical practice. Oxford, United Kingdom; oxford university press; 1992. Pp.82-87

30. Crowe- mj Cooke- em Review of case definitions for nosocomial infection towards a consensus. J. hospital infect 1998;39(1);3-11

31.Glen mayhall. C. Hospital. Epidemiology and infection control.1996; ch 11;154-171

32. Garner js. Guideline for prevention of surgical wound infection.CDC Atlanta 1985 pb 85-92

33. Bengt gastrin, Arild lovestad. Postoperative wound infection- relation to different types of operation and wound contamination categories in orthopeadic surgery.J hosp inf 1989;13 387-393.

34.Berridge d.c Hopkinson br Making s. A bacteriological survey of amputation wound sepsis. J hosp inf 1989 13 167-172

35.Abu hanifah y. Postoperative wound surgical wound infection. Med J Malays 1990;45;293-297

36.Cruse pje Foord r. Epidemiology of wound infection. A ten year prospective study of 62939 wounds. Surg. Clin.North. Am 1980;60;27-40

37.Twum-danso k.Grant c Wosornu l. Microbiology of postoperative wound infection; a prospective study of 1770 Wounds J.hosp infect 1992;21;29-37

38. Kunt tk. Surgical wound infections; an overview. Am J Med. 1981;70;712-718

39.Hansis m. Pathophysiology of infection- a theoretical approach. Injury. 1996;27;s-c5-s-c8.

40. Brook I frazier eh. Aerobic and anaerobic bacteriology of wounds and cutaneous abscess. Arch surg.1990;125;1445-1451.

41. Brook I Finegold sm Aerobic and anaerobic bacteriology of cutaneous abscessis in children. Peadatrics.1981;67(6) 891-895.

42.Meislin hw , Lerner sa, Graves mh, Mcgehee md,Kocka fe, Morella ja, Rosen p. Cutaneous abscesses- Anaerobic and aerobic bacteriology and outpatient management. Ann Intern Med. 1997;87;145-149.

43. Brook I Frazier eh. Aerobic and anaerobic microbiology of infection after trauma. Am J Emerg Med.1998;16;585-591.

44. Brook I Frazier eh. Aerobic and anaerobic microbiology of infection after trauma in children. J Accid Emerg Med.1998;15;162-167

45. Elliot dc, Kufera ja, Myers ram. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. Ann surg. 1996;224;672-683

46.Sachs mk. The optimum use of needle aspiration in the bacteriological diagnosis of cellulitis in adults. Arch Intern Med.1990;150;1907.

47. Sigurdsson. Af. Gudmundsson s. The etiology of bacterial cellulitis as determined by fine needle aspiration. Scand J Infect Dis.1989;21(5) 537-542

48.Bowler p. Davies b. The microbiology of acute and chronic wounds. Wounds 1999;11(4) 72-78

49.Stephens .p.Wall I , Wilson m et al. Anaerobic cocci populating the deep tissues of chronic wounds impair cellular wound healing response in vitro. British Journal of Dermatology 2003;148;456-466.

50.Levenson s Khan Gruber c. Wound healing accelerated by staphylococcus aureus. Archives of Surgery 1983 118;310-320.

51. Tenorio a, Jindrak j, Weiner m, Bella e. Accelerated healing in infected wounds. Surgery Gynaecology and Obstretics 1976;142;537-543

52.Trengove n, Staccy m, mc Gechie dr. Mata s. Qualitative bacteriology and leg ulcer healing. Journal of wound care.1996 5 277-280

53.Fredenburg s, Devalentine m, Loretz l. Clinics in podiatric medicine and surgery 1987 4(2)395-412

54.Joseph ws, Alex da. Clinics in podiatric medicine and surgery 1990 7(3) 467-481

55.Wheat lj, Alled sd, Henry m, Kernek cb- Diabetic foot infections, bacteriological analysis-Archives of Internal Medicine 1986 146(10)1935-1940

56.Capturo gm. Joshi n. Witecamp mr; Foot infections in patients with diabetes. American Family Physician. 1997 56 (1)195-202

57. Diane m Citron Ellie jc Goldstein bacteriology of moderate to severe diabetic foot infections and in vitro activity of antimicrobial agents JCM 2007 45(9) 2819-2828

58.Ravisekar Gadepalli Benu dhawan, Arti kapil. A Clinicomicrobiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetic care 2006 29 1727-1732

59.Lipsky ba Osteomyelitis of foot in diabetic foot patients. Clinical infectious diseases. 1997 25(6) 1318-1326

60.Jones ew Edwards r Fuch r Jeff coate a microbiology study of diabetic foot lesions. Diabetic medicine.1984 2 (3) 213-215

61.Armstrong dg et al. Validation of a diabetic wound classification system. The contribution of depth; infection and ischemia to risk of amputation. Diabetes care 1998 21(5) 855-859

62.Bamberger dm Davs gp Gerding dn. Osteomyletis in the feet of diabetic patients AMJ med 1987;83(4)653-660.

63.Peterson carter fasching clabots. Therapy of lower extremity infections with ciprofloxacin in patients with diabetes. Peripheral vascular disease or both. AMJ med 1989 86 801-808

64.Borrero Rossini . Bacteriology of 100 consecutive diabetic foot infections/ivs of ampicillin/sulbactam vs cefoxitin. 1992 43(4) 357-361

65.Boulton menses Ennis . Diabetic foot ulcers; a framework for prevention and care. Wound Rep Regen 1999;7;7-16

66.Laing p. Diabetic foot ulcers. Am J Surg 1994;167;31-36

67.Gough clapperton Rolando Edmonds . Randomized placebo controlled trial of G-CSF in diabetic foot infections. Lancet 1997 350(9081)855-859

68.Levin . Journal of wound , osteomy and continence nursing. 1998;25(3) 129-146

69.Neil munro . A comparison of two culturing methods for chronic wounds. Osteomy wound management 1997; 43; 20-30

70.Pallua fuchs Hafemann Volpel noah Lutticken. A new technique for bacterial assessment on burn wounds by modified dermabrasion. J Hosp Infect. 1999;42;329-337

71.Johnson lebahn, Peterson gerding . Use of an anaerobic collection and transport swab device to recover anaerobic bacteria from infected foot ulcers in diabetes. Clin Infect Dis 1995;20;289-290

72.Jousmies somer , Finegold . Problems encountered in clinical anaerobic bacteriology. Rev Infect Dis. 1984;6;45-50

73.Summanen baron Citron strong Wexler wadsworth. Anaerobic bacteriology manual. 5th edition Belmont, calif ;1993

74.Popescu doyle . The Gram stain after more than a century. Biotech histochem. 1996 71 145-151

75.Heggers Robson Doran. Quantitative assessment of bacterial contamination of open wounds by slide technique. Trans r Soc Trop Med Hyg . 1969;63;532-534

76.Johnson, Thatcher cox . Techniques for controlling variability in gram staining of obligate anaerobes. J Clin Micro 1995;33; 755-758

77.Bradford . ESBL s in the 21st century; characterization, epidemiology and detection of this important resistance threat. Clinic Micro Rev 2001;14(4) 933-951

78.Rice , Bonomo, A betalactamases which one are clinically important? Drug resistance update.2000;3;178-189

79.Thomson. Controversies about extended spectrum and amp_c betalactamases. Emerging Infectious Dis 2001;7(2) 333-336

80.www.lahey.org maintaining the list of Extended spectrum betalactamases.

81.David Peterson and Robert. ESBL; a clinical update Clinic Micro Rev. 2005;18(4) 657-686

82.Papasian kragel. The microbiology laboratorys role in life threatening infections. Crit care nurse 1997;20;44-59.

83. Konemans color atlas and Text book of diagnostic microbiology, 6th edirion 2006. Lippincott Williams and wilkins..pp945.

. 84.Mackie and Mc Cartney Practical medical microbiology 14th edition 2006 churchill livingstone pp166-169

85. Jonathan osariemen Isibor ,Ashietu Oseni,Adevbo Eyaufe, Rachel Osagie and Ahadu Turay . Incidence of aerobic bacteria and candida albicans in postoperative wound infections, African journal of microbiology research, 2008, vol2, 288-291.

86.Eveline Geubbels, A.Joke Mintjes-de Groot, Jan Maarten J.Van den Berg, Annette S. de Boer.An operating surveillance system of surgical site infections in the Netherlands. Infection Control and Hospital Epidemiology 2000 Vol 21 No.5.

87. Jyoti sonawane ,Narayan Kamath, Rita Swaminathan, kausal Dhosani. Bacterial profile of SSI and their antibiograms in a tertiary care hospital in Navi Mumbai., Bombay hospital journal 2010, 52, 3,

88. Shittu A.O., Kolawole D.O and Oyedepo E.A.R. A study of wound infections in two health institutions in ile-ife, Nigeria, African journal of biomedical research, 5, 3,2002, 97-102

89. Brook I Frazier eh. Aerobic and anaerobic bacteriology of wounds and cutaneous abscess. Arch Surg.1990;125;1445-1451.

91. SP Lilani , N Jangle, A chowdhary, GB Daver. Surgical site infection in clean and contaminated cases, IJMM, 200523, 249-252

92.Mangram AJ , Horan TC, Pearson ML .The Hospital infection control practices advisory committee .Guideline for prevention of surgical site infection.

93.Prabhakar H, Arora S .A bacteriological study of wound infections. Journal of Indian Medical Association 1979 73 145-148.

94.Arti kapil , Benu Dhawan, B K Das, Srujana Mohanty. Bacteriology of orthopaedic wound infections in an Indian tertiary care hospital , Indian Journal of Medical Research, 2005, 121, 784-785.

95.Hayeth kownhar , Esaki Muthu Shankar, Ramachandran Vignesh,Ramalingam Sekar, Vijayakumar Velu, Usha Anandrao. High isolation rate of staphylococcus aureus from SSI in Indian hospital,, Journal of Antimicrobial Chemotherapy,61, 3, 758-760 2008.

96. k Prabhat ranjan , Neelima ranjan, Sathish K Bansal, D R Arora. Prevalence of pseudomonas aeruginosa in post operative wound infection in a referral hospital harayana, India Journal of Laboratory

97. Rezwana Haque M.A Salam .Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh Pak J Med Sci 2010 vol26 no.4 887-891.

98. RN Misra Yogesh Chander, NK Debata, VC Ohri. Antibiotic resistance pattern of isolates from wound and soft tissue infections, MJAFI,56,3,2000.

99.Revathi G,Puri J, Jain BK. Bacteriology of burns, Burns.vol 24 pp347-349

100. Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant Staphylococcus aureus isolates from high risk patients. Indian J Med Microbiol 2001;19:87-90.

101. NP Singh , R Goyal, V Manchanda, S Das, I Kaur,V Talwar. Changing trends in bacteriology of burns in burn unit, Journal of International Society for Burn injuries,2003, 29, 2, 129-132

102. Manjula Mehta , Priya Dutta, Varsha Gupta. Bacterial isolates from burn wound infections and their antibiograms-a eight year study: 2007 | 40 | : 1 | : 25-28

103.Ekrami A Kalanter E. . Bacterial infections in burn patients at a burn hospital in iran. Indian Journal of Medical Research 2007 126 541-544.

104. Shankar srinivasan , Arvind M Vartak, Akanksha Patil, Jovidha Saldhana. Bacteriology of burn wound at the bai jerbia wadia hospital for

children Mumbai-13 year study, Indian Journal of Plastic surgery,2009, 42, 2, 213-218.

105. Herjinder kaur Jyothi Bhat, Anup R Anvikar, Savinder Rao, Vijay Gadge. Bacterial profile of blood and burn wound infections in burns patients, Proceeding of national symposium on tribal health.

106. Agnihotri ,V Gupta, R M Joshi. Aerobic isolates from burn wound infection and their antibiograms-5 year study

107. . Brook I Frazier eh. Aerobic and anaerobic bacteriology of wounds and cutaneous abscess. Arch Surg.1990;125;

108. Brook I Finegold sm Aerobic and anaerobic bacteriology of cutaneous abscess in children. Peadatrics.1981;67(6) 891-895.

109. Fantahun Biadglegne ,Bayeh Abera, Atenaf Alam, Belay Anagaw. Bacterial isolates from wound infections, Ethiopia Journal of Health sciences,2009,19,3,173-177.

110. Sarita yadav , Aparna Yadav, Madhu Sharma , Uma Chaudhary. Prevalence and sensitivity pattern of Staphylococcus aureus in surgical wound infection, International Journal of Pharma and bio sciences,1, 3, 2010

111. Shilpa Arora,Pushpa Devi, Usha Arora, Bimla Devi. Prevalence of methicillin-resistant Staphylococcus Aureus (MRSA) in a Tertiary Care Hospital in Northern India 2010 | : 2 | : 2 | : 78-81

112. Sanjay dhar, K Singh , Saraf R, Raina B. Microbiological profile of chronic burn wounds in burn unit, Journal of medical education and research, JK science,2007, 9 , 4, 182-185.

113. Shampa anupurba ,Amitabh Bhattarcharjee, atul Garg, Malay Ranjan Sen. Antimicrobial susceptibility of pseudomonas aeruginosa in wound infection, Indian Journal of Dermatology, 2006, 51, 4, 286-288.

114. Sachdev D, Amladi S, Nataraj G, Baveja S, Kharkar V, Maharajan S. An Outbreak of Methicillin-resistant Staphylococus aureus (MRSA) infection in dermatology indoor patients. Indian J Dermatol Venereol Leprol 2003;69:377- 80.

115. McDonald M. The epidemiology of methicillin resistant Staphylococcus aureus :Surgical relevance 20 years on . Aust N Z J Surg 1997;67:682-5.

116. Qureshi AH, Rafi S, Qureshi SM, Ali AM. The current susceptibility patterns of methicillin resistant Staphylococcus aureus to conventional anti Staphylococcus antimicrobials at Rawalpindi. Pak J Med Sci 2004;20:361-4

117. Pulimood TB, Lalitha MK, Jesudson MV, Pandian R, Selwyn JJ. The Spectrum of antimicrobial resistance among methicillin resistant Staphylococcus aureus (MRSA) in a tertiary care in India. Indian J Med Res 1996;103:212-5.

118. Majumder D, Bordoloi JN, Phukan AC, Mahanta J.Antimicrobial susceptibility pattern among MRSA in Assam , IJMM 2001 19 138-140

119. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of MRSA in a Tertiary Care Referral Hospital in Eastern Uttar Pradesh, IJMM 2003 21(49-51).

120. K Rajaduaripandi , KR Mani, K Panneerselvam, M Mani, M Bhaskar, P Manikandan. Prevalence and antimicrobial susceptibility pattern of MRSA-a multi centre study, IJMM, 2006, 24, 1, 34-38.

121. Hare Krishna tiwari ,Dharsan Sapkota, Malay Ranjan Sen. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India Infection and Drug Resistance 2008:1

122. Livermore DM. ß-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557–584

123. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, FishmanNO, Bilker WB, et al. Risk factors for increasing multidrugresistance among extended spectrum betalactamase producing Escherichia coli and Klebsiella species. Clin Infect Dis

2005;40:1317-1324.

124. Mathur p , Tatman A, Das B, Dhawan B.Prevalence of ESBL producing bacteria in a tertiary care hospital, Indian journal of medical research 2002 115 153-157

125.C Rodrigues P Joshi, SH Jani, M Alphonse, R Radhakrishnan, A Mehta. Detection of lactamases in nosocomial gram negative clinical isolates IJMM 2004 22 4 247-250

129. Shukla I,Tiwari R, Agarwal M. Prevalence of ESBL producing klebseilla pneumonia in tertiary care hospital, IJMM 2004, 22, 287-291.

130. C.Shanmuganathan , A Ananthakrishnan, SR Jayakeerthi, R Kanungo, A kumar, S Bhattarcharya, S Badrinath. Learning from outbreak: ESBL-the essential points, IJMM 2004,22,4,255-257

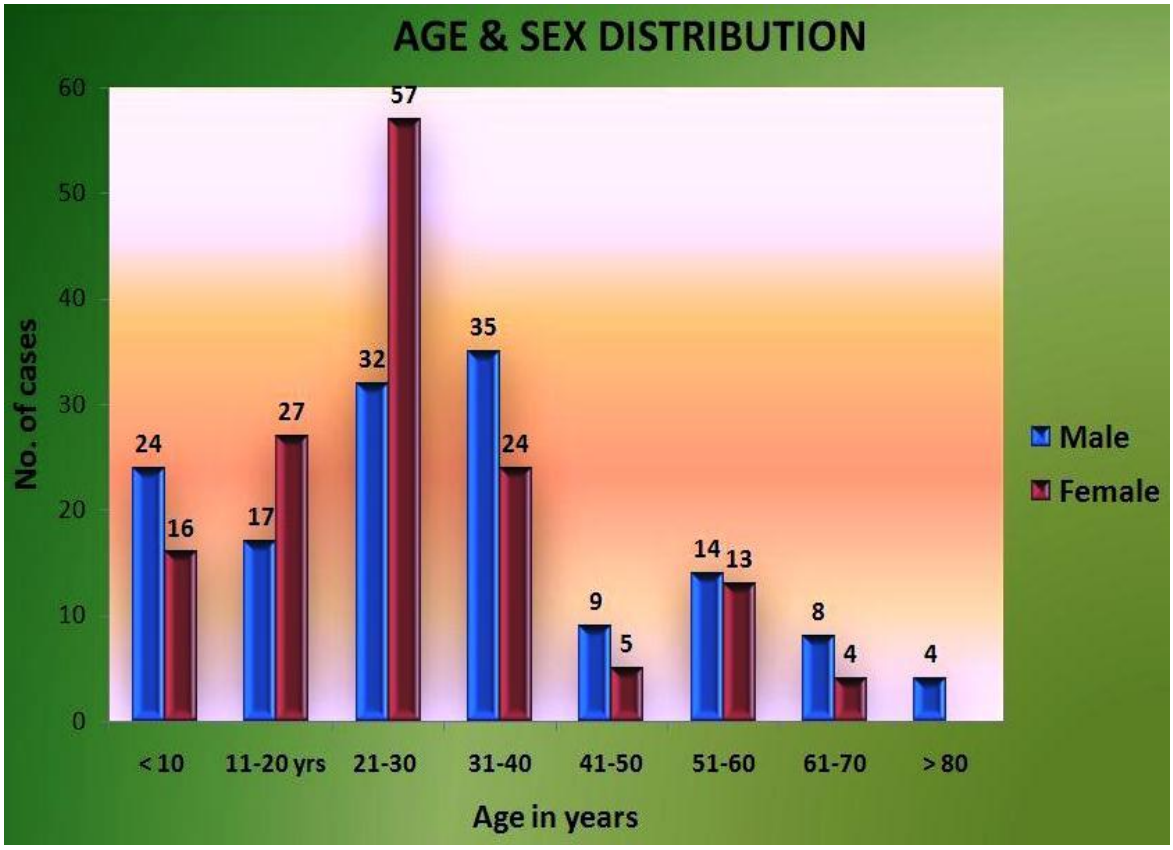
131 . MS Kumar V lakshmi, R Rajagopalan. Occuernce of ESBLs among Enterobacteriaceae isolated at teriary care institute, IJMM, 2006, 24, 3, 208-211.

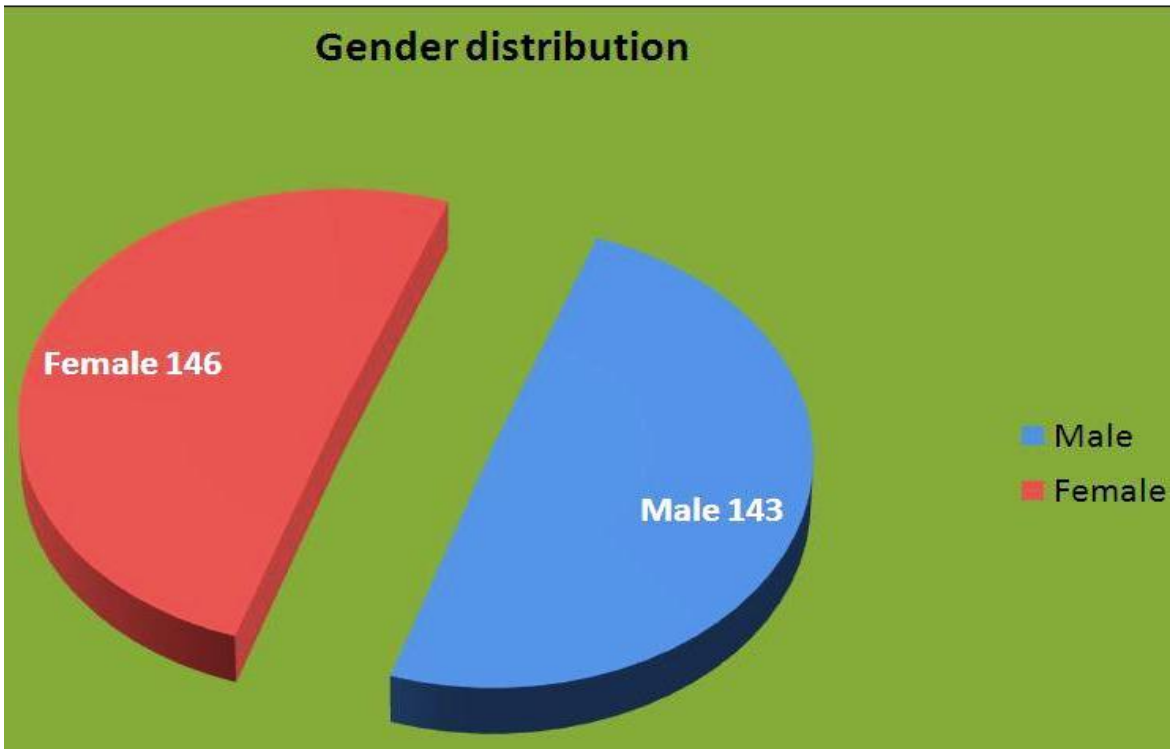
132. Dechen C Tsering , Shyamasree Das, Luna Adhiakari, Ranabil Pal, Takhellambam S K Singh. Extended spectrum beta-lactamase detection in gram-negative bacilli of nosocomial origin Journal of Global Infectious Disease. 2009 1 2 87-92

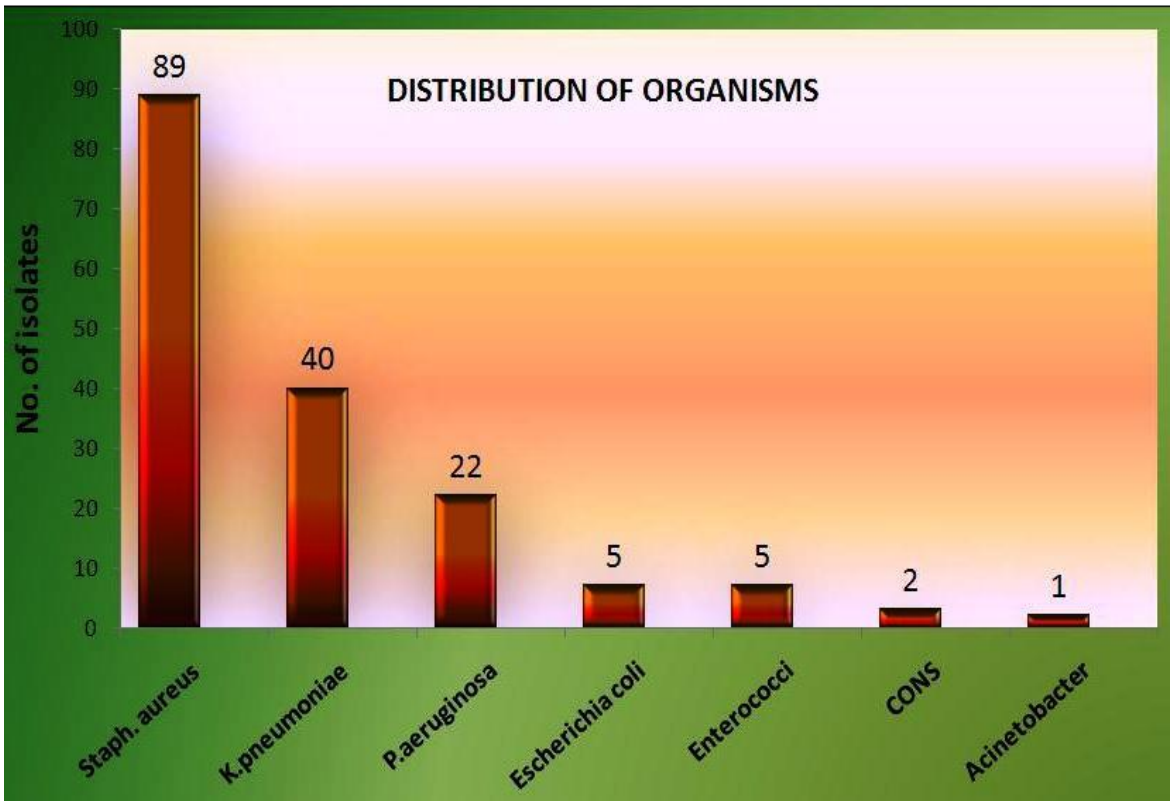
133.S Baby padmini , B Appala Raju, KR M ani. Detection of Enterobacteriaceae producing CTX-M ESBLs from a tertiary care hospital in South India, IJMM 2008 26,2,163-166

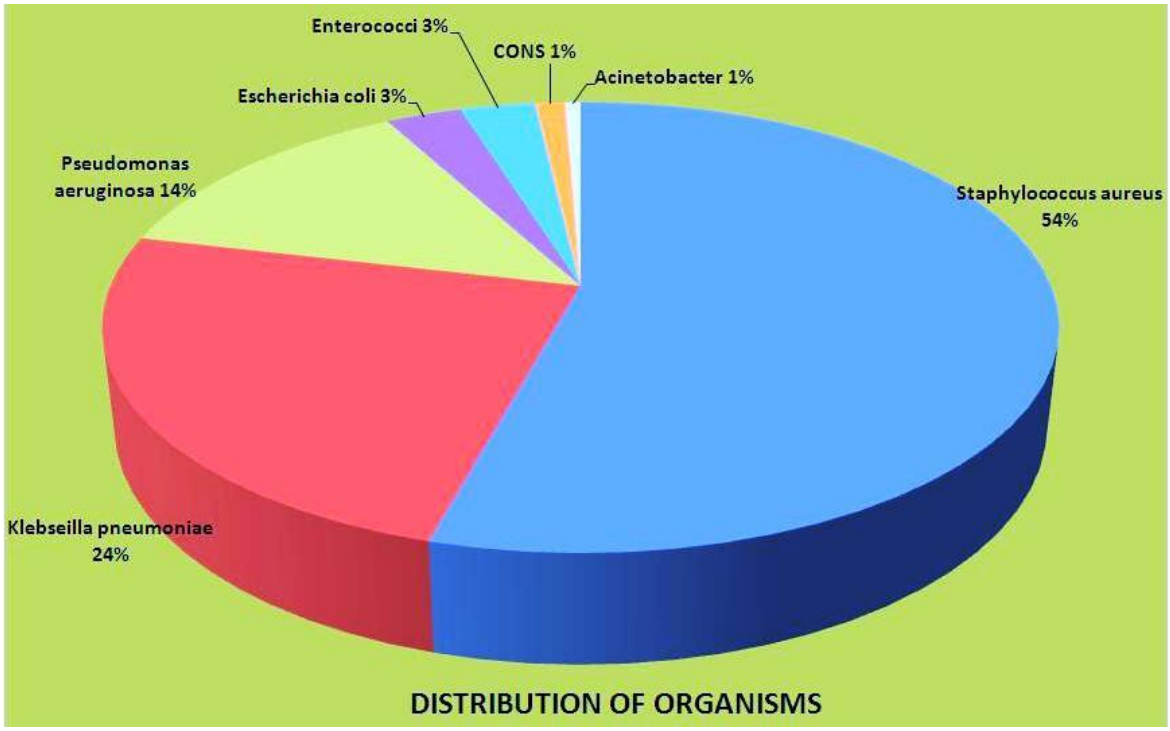
134. Akinjogunla O J,Adegoke A, Mboto, Chukwudebelu, Udokang. Bacteriology of automobile accident wound infection, International Journal of Medicine and Medical Sciences 2009,vol1(2) 23-27

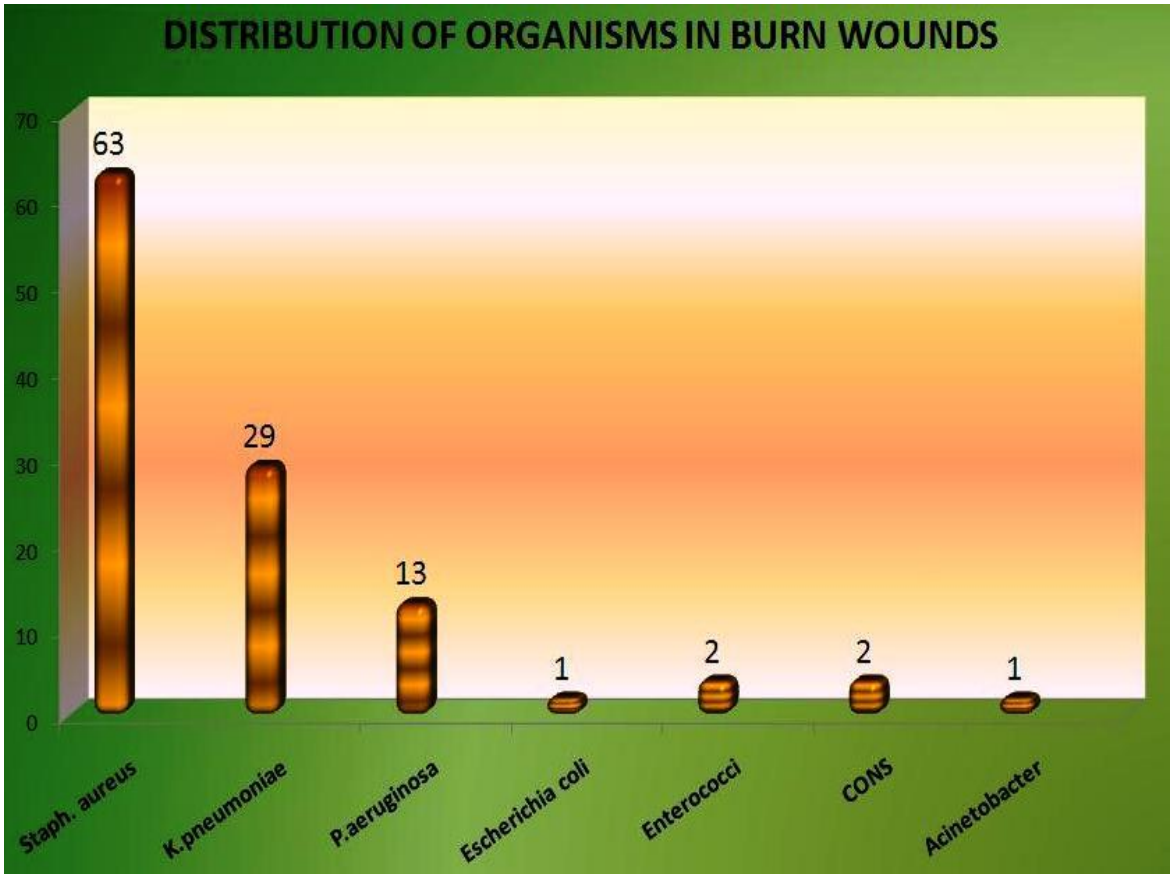
135. Srinivasan S,Sheela D, Shasikala,Mathew R,Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with MRSA, IJMM, 24, 3, 2006, 182-185.

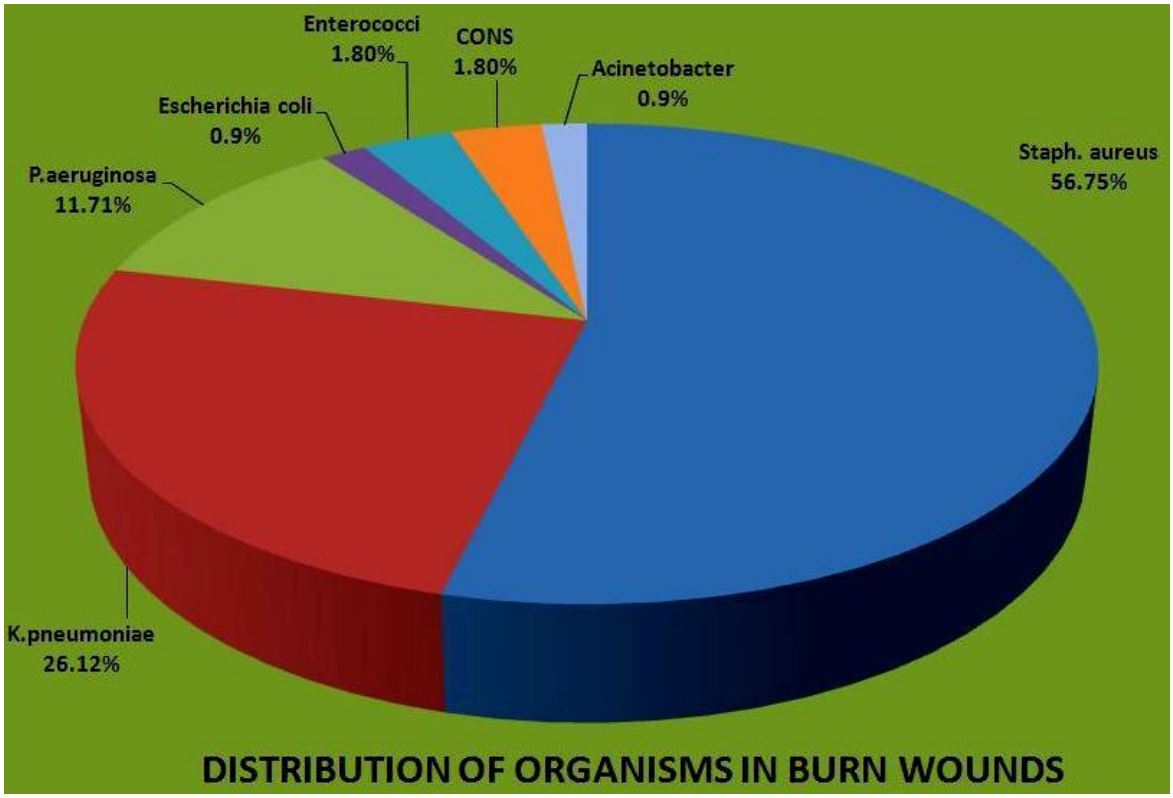

AGE & SEX DISTRIBUTION
No. of cases
60
50
40
30
20
10
0
24
16
17
27
32
57
35
24
9
5
14
13
8
4
4
< 10
11-20 yrs
21-30
31-40
41-50
51-60
61-70
> 80
Age in years
Male
Female

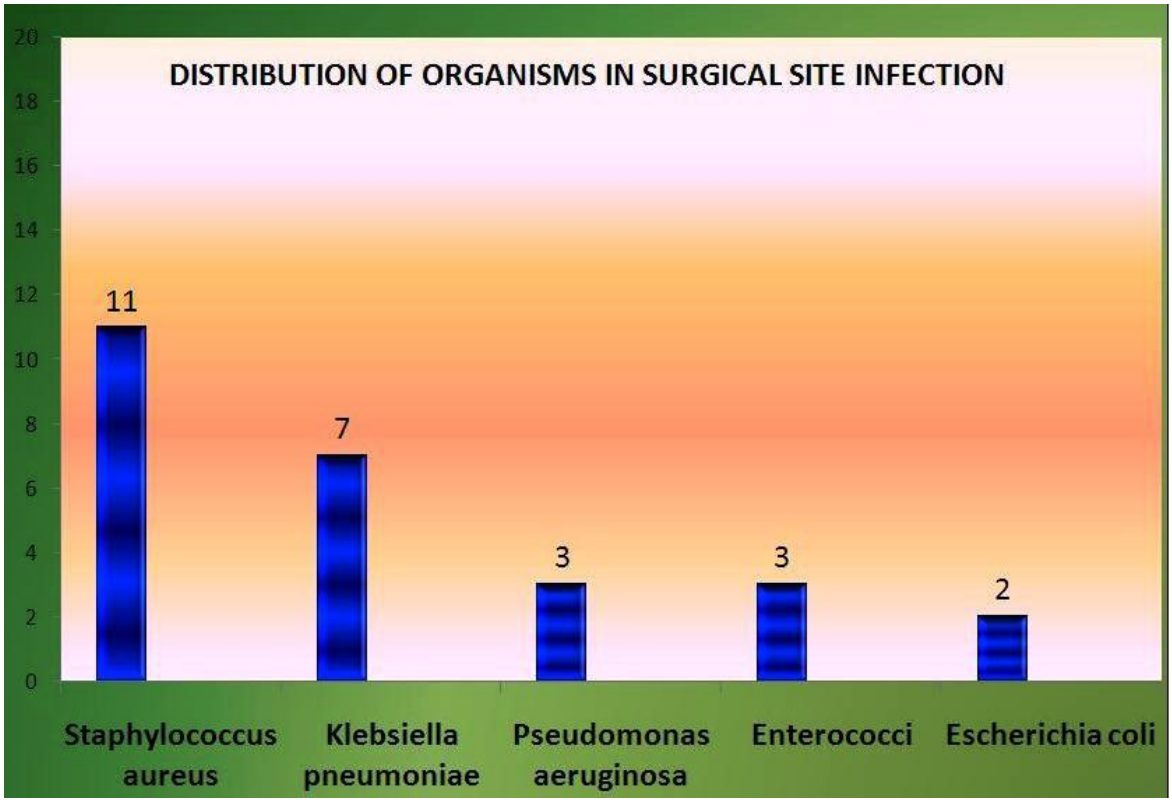

Gender distribution
Female 146
Male 143
Male
Female

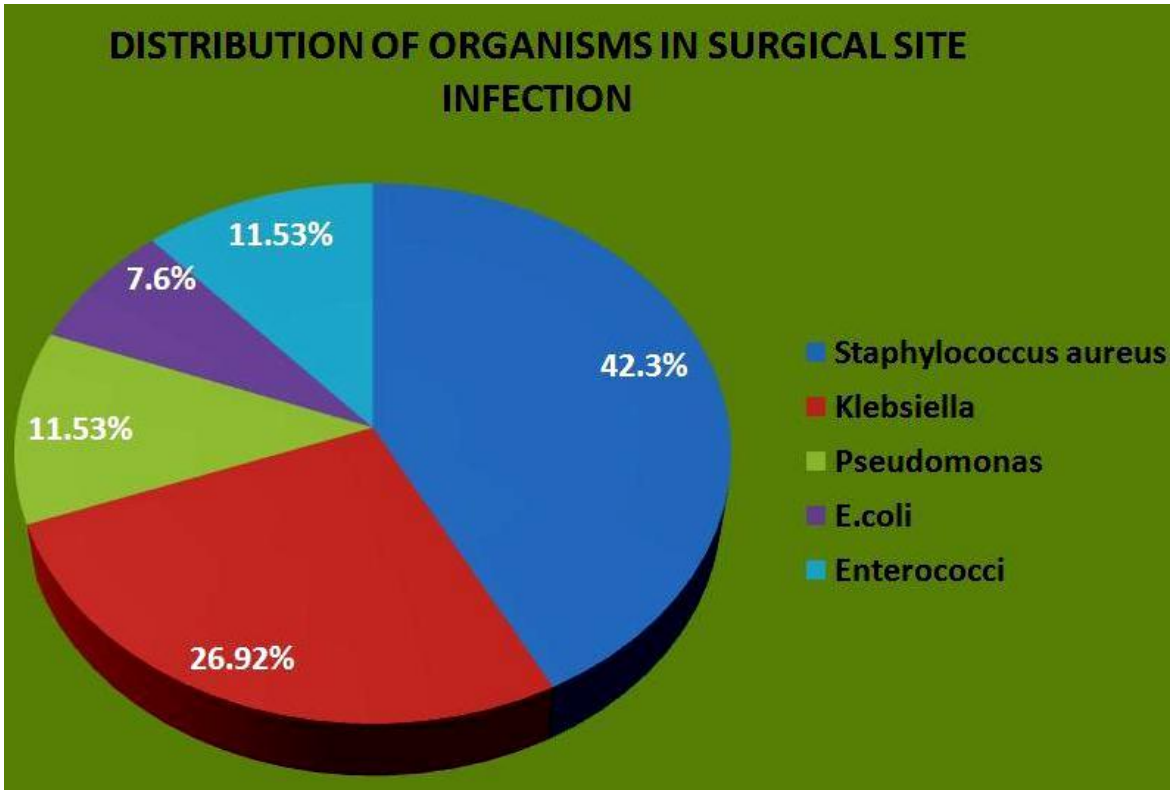

DISTRIBUTION OF ORGANISMS
No. of isolates
100
90
80
70
60
50
40
30
20
10
0
89
40
22
5
5
2
1
Staph. aureus
K.pneumoniae
P.aeruginosa
Escherichia coli
Enterococci
CONS
Acinetobacter

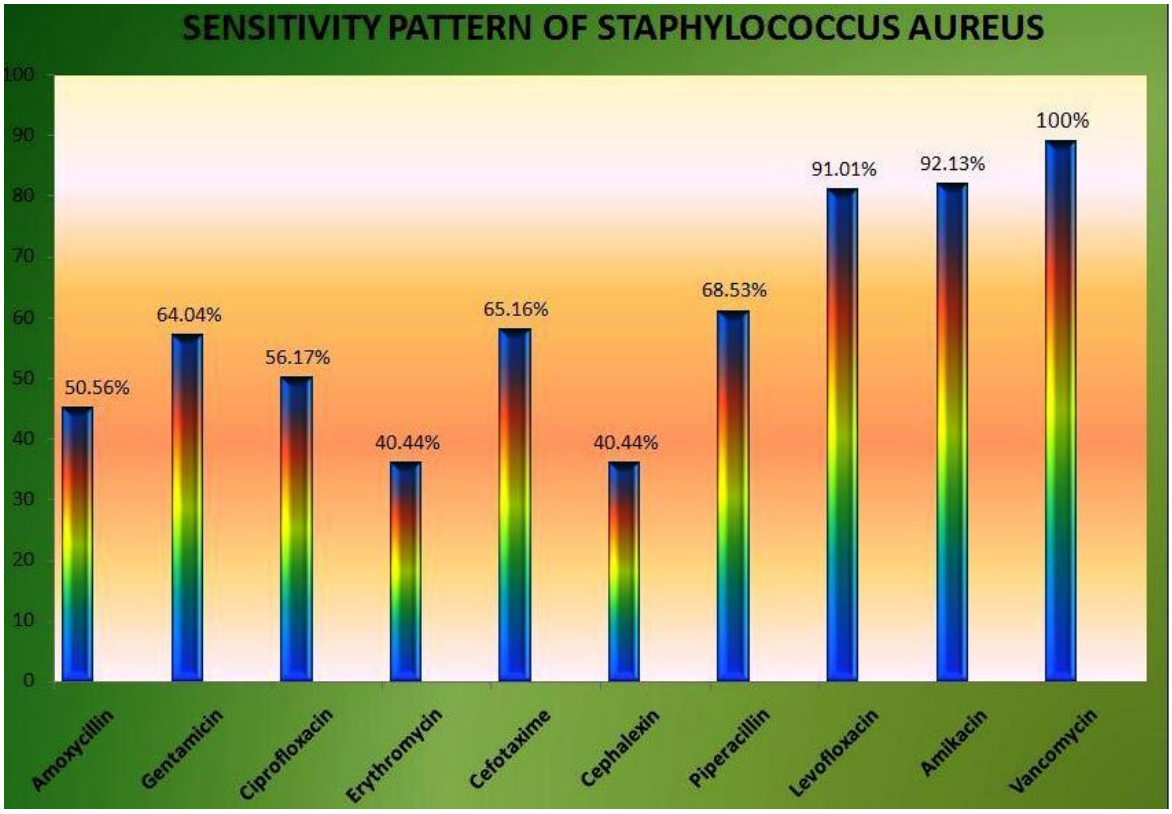

Enterococci 3%
CONS 1%
Acinetobacter 1%
Escherichia coli 3%
Pseudomonas aeruginosa 14%
Staphylococcus aureus 54%
Klebseilla pneumoniae 24%
DISTRIBUTION OF ORGANISMS

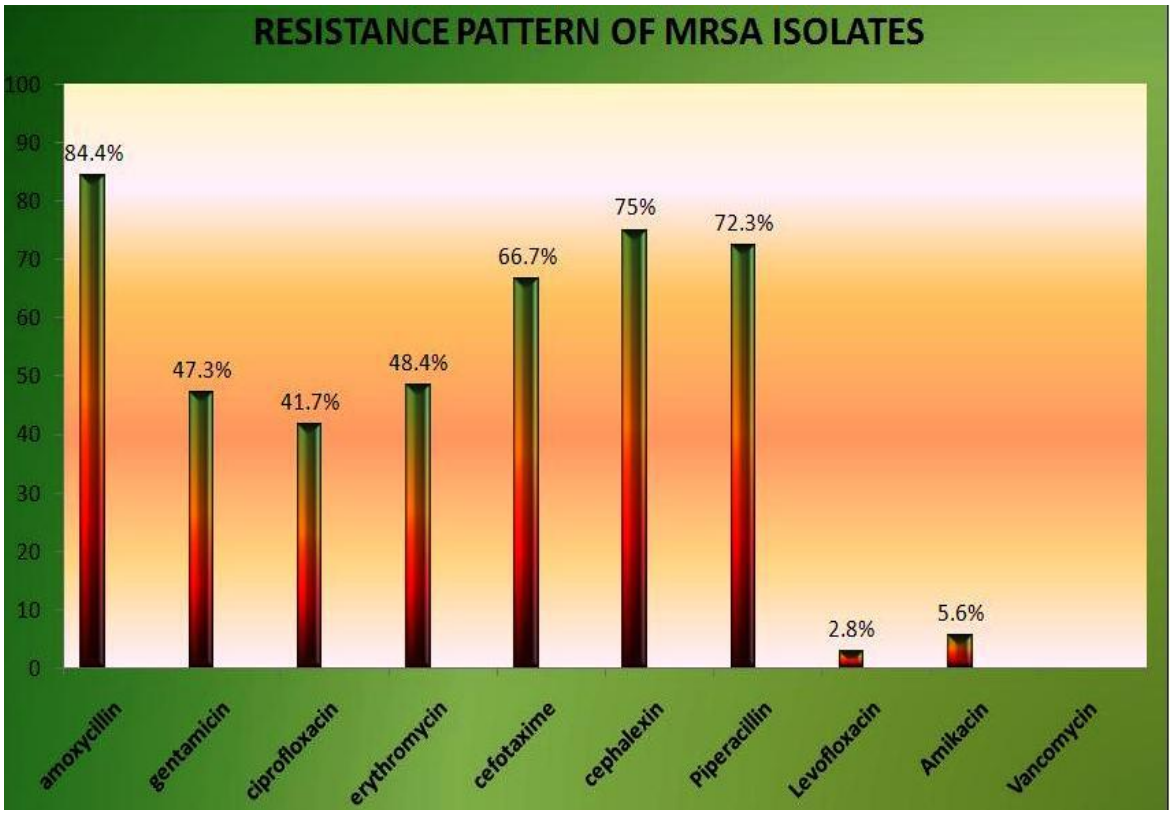

DISTRIBUTION OF ORGANISMS IN BURN WOUNDS
70
60
50
40
30
20
10
0
63
29
13
1
2
2
1
Staph. aureus
K.pneumoniae
P.aeruginosa
Escherichia coli
Enterococci
CONS
Acinetobacter

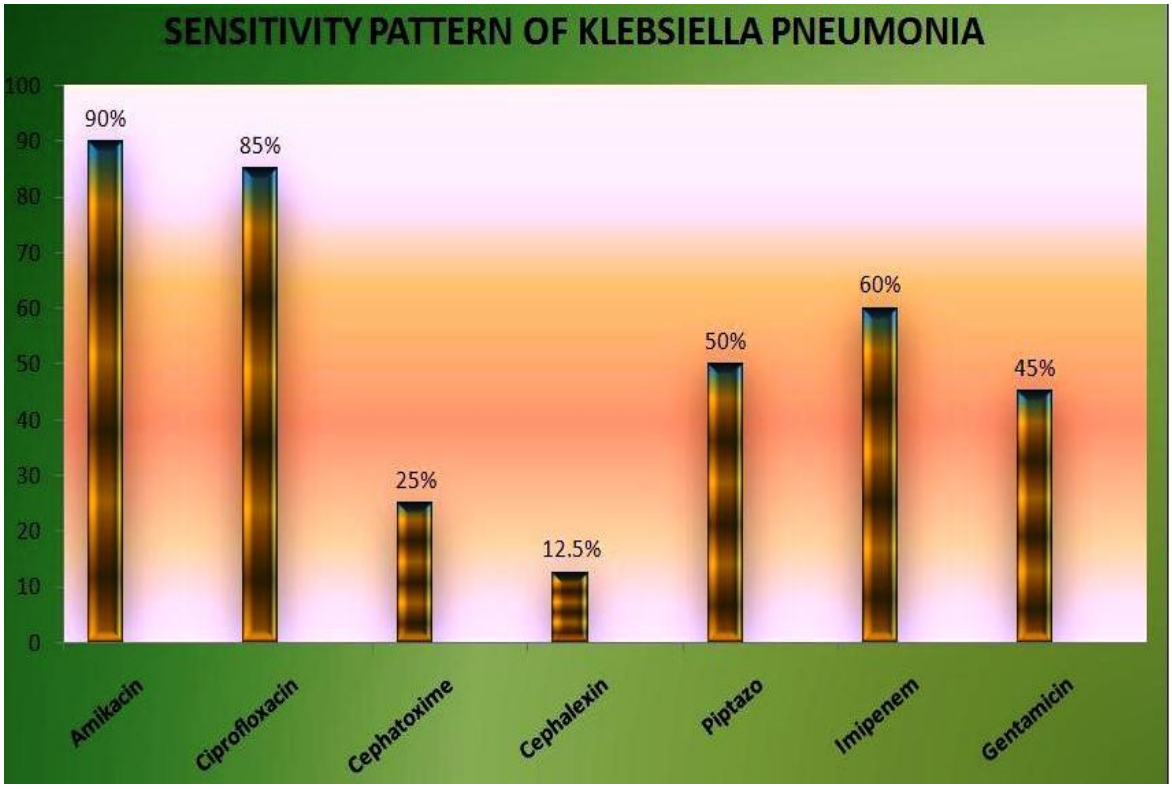

Enterococci
1.80%
CONS
1.80%
Acinetobacter
0.9%
Escherichia coli
0.9%
P.aeruginosa
11.71%
Staph. aureus
56.75%
K.pneumoniae
26.12%
DISTRIBUTION OF ORGANISMS IN BURN WOUNDS

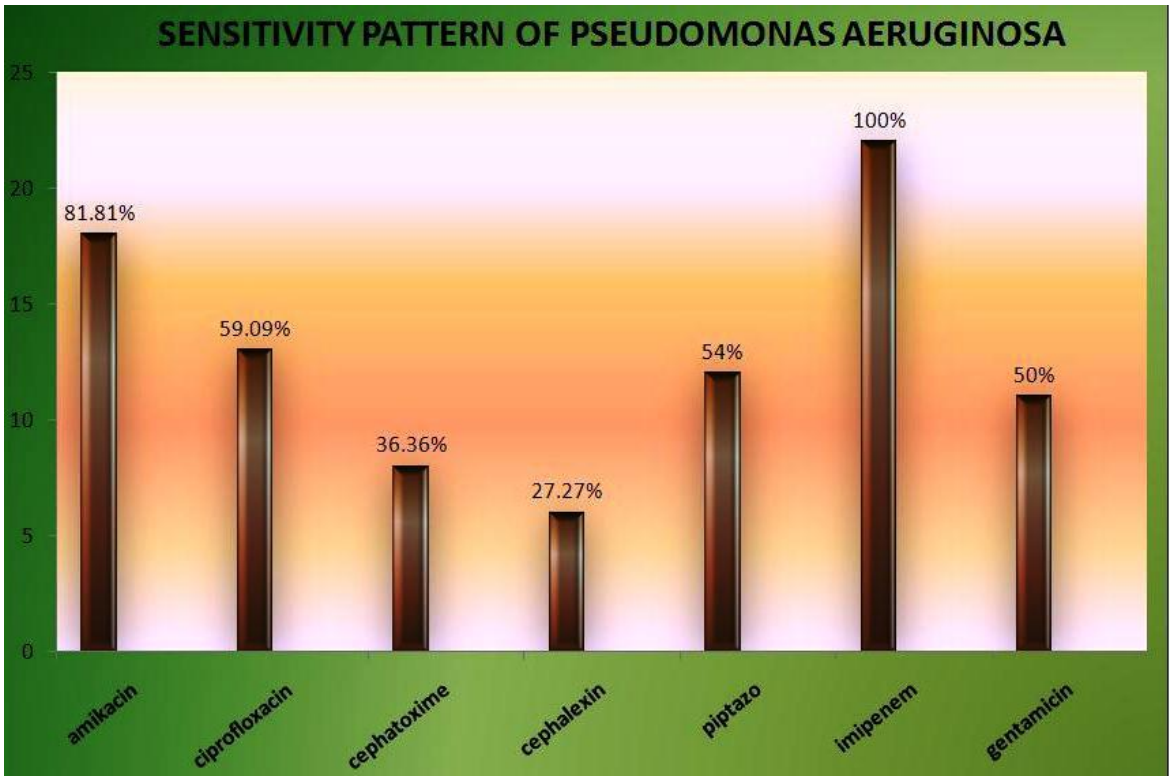

DISTRIBUTION OF ORGANISMS IN SURGICAL SITE INFECTION
20
18
16
14
12
10
8
6
4
2
0
11
7
3
3
2
Staphylococcus aureus
Klebsiella pneumoniae
Pseudomonas aeruginosa
Enterococci
Escherichia coli

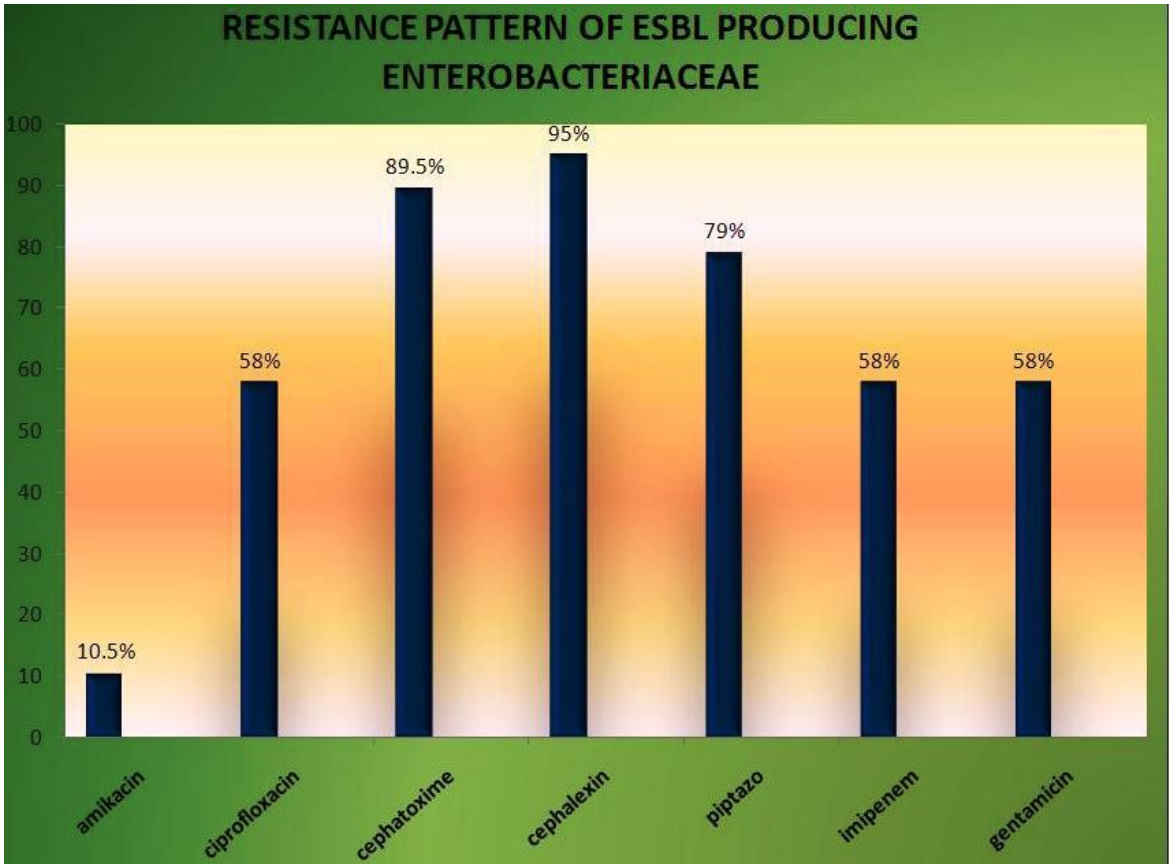

DISTRIBUTION OF ORGANISMS IN SURGICAL SITE INFECTION
11.53%
7.6%
42.3%
11.53%
26.92%
Staphylococcus aureus
Klebsiella
Pseudomonas
E.coli
Enterococci


SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS
100
90
80
70
60
50
40
30
20
10
0
50.56%
64.04%
56.17%
40.44%
65.16%
40.44%
68.53%
91.01%
92.13%
100%
Amoxycillin
Gentamicin
Ciprofloxacin
Erythromycin
Cefotaxime
Cephalexin
Piperacillin
Levofloxacin
Amikacin
Vancomycin


RESISTANCE PATTERN OF MRSA ISOLATES
100
90
80
70
60
50
40
30
20
10
0
84.4%
47.3%
41.7%
48.4%
66.7%
75%
72.3%
2.8%
5.6%
amoxycillin
gentamicin
ciprofloxacin
erythromycin
cefotaxime
cephalexin
Piperacillin
Levofloxacin
Amikacin
Vancomycin


SENSITIVITY PATTERN OF KLEBSIELLA PNEUMONIA
100
90
80
70
60
50
40
30
20
10
0
90%
85%
25%
12.5%
50%
60%
45%
Amikacin
Ciprofloxacin
Cephatoxime
Cephalexin
Piptazo
Imipenem
Gentamicin


SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA
25
20
15
10
5
0
81.81%
59.09%
36.36%
27.27%
54%
100%
50%
amikacin
ciprofloxacin
cephatoxime
cephalexin
piptazo
imipenem
gentamicin


RESISTANCE PATTERN OF ESBL PRODUCING ENTEROBACTERIACEAE
100
90
80
70
60
50
40
30
20
10
0
10.5%
58%
89.5%
95%
79%
58%
58%
amikacin
ciprofloxacin
cephatoxime
cephalexin
piptazo
imipenem
gentamicin

MASTER CHART

S.NO	AGE	SEX	OP/ IP	SPECIMEN	ORGANISM	ESBL	MRSA	Am	G	Cf	E	Ce	Cp	I	Le	Ak	pipt	ox	van
1.	22	F	Ip	Ws	Stap.au			S	S	S						S		S	S
2.	38	F	Ip	Ws	NG														
3.	48	M	Ip	Ws	Stap.au				S							S		S	S
4.	8	M	Ip	Ws	Staph.au				S							S		S	S
5.	17	M	Ip	Ws	Stap.au			S	S	S	S	S	S		S	S		S	S
6.	30	F	Ip	Ws	Kleb.pn	E				S				S		S			
7.	8	M	Ip	Ws	Stap.au									S	S			S	S
8.	14	F	Ip	Ws	NG														
9.	24	M	Ip	Ws	Stap.au		+		S			S	S		S	S		R	S
10.	15	M	Ip	Ws	NG														
11.	32	M	Ip	Ws	Stap.au		+	S	S	S	S	S	S	S	S	S		R	S
12.	32	M	Ip	Ws	Stap.au				S	S					S	S		S	S
13.	22	M	Ip	Ws	Kleb.pn					S						S	S		
14.	7	M	Ip	Ws	Stap.au										S	S		S	S
15.	26	M	Ip	Ws	Stap.au					S					S	S		S	S
16.	14	F	Ip	Ws	Stap.au		+			S					S	S		R	S
17.	23	F	Ip	Ws	Stap.au			S							S	S		S	S
18.	28	F	Ip	Ws	Kleb.pn	E										S	S		
19.	40	M	Ip	Ws	Stap.au										S	S		S	S
20.	20	F	Ip	Ws	Stap.au				S						S	S		S	S
21.	8	F	Ip	Ws	Stap.au				S						S	S		S	S
22.	18	M	Ip	Ws	Stap.au				S	S		S			S	S		S	S
23.	19	F	Ip	Ws	Stap.au		+		S	S	S	S	S	S	S	S	S	R	S
24.	48	M	Ip	Ws	Stap.au				S					S	S	S		S	S
25.	8	F	Ip	Ws	NG														
26.	3	M	Ip	Ws	NG														
27.	60	M	Ip	Ws	NG														
28.	23	F	Ip	Ws	NG														
29.	17	M	Ip	Ws	NG														
30.	21	F	Ip	Ws	NG														
31.	1	M	Ip	Ws	NG														
32.	70	M	Ip	Ws	Stap.au		+			S	S		S		S	S		R	S
33.	38	F	Ip	Ws	Pseudo									S					
34.	24	M	Ip	Ws	NG														

35.	57	F	Ip	Ws	E.coli				S	S		s							
36.	22	M	Ip	Ws	Pseudo				S	S	S	S		S	S	S	S		
37.	25	F	Ip	Ws	Ps.ar									S					
38.	60	M	Ip	Ws	NG														
39.	34	F	Ip	Ws	Stap.au						S	S				S		S	S
40.	19	M	Ip	Ws	Kleb.pn	E								S					
41.	30	M	Ip	Ws	Stap.au		+	S	S	S	S	S	S	S	S	S	S	R	S
42.	61	M	Ip	Ws	NG														
43.	60	M	Ip	Ws	NG														
44.	7	M	Ip	Ws	NG														
45.	30	F	Ip	Ws	NG														
46.	13	M	Ip	Ws	Kleb	E			S	S		S	S	S		S	S		
47.	26	F	Ip	Ws	NG														
48.	2.5	F	Ip	Ws	NG														
49.	31	M	Ip	Ws	NG														
50.	41	F	Ip	Ws	NG														
51.	26	F	Ip	Ws	E.coli				S	S						S			
52.	60	F	Ip	Ws	Stap.au		+			S					S			R	S
53.	7	M	Ip	Ws	NG														
54.	33	F	Ip	Ws	Stap.au				S			S			S			S	S
55.	33	M	Ip	Ws	Stap.au		+		S	S	S	S		S	S	S	S	R	S
56.	7	M	Ip	Ws	Stap.au				S	S	S	S	S	S	S	S	S	S	S
	21	F	Ip	Ws	NG														
57.	4	F	Ip	Ws	NG														
58.	13	M	Ip	Ws	NG														
59.	24	F	Ip	Ws	NG														
60.	20	F	Ip	Ws	Stap.au		+		S	S	S	S	S	S	S	S	S	R	S
61.	29	F	Ip	Ws	E.coli				S					S		S	S		
62.	56	F	Ip	Ws	Stap.au				S	S	S	S	S	S	S	S	S	S	S
63.	65	M	Ip	Ws	NG														
64.	23	F	Ip	Ws	NG			Am	G	Cf	E	Ce	Cp	I	Le	Ak	pipt		
65.	23	M	Ip	Ws	Pseudo				S		S	S	S	S	S	S	S		
66.	64	M	Ip	Ws	Kleb	E			S	S						S			
67.	36	M	Ip	Ws	NG														
68.	35	M	Ip	Ws	Stap.au		+	S	S	S		S	S	S	S	S	S	R	S
69.	21	F	Ip	Ws	NG														
70.	20	F	Ip	Ws	NG														

71.	48	M	Ip	Ws	Stap.au			S	S	S	S		S	S	S	S	S	S	S
72.	70	F	Ip	Ws	NG														
73.	22	M	Ip	Ws	Stap.au		+								S	S		R	S
74.	8	F	Ip	Ws	NG														
75.	20	M	Ip	Ws	NG														
76.	24	F	Ip	Ws	NG														
77.	34	M	Ip	Ws	NG														
78.	25	M	Ip	Ws	NG														
79.	16	M	Ip	Ws	Stap.au			S	S	S	S	S	S	S	S	S	S	S	S
80.	10	M	Ip	Ws	NG														
81.	35	M	Ip	Ws	NG														
82.	27	F	Ip	Ws	PS.ar					S	S	S	S	S	S	S	S		
83.	25	M	Ip	Ws	NG														
84.	55	M	Ip	Ws	Kleb.pn	E			S							S			
85.	24	F	Ip	Ws	Stap.au		+		S	S		S			S			R	S
86.	28	M	Ip	Ws	Stap.au				S	S	S	S	S	S	S	S	S	S	S
87.	78	M	Ip	Ws	Kleb.pn	E			S	S		S				S			
88.	60	M	Ip	Ws	entero					S					S				
89.	36	M	Ip	Ws	Stap.au				S						S	S		S	S
90.	28	M	Ip	Ws	Stap.au		+		S		S				S	S		R	S
91.	56	M	Ip	Ws	Kleb.pn				S	S					S	S			
92.	48	M	Ip	Ws	Kleb.pn					S				S		S	S		
93.	24	M	Ip	Ws	Stap.au				S	S	S	S		S	S	S	S	S	S
94.	22	M	Ip	Ws	Ps.ar				S					S		S			
95.	36	M	Ip	Ws	Stap.au				S	S		S		S	S	S	S	S	S
96.	3	M	Ip	Ws	Stap.au				S	S		S			S	S		S	S
97.	40	M	Ip	Ws	Stap.au		+			S		S		S	S	S	S	R	S
98.	36	M	Ip	Ws	NG														
99.	36	F	Ip	Ws	Stap.au		+			S	S	S		S	S	S	S	R	S
100.	32	F	Ip	Ws	NG														
101.	8	F	Ip	Ws	Stap.au		+			S					S	S		R	S
102.	21	F	Ip	Ws	Ps. ar									S		S			
103.	30	M	Ip	Ws	Kleb.pn									S		S	S		
104.	33	F	Ip	Ws	Kleb.pn	E				S				S		S	S		
105.	20	F	Ip	Ws	Stap.au		+								S	S		R	S
106.	35	M	Ip	Ws	Stap.au										S			S	S
107.	8	F	Ip	Ws	Kleb.pn	E								S			S		
108.	40	F	Ip	Ws	Kleb.pn				S	S		S				S			

109.	55	F	Ip	Ws	NG														
110.	28	F	Ip	Ws	NG														
111.	40	F	Ip	Ws	Stap.au		+		S						S	S		R	S
112.	27	F	Ip	Ws	Kleb.pn				S							S			
113.	35	F	Ip	Ws	NG														
114.	36	M	Ip	Ws	Stap.au		+			S				S	S	S		R	S
115.	30	F	Ip	Ws	NG														
116.	20	F	Ip	Ws	Stap.au		+								S	S		R	S
117.	7	F	Ip	Ws	Stap.au						S				S	S		S	S
118.	33	M	Ip	Ws	Stap.au									S			S	S	S
119.	10	M	Ip	Ws	Stap.au									S		S	S	S	S
120.	55	F	Ip	Ws	NG														
121.	33	F	Ip	Ws	Ps.ar									S		S			
122.	16	F	Ip	Ws	NG														
123.	26	F	Ip	Ws	NG														
124.	7	M	Ip	Ws	NG														
125.	18	F	Ip	Ws	Ps.ar					S				S		S			
126.	32	F	Ip	Ws	Kleb.pn	E										S			
127.	28	F	Ip	Ws	Stap.au										S	S		S	S
128.	4	F	Ip	Ws	Stap.au		+				S				S	S		R	S
129.	22	F	Ip	Ws	Kleb.pn											S	S		
130.	2	F	Ip	Ws	NG														
131.	22	F	Ip	Ws	NG														
132.	67	F	Ip	Ws	Stap.au		+		S	S	S				S	S		R	S
133.	56	F	Ip	Ws	Kleb.pn	E			S	S						S			
134.	57	F	Ip	Ws	Kleb.pn	E			S							S			
135.	45	M	Ip	Ws	E.coli	E										S			
136.	25	F	Ip	Ws	NG														
137.	42	M	Ip	Ws	Stap.au		+			S	S				S	S		R	S
138.	70	M	Ip	Ws	entero			S											
139.	24	F	Ip	Ws	Stap.au		+	S	S						S	S		R	S
140.	15	F	Ip	Ws	NG														
141.	52	M	Ip	Ws	Stap.au				S	S	S	S	S	S	S	S	S	S	S
142.	24	F	Ip	Ws	Stap.au		+		S	S	S	S	S	S	S	S	S	R	S
143.	37	M	Ip	Ws	NG														
144.	32	M	Ip	Ws	Stap.au		+		S						S	S		R	S
145.	25	M	Ip	Ws	NG														
146.	35	F	Ip	Ws	NG														

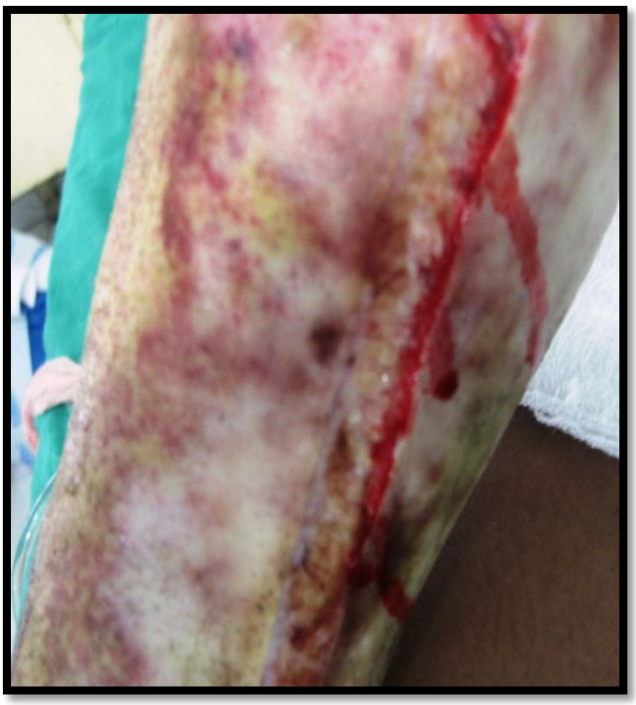
147.	51	F	Ip	Ws	Acineto									S			S		
148.	37	M	Ip	Ws	NG														
149.	11	M	Ip	Ws	CONS				S		S	S	S	S	S	S	S		
150.	24	M	Ip	Ws	Stap.au		+								S	S		R	S
151.	42	M	Ip	Ws	NG														
152.	24	F	Ip	Ws	NG														
153.	25	F	Ip	Ws	NG														
154.	11	F	Ip	Ws	NG														
155.	14	M	Ip	Ws	Stap.au				S				S	S	S	S		S	S
156.	2	M	Ip	Ws	NG														
157.	29	F	Ip	Ws	NG														
158.	29	F	Ip	Ws	NG														
159.	32	F	Ip	Ws	Kleb.pn	E								S		S			
160.	28	F	Ip	Ws	Stap.au				S		S	S	S	S	S	S	S	S	S
161.	75	M	Ip	Ws	Kleb.pn				S	S						S			
162.	36	M	Ip	Ws	Stap.au				S	S				S	S	S		S	S
163.	55	F	Ip	Ws	Kleb.pn	E								S		S			
164.	20	F	Ip	Ws	NG														
165.	25	M	Ip	Ws	Kleb.pn	E								S		S			
166.	18	F	Ip	Ws	Kleb.pn					S						S			
167.	38	F	Ip	Ws	Stap.au			S	S	S	S	S	S	S	S	S	S	S	S
168.	61	M	Ip	Ws	Entero			S		S	S								
169.	37	M	Ip	Ws	Stap.au										S	S		S	S
170.	40	M	Ip	Ws	NG														
171.	17	M	Ip	Ws	Stap.au		+							S	S	S		R	S
172.	35	F	Ip	Ws	Stap.au										S	S		S	S
173.	23	F	Ip	Ws	Stap.au		+							S	S	S		R	S
174.	24	F	Ip	Ws	Stap.au									S	S			S	S
175.	9	M	Ip	Ws	NG														
176.	16	F	Ip	Ws	Kleb.pn				S	S						S	S		
177.	47	M	Ip	Ws	NG														
178.	62	M	Ip	Ws	NG														
179.	19	M	Ip	Ws	Stap.au				S	S					S	S		S	S
180.	35	M	Ip	Ws	Stap.au										S	S		S	S
181.	4	F	Ip	Ws	Kleb.pn				S	S	S	S		S	S	S	S		
182.	9	M	Ip	Ws	Kleb.pn					S						S	S		
183.	6	M	Ip	Ws	Kleb.pn				S	S						S	S		
184.	32	F	Ip	Ws	Stap.au										S	S		S	S

185.	20	F	Ip	Ws	Stap.au		+	S	S	S	S	S	S	S	S	S	S	R	S
186.	55	M	Ip	Ws	Stap.au			S	S	S	S	S	S	S	S	S	S	S	S
187.	55	M	Ip	Ws	Kleb.pn									S			S		
188.	24	M	Ip	Ws	Pseudo				S	S				S		S	S		
189.	40	F	Ip	Ws	Stap.au			S	S	S	S	S	S	S	S	S	S	S	S
190.	24	F	Ip	Ws	NG														
191.	23	F	Ip	Ws	Stap.au		+		S						S	S		R	S
192.	3	M	Ip	Ws	Stap.au				S	S	S	S		S	S	S	S	S	S
193.	14	M	Ip	Ws	Stap.au										S	S		S	S
194.	28	M	Ip	Ws	NG														
195.	27	F	Ip	Ws	Stap.au		+		S						S	S		R	S
196.	10	F	Ip	Ws	Stap.au				S						S	S		S	S
197.	51	M	Ip	Ws	Kleb.pn	E			S	S				S		S			
198.	30	M	Ip	Ws	CONS			S	S	S	S	S	S	S	S	S	S		
199.	34	F	Ip	Ws	NG														
200.	11	M	Ip	Ws	NG														
201.	55	M	Ip	Ws	NG														
202.	40	M	Ip	Ws	Kleb.pn	E				S				S		S			
203.	22	F	Ip	Ws	Kleb.pn									S					
204.	24	F	Ip	Ws	Kleb.pn									S					
205.	22	M	Ip	Ws	NG														
206.	28	M	Ip	Ws	NG														
207.	29	F	Ip	Ws	NG														
208.	24	F	Ip	Ws	NG														
209.	11	M	Ip	Ws	NG														
210.	24	F	Ip	Ws	NG														
211.	45	F	Ip	Ws	Kleb.pn					S				S					
212.	20	F	Ip	Ws	Stap.au		+							S	S	S		R	S
213.	35	M	Ip	Ws	Ps.ar									S					
214.	9	M	Ip	Ws	Stap.au				S							S		S	S
215.	2	M	Ip	Ws	Stap.au				S	S					S	S		S	S
216.	18	F	Ip	Ws	Ps.ar					S				S		S			
217.	9	M	Ip	Ws	Stap.au			S		S						S		S	S
218.	3	M	Ip	Ws	Stap.au			S						S				S	S
219.	57	F	Ip	Ws	E.coli	E			S							S			
220.	27	M	Ip	Ws	NG														
221.	25	F	Ip	Ws	NG														
222.	30	F	Ip	Ws	NG														

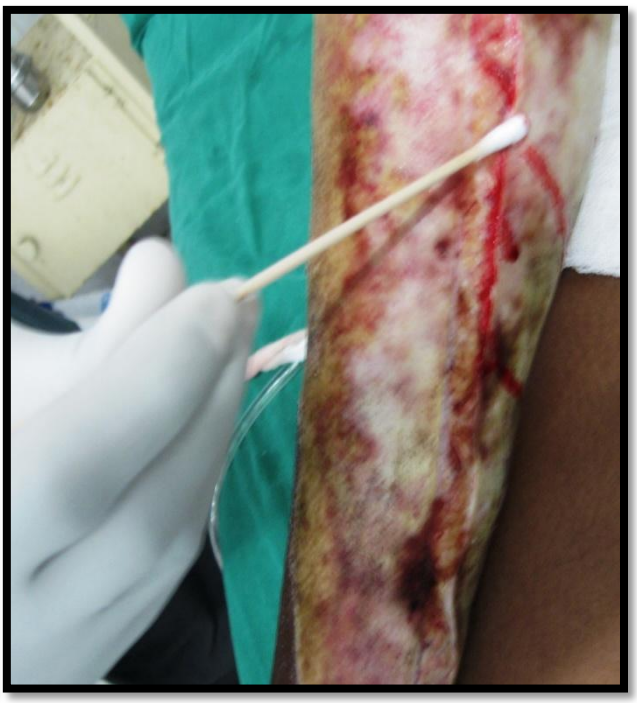
223.	60	M	Ip	Ws	NG														
224.	11	F	Ip	Ws	NG														
225.	22	F	Ip	Ws	NG														
226.	37	F	Ip	Ws	Ps.ar				S	S				S		S			
227.	28	F	Ip	Ws	NG														
228.	47	M	Ip	Ws	NG														
229.	36	F	Ip	Ws	NG														
230.	11	M	Ip	Ws	Stap.au				S	S	S	S	S	S	S	S	S	S	S
231.	58	M	Ip	Ws	Stap.au		+	S		S	S					S		R	S
232.	22	M	Ip	Ws	Ps.ar									S		S	S		
233.	34	F	Ip	Ws	Ps.ar				S					S		S	S		
234.	74	M	Ip	Ws	NG														
235.	36	M	Ip	Ws	Stap.au		+		S	S					S	S		R	S
236.	22	F	Ip	Ws	NG														
237.	21	M	Ip	Ws	Kleb.pn									S		S	S		
238.	2	M	Ip	Ws	NG														
239.	36	M	Ip	Ws	NG														
240.	17	M	Ip	Ws	NG														
241.	28	F	Ip	Ws	NG														
242.	23	F	Ip	Ws	NG														
243.	40	M	Ip	Ws	NG														
244.	5	F	Ip	Ws	NG														
245.	36	M	Ip	Ws	Kleb.pn				S	S				S		S			
246.	17	M	Ip	Ws	NG														
247.	46	F	Ip	Ws	NG														
248.	20	M	Ip	Ws	NG														
249.	20	F	Ip	Ws	Entero				S	S	S					S			
250.	11	F	Ip	Ws	Ps.ar									S			S		
251.	30	M	Ip	Ws	NG														
252.	37	M	Ip	Ws	Kleb.pn					S				S					
253.	60	F	Ip	Ws	Stap.au		+		S	S	S			S	S	S	S	R	S
254.	27	F	Ip	Ws	NG														
255.	25	M	Ip	Ws	Ps.ar					S				S		S			
256.	70	F	Ip	Ws	NG														
257.	40	F	Ip	Ws	NG														
258.	34	F	Ip	Ws	NG														
259.	26	F	Ip	Ws	NG														
260.	56	F	Ip	Ws	NG														

261.	23	M	Ip	Ws	NG														
262.	18	F	Ip	Ws	NG														
263.	60	M	Ip	Ws	Ps.ar				S					S		S			
264.	70	F	Ip	Ws	Entero				S	S	S	S	S	S	S	S	S		
265.	24	M	Ip	Ws	NG														
266.	36	M	Ip	Ws	Ps.ar				S	S				S		S			
267.	28	F	Ip	Ws	Kleb.pn					S				S					
268.	1	F	Ip	Ws	Stap.au										S	S		S	S
269.	30	M	Ip	Ws	NG														
270.	74	M	Ip	Ws	Kleb.pn					S				S		S			
271.	15	F	Ip	Ws	NG														
272.	22	F	Ip	Ws	NG														
273.	32	M	Ip	Ws	NG														
274.	26	F	Ip	Ws	NG														
275.	28	M	Ip	Ws	NG														
276.	20	F	Ip	Ws	Kleb.pn					S				S					
277.	33	M	Ip	Ws	Ps.ar				S	S				S	S	S			
278.	20	M	Ip	Ws	NG														
279.	8	F	Ip	Ws	Ps.ar					S				S		S			
280.	9	M	Ip	Ws	Ps.ar									S			S		
281.	23	F	Ip	Ws	Stap.au				S	S					S	S	S	S	S
282.	25	F	Ip	Ws	NG														
283.	35	M	Ip	Ws	NG														
284.	60	M	Ip	Ws	NG														
285.	18	F	Ip	Ws	NG														
286.	58	M	Ip	Ws	NG														
287.	26	M	Ip	Ws	NG														
288.	42	F	Ip	Ws	NG														
289.	36	M	Ip	Ws	NG														

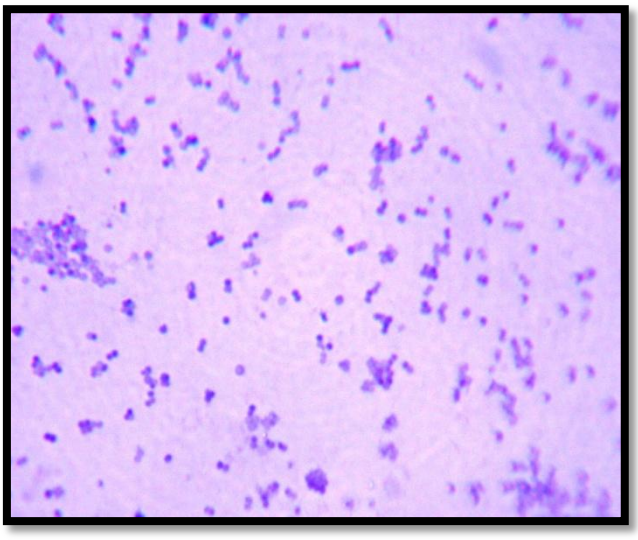
A CASE OF BURN WOUND



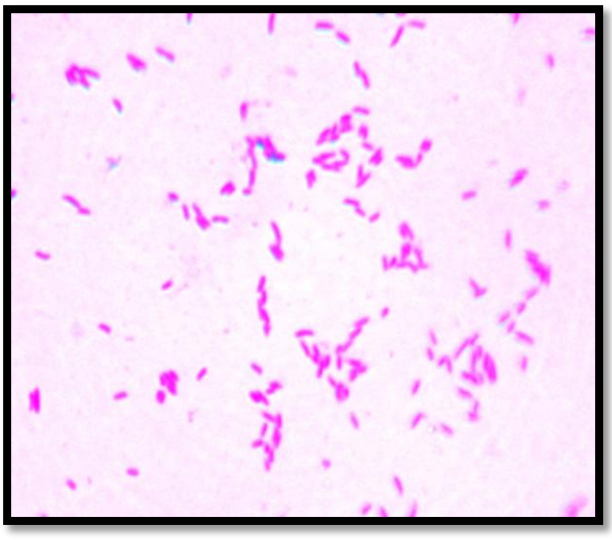
SAMPLE TAKEN BY SWAB



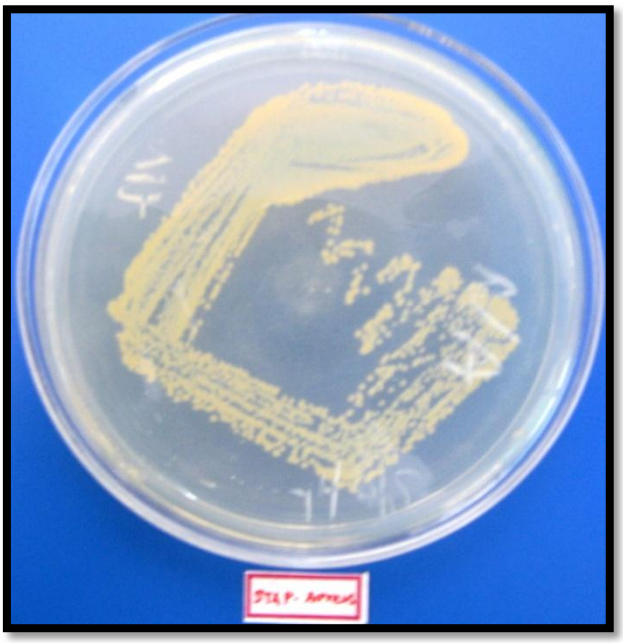
GRAM POSITIVE COCCI IN CLUSTERS



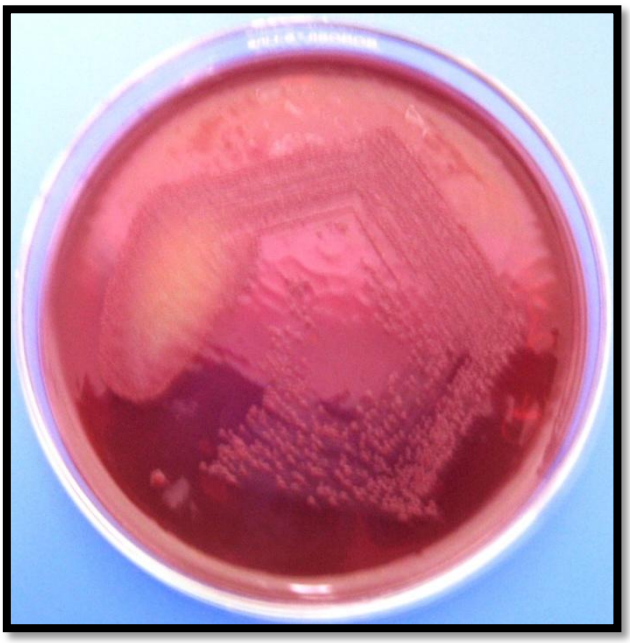
GRAM NEGATIVE BACILLI



STAPH.AUREUS IN NUTRIENT AGAR

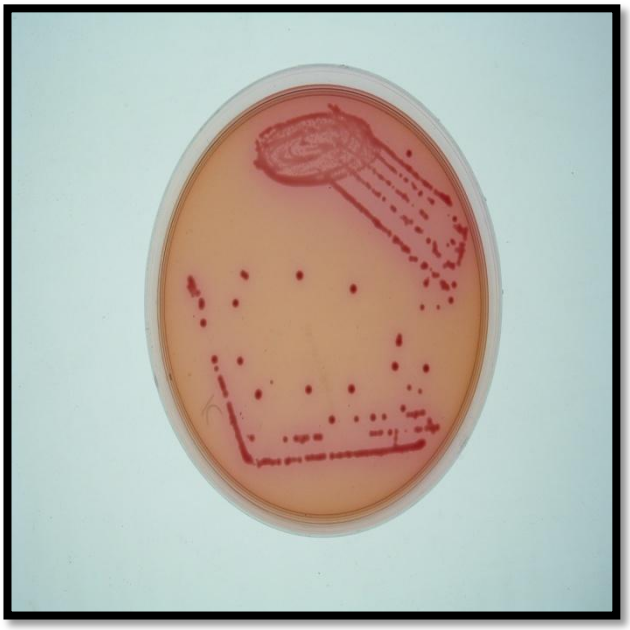


E.COLI IN MACCONKEY AGAR



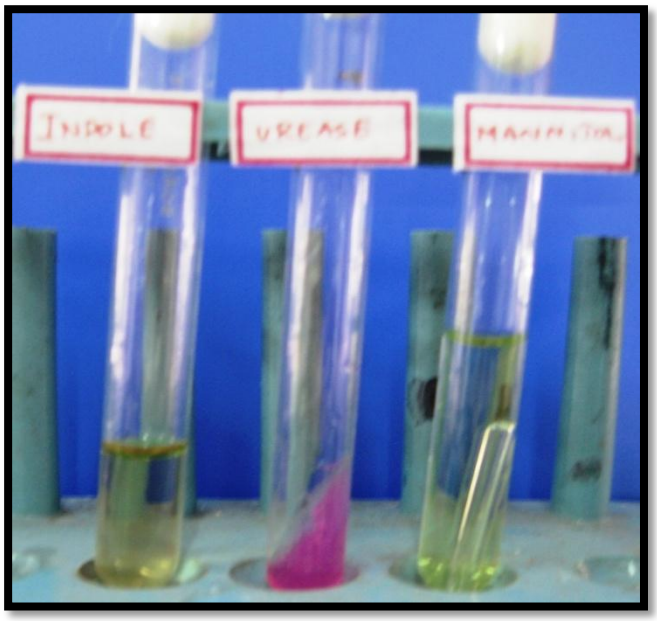
PSEUDOMONAS IN NUTRIENT AGAR

KLEBSIELLA IN MACCONKEY AGAR

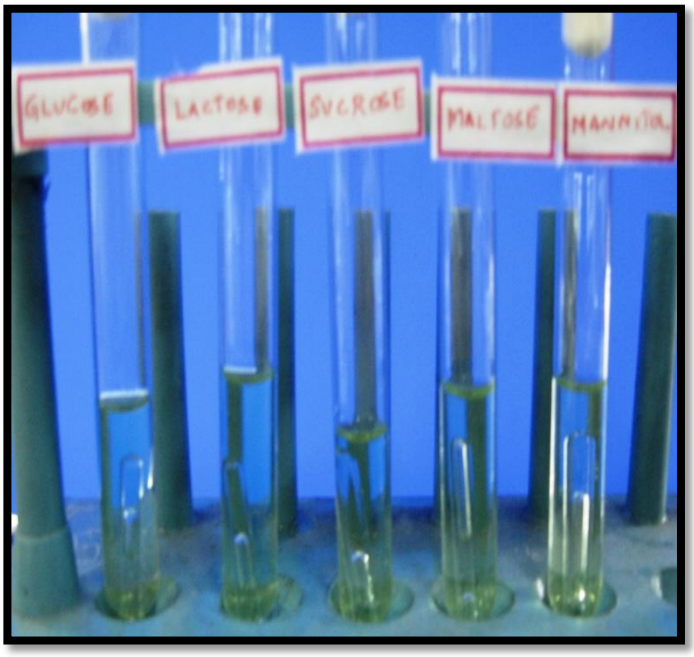


COAGULASE TEST

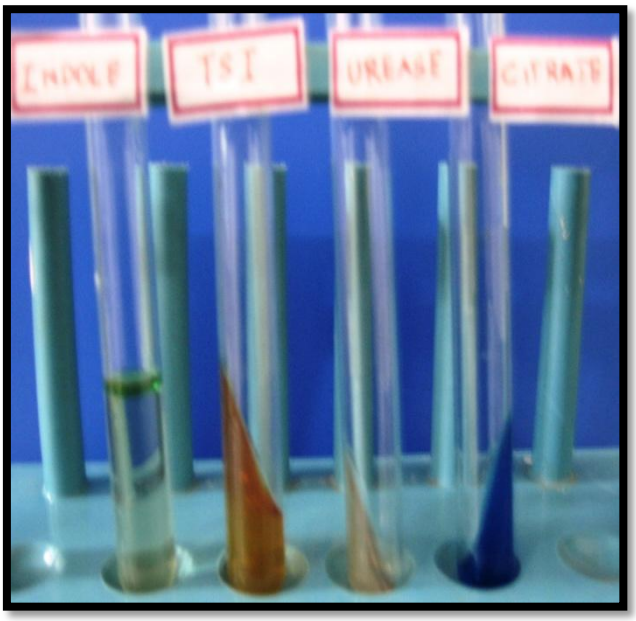
BIOCHEMICAL REACTION STAPH.AUREUS



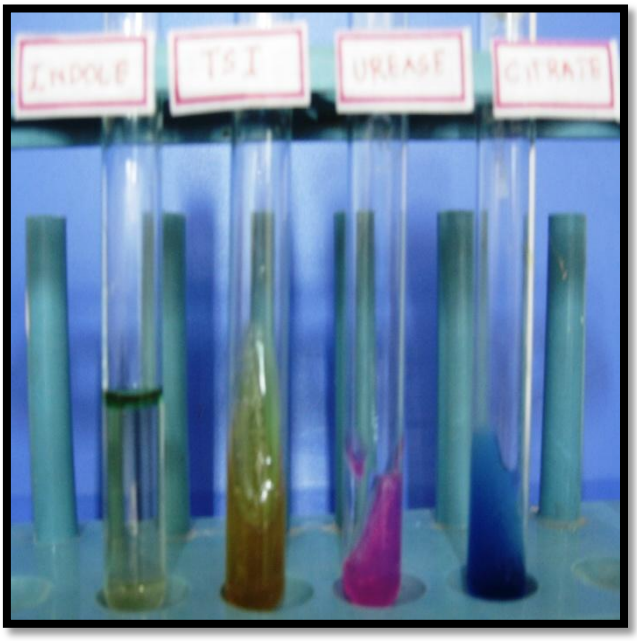
BIOCHEMICAL REACTIONS OF E.COLI



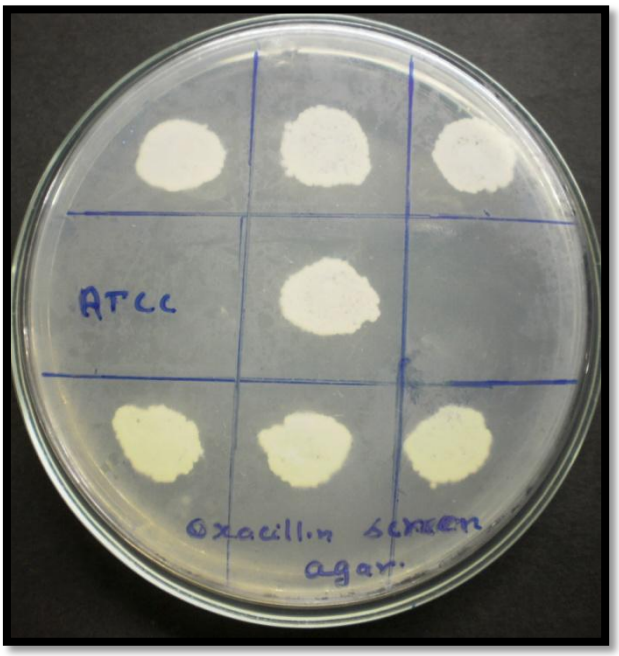
BIOCHEMICAL REACTIONS OF PSEUDOMONAS



BIOCHEMICAL REACTIONS OF KLEBSIELLA



OXACILLIN SCREEN AGAR



DOUBLE DISK SYNERGY TEST

ESBL DETECTION