BACTERIOLOGY OF ABSCESSES WITH SPECIAL REFERENCE TO PHENOTYPING AND GENOTYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL, CHENNAI.

Dissertation submitted in
THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
in partial fulfilment of the regulations
for the award of the degree of

M.D. (MICROBIOLOGY)
BRANCH - IV

GOVERNMENT STANLEY MEDICAL COLLEGE

& HOSPITAL

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

CHENNAI

MARCH 2010

CERTIFICATE

This is to certify that this dissertation entitled "**BACTERIOLOGY OF ABSCESSES WITH SPECIAL REFERENCE TO PHENOTYPING AND GENOTYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN GOVT STANLEY MEDICAL COLLEGE AND HOSPITAL, CHENNAI"** is the bonafide work done by **Dr.S.PRAMODHINI** in the Department of Microbiology, Govt. Stanley Medical College & Hospital, Chennai, in partial fulfillment of the regulation for **M.D. (Branch - IV) Microbiology** examination of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, to be held in March 2010.

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ACKOWLEDGEMENT

My sincere thanks to **Dr.S.Chitra, M.D.,** Dean, Government Stanley Medical College and Hospital for giving me permission to commence this dissertation and use the resources of this institution.

I owe my gratitude to **Dr.P.R.Thenmozhi Valli, M.D.,** Professor and Head Department of Microbiology for her unflinching interest, relentless efforts, valuable advice, excellent guidance and encouragement given to me throughout this study.

I am thankful to **Dr.R.Selvi M.D.,** Associate Professor of Microbiology for her perpetual support, encouragement, valuable advice and guidance in my dissertation work.

My sincere thanks to **Dr.Thyagarajan Ravinder, M.D.,** and **Dr.Devasena ,M.D**, former Additional and Associate Professors of microbiology for their encouragement and valuable advices given to me.

I am extremely thankful to **Dr.C.P.Ramani, M.D., and Dr.C.Usha, M.D.,** former Assistant Professors of Microbiology for keeping up my moral and for the enduring support and guidance provided when most needed.

I extend my sincere thanks to Assistant Professor **Dr.V.Dilli Rani M.D, Dr.A.Vasumathi,M.D, Dr.Ushakrishnan M.D, Dr.David Agatha** and **Dr.Eunice Swarna Jacob, M.D** of Department of Microbiology for their help, support, interest and valuable hints.

I also thank all my department colleagues for their timely help, cooperation and support.

I express many thanks to all the technical staff and other staff members of the Department of Microbiology & Immunology for their kind cooperation to carry out this work successfully.

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

DECLARATION

I, **Dr.S.PRAMODHINI,** solemnly declare that this dissertation **"BACTERIOLOGY OF ABSCESSES WITH SPECIAL REFERENCE TO PHENOTYPING AND GENOTYPING OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN GOVT STANLEY MEDICAL COLLEGE AND HOSPITAL, CHENNAI"** is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof.Dr.P.R.THENMOZHI VALLI, M.D.,** Professor of Microbiology, Government Stanley Medical College, Chennai-600 001.

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 March 2010.

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INTRODUCTION

An abscess is a localized collection of purulent inflammatory tissue caused by suppuration deep within a tissue, an organ or a confined space. It is produced by deep seeding of pyogenic bacteria into a tissue. It may involve skin, dermis, fasciae, muscles, and even bones.[58] They occur in many parts of the body as superficial infections or as deep-seated infections associated with any internal organ. Any organism isolated from them may be of significance.

Abscesses that develop as a result of introduction of the normal flora into a normally sterile body site are often polymicrobial in nature [25] Flora can gain access to the sterile site by direct extension or secondary to laceration or perforation. Because of the uniqueness of the normal flora at various body sites, the microbiology of such abscesses is generally predictable by their location.

Organisms may enter the tissue by direct implantation (eg, penetrating trauma with a contaminated object); spread from an established, contiguous infection; dissemination via lymphatic or hematogenous routes from a distant site; or migration from a location where there are resident flora into an adjacent, normally sterile area because of disruption of natural barriers

Staphylococcus aureus and Group A ß-haemolytic streptococci are the most prevalent aerobes in skin and soft tissue abscesses and are isolated at all body sites[7].In contrast, organisms that colonize the mucous membranes predominated in infections adjacent to these membrane[6,17] In this fashion, organisms of the gastrointestinal and cervical flora (enteric Gram-negative bacilli and Bacteroides fragilis group) were found most often in intra-abdominal and buttock and leg lesions.[9] Group A ß-haemolytic streptococci, pigmented Prevotella and Porphyromonas spp., and Fusobacterium spp. were most commonly found in lesions of the mouth, head, neck and fingers[8] .

Appropriate management of mixed aerobic and anaerobic infections requires the administration of antimicrobials that are effective against both aerobic and anaerobic components of infection in addition to surgical correction and drainage of pus. A number of factors should be considered when choosing appropriate antimicrobial agents. They should be effective against all target organisms, induce little or no resistance, achieve sufficient level in infected site, have minimal toxicity and have maximal stability and longevity.

Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections.[48] The organism has a differential ability to spread and cause outbreaks in hospitals.[62] Infections causes by S.aureus used to respond to beta lactam and related group of antibiotics. However, due to development of methicillin resistance amongst S.aureus isolates (MRSA); treatment of these infections has become problematic. Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, carriage of MRSA in nose are few important risk factors for MRSA acquisition[31]. Burns and Orthopaedics are two such high risk

units where patients are on multiple antibiotics and have a long stay in hospital[59]. Currently, the treatment options for MRSA infections are limited to very few and expensive drugs like Teicoplanin and Vancomycin. Thus, control of MRSA is essential to curtail the introduction and spread of infection.

This study was aimed to determine the bacteriological profile,the antibiotic susceptibility patterns of organisms isolated and the incidence of methicillin resistant staphylococcus aureus in abscesses.

AIMS AND OBJECTIVES

- To study the prevalence of aerobes and anaerobes in abscess
- To characterize the aerobes and anaerobes
- Phenotypic and Genotypic characterization of antibiotic susceptibility of isolated organism
- To study the incidence of methicillin resistant Stapylococcus aureus among the isolate
- To monitor antibiotic sensitivity pattern of MRSA and to formulate definite antibiotic policy to reduce the incidence of MRSA infection.

REVIEW OF LITERATURE

An abscess is a collection of pus that has accumulated in a cavity formed by the tissue on the basis of an infectious process or other foreign materials . It is a defensive reaction of the tissue to prevent the spread of infectious materials to other parts of the body.The organisms or foreign materials destroys the adjacent cells, resulting in the release of cytokines. The cytokines trigger an inflammatory response, which draws large numbers of leucocytes to the area and increases the regional blood flow.

Abscess wall or capsule is formed by the adjacent healthy cells in an attempt to keep the pus from infecting neighboring structures. The final structure of the abscess tends to prevent immune cells from attacking bacteria in the pus, or from reaching the causative organism or foreign object.

Abscesses can develop in many parts of the body, but they usually involve the skin surface. Common sites affected in superficial abscess include the armpits, groin, rectal area (perirectal abscess), the external vaginal area (Bartholin abscess), and along the tailbone (pilonidal abscess). Deep seated abscesses affect the brain, kidneys, liver (hepatic abscess), lungs, teeth (dental abscess), and tonsils (peritonsillar abscess)[24]

Immunopathogenesis of abscess formation

Bacterial Pathogens Induce Abscess Formation by $CD4^{+}$ T-Cell Activation via the CD28-B7-2 Costimulatory Pathway .The mechanism by which T cells mediate abscess formation requires the role of T-cell activation and the contribution of antigen-presenting cells via CD28-B7 costimulation. Blockade of T-cell activation via the CD28-B7 pathway in animals with CTLA4Ig prevented abscess formation following challenge with different bacterial pathogens. Abscess formation in vivo and T-cell activation in vitro required costimulation by B7-2 but not B7-1. These results demonstrate that abscess formation by pathogenic bacteria is under the control of a common effector mechanism that requires T-cell activation via the CD28-B7-2 pathway[2]

Abscess Causes

Abscesses are typically caused by either an inflammatory reaction to an infectious process or less commonly, to a foreign substance within the body, which triggers the body's immune system to form a cavity or capsule to contain the infection and prevent it from spreading to other parts of the body. The interior of the abscess liquefies, and pus develops (which contains dead cells, bacteria, and other debris). This area then begins to expand, creating increasing tension and inflammation of the overlying skin.

Individuals with any of the following conditions are at higher risk for developing abscesses :

Chronic steroid therapy, Chemotherapy, Diabetes, Cancer,Dialysis for kidney failure, AIDS, Sickle cell disease, Leukemia, Peripheral vascular disease, Crohn's disease, Ulcerative colitis, Severe burns, Severe trauma

Abscess Symptoms

The symptoms of an abscess vary depending on the location of the abscess, but in general, individuals will experience the following:

- -Most often, an abscess becomes a painful, compressible mass that is red, warm to the touch, and tender.
- -As an abscess progresses, it may "point" and come to a head. Pustular drainage and spontaneous rupture may occur.
- -Most abscesses will continue to get worse without care and proper incision and drainage. The infection can potentially spread to deeper tissues, and even into the bloodstream.
- -If the infection spreads, fever, nausea, vomiting, increasing pain, and increasing skin redness may develop.

Manifestations of abscess:

The cardinal symptoms and signs of any kind of inflammatory process are redness (rubor), heat (calor), swelling (tumor), pain (dolor) and loss of function [27]. Major complications are spreading of the abscess material to adjacent or remote tissues and extensive regional tissue death (gangrene). Abscesses in most parts of the body rarely heal themselves, so prompt medical attention is indicated at the first suspicion of an abscess. An abscess could potentially

be fatal (although this is rare) if it compresses vital structures such as the trachea in the context of a deep neck abscess

Abscesses that develop as a result of introduction of the normal flora into a normally sterile body site are often polymicrobial in nature[25]. Most deep seated abscess and necrotizing lesions involving anaerobes are polymicrobial and may include obligate anaerobes, facultative anaerobes or microaerophiles as concomitant microorganisms .These microorganisms acting in concert with trauma, vascular stasis or tissue necrosis, lower oxygen tension and oxidation reduction potential in tissues and provide favourable conditions for obligate anaerobe to multiply [32].

Mechanisms of Microbial Synergy:

Microbial synergy may increase the net pathogenic effect and hence the severity of infection in several ways:

(i) oxygen consumption by aerobic bacteria induces tissue hypoxia and a lowering of the redox potential, which favors the growth of anaerobic bacteria;

(ii) specific nutrients produced by one bacterium may encourage the growth of fastidious and potentially pathogenic cohabiting microorganisms; and

(iii) some anaerobes are able to impair host immune cell function and thus provide a competitive advantage for themselves as well as for other, cohabiting, microorganisms [46]

Mixed anaerobic infections

Anaerobiasis was discovered in 1861 by Louis Pasteur who introduced the terms aerobes and anaerobes to designate, respectively, microorganism that live in the presence and absence of oxygen within the next 30 years,many diverse type of anaerobic bacteria were isolated and described . the association of non sporing anaerobes with infections in humans was recognized as early as 1897[1].

"Anaerobic bacteria are organisms that require reduced oxygen tension for growth, failing to grow on the surface of solid media in 10% CO_2 in air. (In contrast, microaerophilic bacteria can grow in an atmosphere of 10% CO_2 in air or under anaerobic or aerobic conditions, although they grow best in the presence of only a small amount of atmospheric oxygen, and facultative bacteria can grow in the presence or absence of air.

Anaerobes can infect normal hosts and those with compromised resistance or damaged tissues. Symptoms depend on site of infection. Anaerobes are often accompanied by aerobic organisms[23]. Hundreds of species of nonsporulating anaerobes are part of the normal flora of the skin, mouth, GI tract, and vagina. If this commensal relationship is disrupted (eg, by surgery or other trauma, poor blood supply, tissue necrosis), a few of these species can cause infections with high morbidity and mortality. After becoming established in a primary site, organisms can spread hematogenously to distant sites. Because aerobic and anaerobic bacteria frequently are found in the same infected site, appropriate procedures for isolation and culture are necessary to keep from overlooking the anaerobes. Anaerobes can be the major cause of infection in the pleural spaces and lungs; in intra-abdominal, gynecologic, CNS, upper respiratory tract, and cutaneous diseases; and in bacteremia.

The principal anaerobic gram-positive cocci that produce disease are the peptococci and the peptostreptococci, which are part of the normal flora of the mouth, upper respiratory tract, and large intestine. The principal anaerobic gram-negative bacilli include Bacteroides fragilis , Prevotella melaninogenica, and Fusobacterium sp. The B. fragilis group is part of the normal bowel flora and includes the anaerobic pathogens most frequently isolated from intra-abdominal infections. Organisms in the Prevotella group and Fusobacterium sp are part of the indigenous oral flora [23,25].

Anaerobic infections can usually be characterized by the following features:

- They tend to occur as localized collections of pus or abscesses
- The reduced O_2 tension and low oxidation-reduction potential that prevail in avascular and necrotic tissues are critical for their survival
- When bacteremia occurs, it usually does not lead to disseminated intravascular coagulation (DIC) and purpura.

Some anaerobic bacteria possess distinct virulence factors. Those of B. fragilis probably account for its frequent isolation from clinical specimens despite its relative rarity in normal flora. This organism has a polysaccharide capsule that apparently stimulates abscess formation. An experimental model of intra-abdominal sepsis has shown that B. fragilis alone can cause abscesses, whereas other Bacteroides sp require the synergistic effect of another organism[25]. Another virulence factor, a potent endotoxin, is implicated in septic shock associated with severe Fusobacterium pharyngitis.

Because of their fastidiousness, they are difficult to isolate and are often overlooked. Their isolation requires appropriate methods of collection, transportation, and cultivation of specimens. Treatment is complicated by factors which include slow growth, increasing resistance to antimicrobial agents, and the polymicrobial synergistic nature of the infection. Morbidity and mortality are as great from anaerobic and mixed bacterial sepsis as from sepsis caused by a single aerobic organism. Anaerobic infections are often complicated by deep-seated tissue necrosis.

Bacteria causing cutaneous abscesses are typically indigenous to the skin of the involved area. For abscesses on the trunk, extremities, axillae, or head and neck, the most common organisms are Staphylococcus aureus and Streptococci .

Staphylococcus aureus is a medically important bacterial pathogen that is a common cause of superficial and deep-seated abscesses in humans. Most S. aureus isolates produce either a serotype 5 or 8 capsular polysaccharide (CP) that has been shown to enhance bacterial virulence [2].

In an elegant series of clinical observations and laboratory studies published in 1880 and 1882, Ogston described staphylococcal disease and its role in sepsis and abscess formation. More than 100 years later, Staphylococcus aureus remains a versatile and dangerous pathogen in humans. The frequencies of both community-acquired and hospital-acquired staphylococcal infections have increased steadily, with little change in overall mortality [18]. Treatment of these infections has become more difficult because of the emergence of multidrug-resistant strains.

Staphylococcal Components and Products

S. aureus is a member of the Micrococcaceae family . The genus Staphylococcus has at least 35 species.On microscopical examination, the organisms appear as gram-positive cocci in clusters. S. aureus is distinguished from other staphylococcal species on the basis of the gold pigmentation of colonies and positive results of coagulase, mannitol-fermentation, and deoxyribonuclease tests. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins [28].

Genome

The staphylococcal genome consists of a circular chromosome (of approximately 2800 bp, with prophages, plasmids, and transposons. Genes governing virulence and resistance to antibiotics are found on the chromosome, as well as the extrachromosomal elements [38] .These genes are transferred between staphylococcal strains, species, or other gram-positive bacterial species through the extrachromosomal elements [47] .

Antigenic Structure

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of infection: It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it

can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement.

Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid antibodies detectable by gel diffusion may be found in patients with active endocarditis due to S aureus [28].

Protein A is a cell wall component of many S aureus strains that binds to the Fc portion of IgG molecules except IgG_3. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic laboratory technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen will agglutinate bacteria that have that antigen ("coagglutination").

Some S aureus strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. Most strains of S aureus have coagulase, or clumping factor, on the cell wall surface; coagulase binds nonenzymatically to fibrinogen, yielding aggregation of the bacteria.

Various Enzymes and Toxins Produced by Staphylococci [40]

- β--lactamase - Breaks down penicillin

- Catalase - Converts hydrogen peroxide into water and oxygen and reduces killing by phagocytosis.

- Coagulase - Reacts with prothrombin to form a complex that can cleave fibrinogen and

cause the formation of a fibrin clot; fibrin may also be deposited on the surface of staphylococci, which may protect them from destruction by phagocytic cells; coagulase production is synonymous with invasive pathogenic potential.

- DNase - Destroys DNA.

- Enterotoxins - Are divided into heat-stable toxins of six known types (A, B, C1, C2, D, E); responsible for the gastrointestinal upset typical of food poisoning.

- Exfoliative toxins (A and B) -Causes loss of the surface layers of the skin in scalded-skin syndrome.

- Hemolysins- Alpha hemolysin destroys erythrocytes and causes skin destruction , Beta hemolysin destroys erythrocytes and sphingomyelin around nerves, Gamma hemolysin and Delta hemolysin destroys erythrocytes.

- Hyaluronidase -Also known as spreading factor; breaks down hyaluronic acid located between cells, allowing for penetration and spread of bacteria.

- Panton Valentine leukocidin - Inhibits phagocytosis by granulocytes and destroy these cells by forming pores in the phagosomal membranes.

- Lipases - Break down lipids.

- Nuclease- Breaks down nucleic acids.

- Protein A - Is antiphagocytic by competing with neutrophils for the Fc portion of

specific opsonins.

- Proteases - Break down proteins.
- Toxic shock syndrome toxin-1- Is associated with the fever, shock, and multisystem syndrome involvement of toxic shock syndrome.

Genetic Regulation of Virulence-Determinant Expression

The expression of staphylococcal virulence determinants is regulated by several systems that are sensitive to environmental signals. These systems consist of two proteins (two component systems), a sensor kinase, and a response regulator [28] .There are several well-described two-component regulatory systems in S aureus. These include agr, the best described, sae RS, srrAB, arlSR, and lytRS.

The accessory gene regulator (agr) is essential in quorum-sensing control of gene expression. It controls the preferential expression of surface adhesins (protein A, coagulase, and fibronectin binding protein) and production of exoproteins (toxins such as TSST-1) depending upon the growth phase (and hence bacterial density).

The most extensively studied gene, agr, induces the expression of exoprotein (extracellular protein) while suppressing the expression of surface protein through a bacterial-density–sensing octapeptide. Surface proteins are predominantly synthesized during the exponential growth phase, and the secreted proteins are synthesized during the stationary phase . This sequential expression of genes may have clinical importance.

Different stages of staphylococcal infection appear to require different panels of virulence determinants. During the initial stages of infection, the expression of surface proteins that bind extracellular-matrix molecules favors successful colonization of host tissues, whereas the synthesis of exoproteins favors the spread to adjacent tissues.

Epidemiology of Staphylococcal Disease

Humans are a natural reservoir of S. aureus. Thirty to 50 percent of healthy adults are colonized, with 10 to 20 percent persistently colonized[10]. Persons colonized with S. aureus are at increased risk for subsequent infections[45]. Rates of staphylococcal colonization are high among patients with type 1 diabetes, intravenous drug users, patients undergoing hemodialysis, surgical patients, and patients with the acquired immunodeficiency syndrome.

Transmission

Persons colonized with S. aureus strains are at increased risk of becoming infected with these strains. Most cases of nosocomial infection are acquired through exposure to the hands of health care workers after they have been transiently colonized with staphylococci from their own reservoir or from contact with an infected patient.

Temporal Trends in S. aureus Disease

Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections. The organism has a differential ability to spread and cause outbreaks in hospitals. Infections causes by S.aureus used to respond to beta lactam and related group of antibiotics. However, due to development of methicillin resistance amongst S.aureus isolates (MRSA); treatment of these infections has become problematic. Indiscriminate use of multiple

antibiotics, prolonged hospital stay, intravenous drug abuse, carriage of MRSA in nose are few important risk factors for MRSA acquisition [31].

The epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago. Initially, there were sporadic reports of methicillin resistance amongst nosocomial S. aureus isolates but later MRSA became a well established hospital acquired pathogen with few reports of community acquired isolates. Recent studies report an increased prevalence of community acquired MRSA with different risk factors compared to the earlier investigations from Detroit which first reported community acquired MRSA[59].

Pathogenesis of Staphylococcal Disease

The prototype of a staphylococcal lesion is the furuncle or other localized abscess[28]. Groups of S aureus established in a hair follicle lead to tissue necrosis (dermonecrotic factor). Coagulase is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue. Within the center of the lesion, liquefaction of the necrotic tissue occurs (enhanced by delayed hypersensitivity), and the abscess "points" in the direction of least resistance. Drainage of the liquefied center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing.

Focal suppuration (abscess) is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body. Suppuration within veins, associated with thrombosis, is a common feature of such dissemination. In osteomyelitis, the primary focus of S aureus growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration. S aureus may cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. Staphylococci of low invasiveness are involved in many skin

infections (eg, acne, pyoderma, or impetigo). Anaerobic cocci (peptostreptococcus) participate in mixed anaerobic infections.

Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection. Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxins. Toxic shock syndrome is associated with TSST-1 [28].

The virulence of S. aureus infection is remarkable, given that the organism is a commensal that colonizes the nares, axillae, vagina, pharynx, or damaged skin surfaces. Infections are initiated when a breach of the skin or mucosal barrier allows staphylococci access to adjoining tissues or the bloodstream. Whether an infection is contained or spreads depends on a complex interplay between S. aureus virulence determinants and host defense mechanisms.

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Host Response to Infection

The typical pathological finding of staphylococcal disease is abscess formation. Leukocytes are the primary host defense against S. aureus infection. The migration of leukocytes to the site of infection results from the orchestrated expression of adhesion molecules on endothelial cells. This cytokine-mediated process is triggered by bacteria and tissue-based macrophages. After infection, cytokines are first demonstrable within vessels, extending into tissues as inflammatory cells migrate to the sites of infection.S. aureus–infected endothelial cells also express intercellular adhesion molecule 1 (CD54), vascular-cell adhesion molecule 1 (CD106), and MHC class I molecules and probably contribute to this process

Mechanisms of Resistance to Antimicrobial Agents

Staphylococci are variably sensitive to many antimicrobial drugs. Resistance falls into several classes: [28]

1. β--lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins (penicillin G, ampicillin, ticarcillin, piperacillin, and similar drugs). The plasmids are transmitted by transduction and perhaps also by conjugation.

2. Resistance to nafcillin (and to methicillin and oxacillin) is independent of beta -lactamase production. The mecA gene for nafcillin resistance resides on the chromosome, and the gene encodes a low-affinity penicillin binding protein (PBP2 or PBP2a).

3. In the United States, S aureus and S lugdunensis are considered to be susceptible to vancomycin if the minimum inhibitory concentration (MIC) is 2 µg/mL; of intermediate susceptibility if the MIC is 4–8 µg/mL; and resistant if the MIC is 16 µg/mL.

The mechanism of vancomycin resistance is associated with increased cell wall synthesis and alterations in the cell wall and is not due to the van genes found in enterococci. S aureus strains of intermediate susceptibility to vancomycin "VISA." usually are nafcillin-resistant but generally are susceptible to oxazolidinones and to quinupristin/dalfopristin.

4. Since 2002, several isolates of vancomycin-resistant S aureus (VRSA) strains were isolated from patients in the United States. The isolates contained the vancomycin resistance gene vanA from enterococci and the nafcillin resistance

gene mecA Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in S aureus is of major concern worldwide.

5. Plasmid-mediated resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs is frequent in staphylococci.

6. "Tolerance" implies that staphylococci are inhibited by a drug but not killed by it —ie, there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug. Patients with endocarditis caused by a tolerant S aureus may have a prolonged clinical course compared with patients who have endocarditis caused by a fully susceptible S aureus. Tolerance can at times be attributed to lack of activation of autolytic enzymes in the cell wall.

SPECIMEN SAMPLING METHODS:

SPECIMEN:

In clinical microbiology a clinical specimen represents a portion or quantity of human material that is tested, examined, or studied to determine the presence or absence of particular microorganisms.[40]

Important concerns regarding specimens need emphasis:

1. The specimen selected should adequately represent the diseased area and also may include additional sites (e.g.,liver and blood specimens) in order to isolate and identify potential agents of the particular disease process.

2. A quantity of specimen adequate in amount to allow a variety of diagnostic testing should be obtained.

3. Attention must be given to specimen collection in order to avoid contamination from the many varieties of microorganisms indigenous to the skin and mucous membranes .

4. The specimen should be forwarded promptly to the clinical laboratory.

5. If possible, the specimen should be obtained before antimicrobial agents have been administered to the patient.

SPECIMEN TRANSPORT:

Skin or mucus membranes were decontaminated using alcohol or povidone iodine. The specimens were purulent exudate aspirated from abscesses Aspirates of purulent fluid and tissue samples are considered to be preferable to swabs because they will maintain the conditions required to sustain microbial viability. Prompt delivery of the specimen to the laboratory is considered to be of utmost importance , particularly if anaerobic bacteria are being investigated. The material is aspirated with a needle and syringe. Most of the time it is practical to remove the needle, cap the syringe with its original seal, and bring the specimen directly to the clinical laboratory. For specimens that cannot be transferred to the laboratory within 1-2 hours, storage at room temperature is considered to be appropriate for the maintainance of aerobic and anaerobic microorganisms [32] .

SPECIMEN PROCESSING:

Gram Stain

Despite being used for over a century Gram's stain is still the most important stain in microbiology and is widely used as a rapid technique for guiding antibiotic therapy. Meislin et al .[36] reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure Staphylococcus aureus. Similarly, this procedure may also facilitate

identification of the etiological agent where there is a higher probability of one microorganism being involved

CULTURE OF SPECIMEN & ANTIBIOGRAM:

Routine analysis of pus specimens normally involves the use of selective and non selective agar media to culture aerobic and anaerobic bacteria For anaerobic culture, the specimens were collected in cooked meat broth (CMB, Hi-Media) and incubated at 37ºC for 48 hours. The media used for aerobic incubation were 5% sheep blood agar, MacConkey agar, 7% salt agar and chocolate agar. Chocolate agar was incubated aerobically with 5% to 10% CO_2*. The media used for anaerobic incubation were Blood agar, Neomycin Blood agar, Bacteroides Bile esculin agar. Anaerobic incubation was done with P. aeruginosa as a biological indicator and alkaline methylene blue glucose as a chemical indicator.* [4]

Following incubation under aerobic or anaerobic conditions for 24 to 48hours, qualitative and semiquantitative assessments of the cultures are normally made. Aerobes were identified using standard microbiological methods and anaerobes were processed upto identification level.

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, anaerobes should be suspected and investigated more thoroughly.

METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Methicillin-resistant Staphylococcus aureus (MRSA) were first reported in 1961 in UK and have since become a major nosocomial pathogen worldwide. The reservoir of MRSA is infected and colonized patients, and the major mode of transmission from patient to patient is on the contaminated hands of healthcare workers. It is axiomatic that the sooner an MRSA infection is diagnosed, and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling and preventing MRSA infections.[15].

The organism is often sub-categorized as Community-Associated MRSA (CA-MRSA) or Hospital-Associated MRSA (HA-MRSA) depending upon the circumstances of acquiring disease About 75 percent of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively; however CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional HA-MRSA infections, which can affect vital organs and lead to widespread infection (sepsis), toxic shock syndrome and necrotizing pneumonia

Methicillin resistance in staphylococci is due to the acquisition of the mecA gene, which encodes the low-affinity penicillin-binding protein(2a or 2'). Presence of the mecA gene defines the Staphylococcus as methicilin resistance(MR), while absence of the gene from a staphylococcal strain indicates methicillin susceptibility (MS). Methicillin resistance can be difficult to detect because mecA-positive strains can differ in their level of expression of resistance. Resistance is typically heterogeneous with only a few cells, one in 10^{4} or 10^{6} , expressing the phenotype. [11]

In vitro testing condition can be modified to enhance the expression of oxacillin

resistance,they are as follows,[13]

a) preparation of inocula using the direct inoculum suspension procedure.

b) incubation of test at temperature no greater than 35 degree Celsius

c) obtaining final test reading after a full 24 hours of incubation .

d) supplementation of Muller Hinton Agar with 2% NaCl

The extended incubation allows the slower growing resistant subpopulation sufficient time to grow to detectable number. In addition zone of inhibition must be examined using transmitted light and growth is considered significant.

In the routine laboratories, phenotypical methods are preferred in detecting methicilin resistance, but it is time consuming and there are some difficulties in detecting all of the resistant isolates. Many factors including inoculum size, incubation time, incubation temperature, beta lactam antibiotic being tested, pH of the culture medium and NaCl concentration have a major effect on the expression and therefore the detection of resistance. Hence, the tests based on detection of genotype are more accurate than phenotypic methods.

More recently, PCR-based methods have been used routinely by reference laboratories as their standard method for detecting the mecA gene[15] Occasional susceptible strains carrying a non-functional or non-expressed mecA, will also be detected, but the presence of mecA is generally considered to indicate a potential for resistance and is used as a marker to identify MRSA.

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS:

Disk diffusion methods:

The disk agar diffusion was performed by following the recommendations of the Clinical Laboratory Standards [13]. 1 µg oxacillin disks were applied onto each plate. After incubation at 35 °C for 24 hours, the zone diameters were documented in millimeters.

Cefoxitin is a potent inducer of the mecA regulatory system and an accurate surrogate marker for the detection of MRSA in routine susceptibility testing for disk diffusion [52].Testing a surrogate marker of resistance may provide a more accurate indication of oxacillin resistance than testing oxacillin itself. This is because cefoxitin serves to induce greater expression of PBP2a in mecA containing strains of staphylococci and also function as a test reagent to detect resistance. It is suggested that no special medium or incubation temperature is required with cefoxitin .

Cefoxitin disk diffusion testing is now recommended by CLSI as a preferred method of detection of oxacillin resistance in staphylococci; however, it is important to report the findings from cefoxitin disc diffusion test as indicative of either oxacillin susceptibility or resistance; cefoxitin report should not be reported.

Oxacillin agar screening methods

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. Plates were spot inoculated with 0.5 Mc Farland suspension of the isolate and incubated at 35 0 C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant [52]

Quality control strains - methicillin sensitive S. aureus (MSSA) ATCC 25923 and methicillin resistant S. aureus (MRSA) ATCC 43300 - were used as negative and positive controls, respectively.

Molecular methods :

The fact that high-level resistance to penicillinase-resistant penicillins is generally related to the presence of the mecA gene means that a genotypic method for the detection of mecA allows rapid and unambiguous characterization of this resistance mechanism. The earliest molecular methods for the detection of mecA relied on either radiolabelled or digoxigenin (DIG)-labelled DNA probes.[3] The non-radioactive DIG-labelled probe performed as well as the radioactive label, enabling the safer utilization of the test system in a diagnostic laboratory.

More recently, PCR-based methods have been used routinely by reference laboratories as their standard method for detecting the mecA gene.[18] Occasional susceptible strains carrying a non-functional or non-expressed mecA, will also be detected, but the presence of mecA is generally considered to indicate a potential for resistance and is used as a marker to identify MRSA.

Generally speaking, MRSA PCR assays that use a single amplification step are both robust and simple to perform. However, simple assays of this type are vulnerable to the presence of inhibitors, which will lead to a false-negative result, and the addition of a second set of primers to amplify a gene which is always present within staphylococci has been a very common control method. Primers directed against the nuc, coa and gyrA genes have been used for this purpose..[15]

Treatment of S. aureus Infection

Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy.[28] Acute hematogenous osteomyelitis responds well to antimicrobial drugs. In chronic and recurrent osteomyelitis, surgical drainage and removal of dead bone is accompanied by long-term administration of appropriate drugs, but eradication of the infecting staphylococci is difficult. Hyperbaric oxygen and the application of vascularized myocutaneous flaps have aided healing in chronic osteomyelitis.

Bacteremia, endocarditis, pneumonia, and other severe infections due to S aureus require prolonged intravenous therapy with a β- -lactamase-resistant penicillin. Vancomycin is often reserved for use with nafcillin-resistant staphylococci. If the infection is found to be due to non-β--lactamase-producing S aureus, penicillin G is the drug of choice, but only a small percentage of S aureus strains are susceptible to penicillin G.[28]

Patients unable to tolerate vancomycin have been treated with fluoroquinolones, trimethoprim–sulfamethoxazole, clindamycin, or minocycline. Quinolones with enhanced antistaphylococcal activity have recently become available, but their use may also be limited by the development of resistance during therapy.

Newer antimicrobial agents such as linezolide, daptomycin, and quinupristin/dalfopristin [28] are generally reserved for patients with serious staphylococcal or enterococcal infections that are resistant to the more traditional agents, who are failing clinically or who are highly allergic.

Prevention of Staphylococcal Disease

The use of topical agents to eliminate staphylococcal colonization in high-risk groups, such as patients undergoing hemodialysis or surgery, has been shown to reduce the incidence of subsequent infections. Mupirocin, a topical antistaphylococcal agent that inhibits RNA and protein synthesis, eliminates nasal colonization in carriers and can reduce the incidence of wound infections.[41] In healthcare environments, MRSA can survive on surfaces and fabrics, including privacy curtains or garments worn by care providers. Complete surface sanitation is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures. Testing patients for MRSA upon admission, isolating MRSA-positive patients, decolonization of MRSA-positive patients, and terminal cleaning of patients' rooms and all other clinical areas they occupy is the current best practice protocol for nosocomial MRSA

At present, prevention of the spread of infection relies on the application of appropriate principles of infection control. These approaches have been effective in reducing the nosocomial spread of staphylococcal infection.

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MATERIALS AND METHODS

STUDY DESIGN: Prospective Cohort study

The present study was carried out in department of microbiology in Govt. Stanley Medical College and Hospital ,Chennai.

STUDY PERIOD:

Over a period of one year from May 2008 to June 2009.

MATERIALS:

Pus sample taken from a case of total 120 patients with abscess, superficial and deep attending surgical and allied surgical departments as outpatients and inpatients in Stanley Medical College and Hospital, Chennai were included in the study.

METHODOLOGY:

SPECIMEN COLLECTION & TRANSPORT:

The specimen were collected by aspiration in sterile syringe and needle, tip of the needle sealed with sterile rubber cork, transported to the laboratory and processed immediately within 1-2 hours of collection..

SPECIMEN PROCESSING:

Once the specimen reached the laboratory, smears were prepared by smearing the purulent material on clean glass slide. Gram staining of the smear done ,examined under microscope and findings were recorded..

For aerobic culture specimens were inoculated onto Blood agar, Mac conkey agar plate and incubated aerobically at 37 degree Celsius for 18 to 24 hours.[6]

For anaerobic culture specimen were inoculated onto freshly prepared Blood agar, Neomycin Blood agar and Bacteroides Bile Esculin agar plate. Inoculated plates were kept in anaerobic jar with media facing upwards. Commercially available gas pack was cut open in the corner and placed inside the jar and the lid of the jar was closed immediately and kept for incubation for 48 hours at 37ºC. A plate inoculated with pseudomonas was put in the jar which served as a control to check the maintanence of anaerobiasis.[6]

Each specimen was also inoculated into Robertson cooked meat media and incubated at 37 ºC for 48 hours. [67]

On the second day, the aerobically incubated plates were examined for any growth. If growth was seen organism were identified by gram staining, motility, colony morphology and biochemical reactions.

GPC were identified on the following media as mentioned in appendix (6).

Differentiating characters of gram negative bacilli were identified based on biochemical reaction as mentioned in appendix (7)

On the third day anaerobic jar was opened Blood agar, Neomycin Blood agar and Bacteroides Bile Esculin agar plates were examined for any growth. All different types of colony were marked.

From each colony the following were done:

i) Gram stain and the findings were recorded.

ii) Inoculated onto Blood agar incubated aerobically at 37 ºC to check for aerotolerance.

iii) Inoculated onto Blood agar and incubated anaerobically at 37 ºC for 48 hours.

From RCM which was incubated for 48 hours the following were done

i. Gram stain and the findings were recorded.

ii. Blood agar, Mac conkey agar plate were inoculated and incubated aerobically at 37 ºC for 18 to 24 hours.

iii. Blood agar, Neomycin Blood agar and Bacteroides Bile Esculin agar plate were inoculated and incubated anaerobically in jar at 37 ºC s for 48hrs.

On fourth day, the Blood agar plate put up to check aerotolerance was examined for any growth and findings recorded. Any colony which showed aerotolerant negative was considered as obligate anaerobe. From RCM subcultured aerobic plates were examined for any growth. Growth was identified with the help of biochemical reactions.

On fifth day anaerobic jar was opened and the blood agar plates where individual colonies were subcultured was examined for growth. If the corresponding blood agar plate kept for aerotolerance did not show any growth, then the bacteria was an anaerobe and appropriate test was put up for identification.

Level I Identification

Gram stain and colonial morphology of a pure isolate provides presumptive identification of anaerobic organisms [6]

Organisms	Cell shape	Gramreaction	Aerotolerance	Distinguishing characteristics
Bacteroides fragilis	B	-	-	Growth on BBE with colony size >1mm in diameter.
Pigmented Bacteroides species	CB B	-	-	Dark pigmenting or brickred fluorescing colony.
B. ureolyticus like group.	.B	-	-	Pitting colonies
F. nucleatum (presumptive)	B	-	-	Slender bacillus with pointed ends; bread crumbs speckling colony.
Anaerobic gram negative bacillus.	B CB	-	-	
Anaerobic gram negative coccus	C	-	-	
Anaerobic gram positive coccus	C	+	-	
C.perfringens (presumptive)	B	+	-	Double zone of beta hemolysis; box car shaped cells.
Other clostridium species	B	+	±	Spores seen on Grams stain
Anaerobic gram positive bacillus	B	+	±	No boxcar shaped cells;no spores

B - BacillusCB - Coccobacillus

± - Most strains negative ,some weakly positive.

Antibiotic identification disc:

Many anaerobes have characteristic susceptibility pattern to colistin (10µg), vancomycin(5 µg) and kanamycin (1 mg) disk as mentioned in appendix (11). The pattern generated will usually confirm a dubious gram stain reaction and aid in subdividing the anaerobic gram negative bacilli into groups.[6]

STAPHYLOCOCCUS AUREUS:

Staphylococcus aureus was identified by its gram stain ,colony morphology , catalase test and coagulase test [37]

Gram stain:

On gram staining, Gram positive cocci in clusters was seen.

Colony morphology:

Nutrient agar: Showed 1 to 3mm diameter, circular, smooth, low convex, glistening, densely opaque colonies with golden yellow pigmentation.

Blood agar: Showed colonies surrounded by narrow zone of beta hemolysis

Mac conkey agar: Showed small size pink coloured colonies .

Further confirmation was done by slide and tube coagulase test , growth on mannitol salt agar and DNase test by standard microbiological technique as recommended by CLSI guidelines.

Slide coagulase test:

A staphylococcal colony was emulsified in a drop of saline to form a smooth milky suspension. Similar suspension was made with a positive and negative control strains. To the suspension, a loopful of plasma was added. Coarse clumping of cocci visible to naked eye within 10 seconds was considered positive and absence of clumping as negative. [37]

Tube coagulase test:

Staphylococcus to be tested was grown in brain heart infusion broth overnight at 37ºC . To 0.1ml of this culture , 0.5 ml of undiluted plasma was added and incubated at 37ºC in a water bath for upto 4 hours. Tubes were examined at 1,2,and 4 hrs for clot formation by tilting the tube at 90º. Any degree of clot formation was considered positive. If the plasma remained wholly liquid it was considered negative [37]

Deoxyribonuclease test:

DNase was detected by heavily spot inoculating several colonies of the organism on DNase test medium. After 24 hours of incubation at 37 ºC, the plate is flooded with 3.6% hydrochloric acid. After few minutes, the medium under and around the inoculum became clear indicating hydrolysis of DNA[37]

Mannitol salt agar:

The organism was inoculated in mannitol salt agar and incubated at 37 ºC for 24 hours. S.aureus produced yellow coloured colonies. A positive control was put using S.aureus ATCC 25923.

Biochemical reactions

Indole	Negative
MR	Positive
VP	Positive
Mannitol	Fermented
Urease	Positive

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Disk diffusion test:

Reagents for the Disk Diffusion Test

Müeller-Hinton Agar Medium containing 2% NaCl

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), was used. A $BaSO_4$ 0.5 McFarland standard was prepared as follows:

A 0.5-ml aliquot 1.175% w/v $BaCl_2$ is added to 99.5 ml of 1% H_2SO_4 with constant stirring to maintain a suspension [17]

Inoculum Preparation

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium. The broth culture was incubated at 35℃ until it achieved the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours) .The turbidity of the actively growing broth culture was adjusted with sterile McFarland standard. This results in a suspension containing approximately 1 x 10^8CFU/ml

Inoculation of Test Plates

The dried surface of a Müeller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. [17]

Application of Discs to Inoculated Agar Plates

The predetermined battery of antimicrobial discs which included Penicillin(10u), Ampicillin (10µg), Cefotaxime(30µg), Amikacin(30 µg), Erythromycin (15 µg), Ciprofloxacin(5µg), Oxacillin (1µg) was dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface.

Reading Plates and Interpreting Results

The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted petri plate. Transmitted light (plate held up to light) was used to examine the for light growth of methicillin-resistant colonies, within apparent zones of inhibition[17]. Any discernable growth within zone of inhibition was indicative of methicillin resistance.

The sizes 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 as mentioned in appendix(14)

Oxacillin disk diffusion method

Oxacillin disk (1 µg) diffusion method was carried out on Mueller-Hinton agar supplemented with 2% NaCl to detect MRSA according to the CLSI guidelines. The plates were incubated at 35°C and results were recorded after 24 hrs of incubation. Isolates were considered resistant when the diameter of inhibition was ≤10 mm, intermediate resistance when the diameter was 11-12 mm and sensitive when the diameter was ≥13 mm. .[16]

Cefoxitin disc diffusion test

Cefoxitin is a potent inducer of the *mecA* regulatory system and an accurate surrogate marker for the detection of MRSA in routine susceptibility testing for disk diffusion The Clinical and Laboratory Standards Institute (CLSI) guidelines (2006) has recommended cefoxitin disc diffusion method for the detection of MRSA. This is performed by using a 30 µg cefoxitin disc and an inhibition zone diameter of ≤21 mm is reported as methicillin resistant and ≥22 mm is considered as methicillin sensitive. [16]

Quality control strains - methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC 43300 - were used as negative and positive controls, respectively.

Oxacillin screen agar:

Muller-Hinton agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. To perform oxacillin screen test, a swab dipped in 0.5 Mc Farland suspension of the isolate was deposited as a spot on agar surface and incubated at 35 ^{0} C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant.[59]

MOLECULAR METHODS:

PCR assay for detection of mecA gene:

Extraction of DNA:

- 1.5 ml of overnight bacterial culture was taken & centrifuged at 12000 rpm at 4°C for 5 min.

- To the pellet 330µl of TE buffer and 4 µl of Lysozyme was added.

- About 16.7 µl of SDS was added to the sample and incubated for 10 min at 50 °C

- After incubation 17 µl of 0.5 M EDTA was added and incubated for 10 min at 50°C

- Then 10 µl of proteinase K was added to the sample and incubated for 3 hours at room temperature .

- Equal volume of phenol-chloroform was added to the sample and mixed well
- The sample was centrifuged for 10,000 rpm for 10 minutes
- The supernatant was transferred to new tube and 0.1 volume of sodium acetate and two volume of 95% ethanol was added
- Tube was incubated at -20 °C overnight
- The sample was centrifuged for 10,000 rpm for 10 minutes
- The pellet was washed with 70% ethanol
- The pellet was allowed to dry and resuspended in 20 µl of TE buffer

MASTER MIX

dNTP mix 20nM

Taq buffer

Taq polymerase enzyme

Forward primer (5'CAT TTT GAG TTC TGC ACT ACC 3')

Reverse primer (5'GCA ATA CAA TCG CAC ATA CAT TAA TAG 3')

An optimal negative control was employed using 1 µl molecular grade water

PCR AMPLIFICATION

The following were the conditions adopted for PCR amplification

- Initial denaturation at 94° C for 5 min
- Cycle denaturation at 94°C for 30 secs
- Primer annealing at 60 °C for 1min 20 secs
- Extension 72 °C for 1min 20 secs
- The PCR was carried out for35 cycles
- A final extension at 72°C for 7 min

ANALYSIS BY GEL ELECTROPHORESIS [48]

Preparation of agarose gel

- To prepare 1% agarose gel 0.5gms agarose powder was mixed with 50ml of electrophoresis buffer, then heated in a microwave oven, mixed well Until the agarose was uniformly dissolved.

- After cooling to about 60°C ethidium bromide* was added to the gel(final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis. After cooling the solution, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature

- After the gel hardens enough, the gel was mounted in electrophoresis tank.

- Electrophoresis buffer is poured into the electrophoresis tank so that the gel was completely immersed

- the comb carefully removed

- Ethidium Bromide is mutagenic and should be handled with extreme caution. Dispose of the contaminated tip into a dedicated ethidium bromide waste container.

Gel electrophoresis

- Electrical leads connected.As the DNA amplified by PCR was charged negative, it migrates from cathode to anode.

- 2.0 µ l of 6 x loading buffer was addedto each tube containing the PCR reactant and mixed.

- The mixture was slowly loaded into the slots of the submerged gel using a Micropipette.

- Marker DNAs of known size was loaded into slots

- Constant voltage of 50-150 V was applied to allow the gel run until the Bromophenol Blue have migrated ¾ the length of the gel.

All PCR products were analysed in 1.5% agarose gel, stained with ethidium bromide and observed under UV transilluminator. Amplicons of 310 bp[58] were consistent with *mecA* gene amplification

RESULTS

The study was performed during May 2008 to June 2009 at Department of Microbiology & Surgery, which included 120 cases of superficial and deep abscesses Govt Stanley Medical College and Hospital This study was done to find the bacteriological profile, antibiotic susceptibility pattern and the incidence of MRSA in the abscesses. The results were analysed as followed

TABLE – 1

AGE AND SEX DISTRIBUTION OF CASES (n = 120 CASES)

Age in years	Male	Female	Total
1- 20	17	8	25(20.83%)
21 - 40	25	22	47(39.17%)
41 - 60	31	11	42(35%)
61 – 80	3	3	6(5%)
Total	**76**(63.33%)	**44**(36.67%)	**120**

Out of 120 cases taken for study, 25 (20.83%) were between 1-20 years, 47 (39.17%) were between 21-40 years, 42 (35%) were between 41-60 years and 6 (5%) were between 61 – 80 years. Maximum cases were recorded between 21-40 years followed by 41-60 years age group. The study group included 76 (63.33%) males and 44 (36.67%) females. In all age group the sex distribution was predominantly male.

TABLE – 2

DISTRIBUTION OF SAMPLE SOURCE (n = 120)

Speciality	No. of Cases	Percentage
General Surgery	64	53.34
Orthopaedics	34	28.33
ENT	7	5.83
GastroEnterology	9	7.5
Paediatric Surgery	3	3
Obst.&Gynaecology	3	2.5
Total	**120**	**100**

Out of 120 specimen 64 (53.34%) cases were from General surgery , 34 (28.33%).cases from Orthopaedic department, 7 (5.83%)cases from ENT department, 9 (7.5 %) cases from GastroEnterology department, 3 (3%) case from Paediatric Surgery and 3 (2.5%) case from Obst. & Gynaecology department. Maximum cases were recorded from General surgery followed by Orthopaedic department

TABLE - 3

DISTRIBUTION OF ABSCESS AS PER SITE (n=120)

S.NO	Location of Abscess	No of cases	Type of Abscess	
			Superficial Abscess	Deep Abscess
1	Neck	5	4	1
2	Breast	13	13	-
3	Axilla	7	7	-
4	Arm	5	2	3
5	Hand	11	7	4
6	Inguinal	2	2	-
7	Gluteal	11	1	10
8	Perianal	10	10	-
9	Thigh	2	-	2
10	Leg	4	4	-
11	Feet	7	6	1
12	Chestwall	3	3	-
13	Bone & Joint	5	-	5
14	Liver	7	-	7
15	Gall Bladder	2	-	2
16	Abd. Wall	7	-	7
17	Appendix	3	-	3
18	Ear	7	3	4
19	Lung	4	-	4
20	Genital	5	2	3
	Total (%)	**120(100%)**	**64(53.33%)**	**56(46.67%)**

Out of 120 pus samples taken, 64 (53.33%) were from superficial abscess and 56 (46.67%) were from deep abscess.

TABLE - 4

MICROBIOLOGIC CHARACTERISTICS OF ABSCESSES BY LOCATION (n = 120)

S.No.	Abscess Location	No. of Cases	Aerobic	Anaerobic	Both
1	Neck	5	4	1	0
2	Breast	13	7	6	0
3	Axilla	7	5	2	0
4	Arm	5	4	1	0
5	Hand	11	8	2	1
6	Inguinal	2	1	0	1
7	Gluteal	11	9	0	2
8	Perianal	10	7	2	1
9	Thigh	2	2	0	0
9	Leg	4	4	0	0
10	Foot	7	6	1	0
11	Chestwall	3	3	0	0
12	Bone & Joint	5	5	0	0
13	Liver	7	4	3	0
14	Gall Bladder	2	2	0	0
15	Abd. Wall	7	3	4	0
16	Appendix	3	2	0	1
17	Ear	7	4	3	0
18	Lung	4	4	0	0
19	Genital	5	5	0	0
	Total(%)	**120(100%)**	**89(74.17%)**	**25(20.83%)**	**6(5%)**

Out of 120 pus samples, samples with aerobic isolates only were 89 (74.17%), samples with anaerobic isolates only were 25 (20.83%) and samples with both aerobic and anaerobic isolates were 6 (5%).

TABLE - 5

PREDOMINANT ISOLATES OF THE ABSCESS (n=120)

S. No	Location of Abscess	No. of Cases	Isolates						
			Aerobic					Anaerobic	
			Staph. Aureus (%)	Strep. Pyogenes (%)	Escherichia coli (%)	Klebsiella sp. (%)	Proteus sp. (%)	Peptostreptococcus (%)	Bacteroides fragilis(%)
1	Neck	5	4					1	
2	Breast	13	7					6	
3	Axilla	7	5					2	
4	Arm	5	4					1	
5	Hand	11	6		1	2		3	
6	Inguinal	2	1				1		1
7	Gluteal	11	4		5	2	2		2
8	Perianal	10	1		7		2		3
9	Leg	6	4	1	1		1		
10	Foot	7	4	1	1			1	
11	Chestwall	3	3						
12	Bone & Joints	5	3	1		2			
13	Liver	7			3	2			3
14	Gall Bladder	2			1	1			
15	Lung	4	1	1		2			
16	Ear	7	4					3	
17	Abd. Wall	7	2	1				2	2
18	Appendix	3			3				1
19	Genito Urinary	5	2		2	2			
	Total	**120**	**55**	**5**	**24**	**13**	**6**	**19**	**12**

Out of 134 isolates, 55 (41.04%) were Staphylococcus aureus, 5 (3.73%) were Streptococcus pyogenes, 24 (17.91%) were Escherichia coli, 13 (9.7%) were Klebsiella sp, 6 (4.48%) were Proteus sp, 19 (14.18%) were Peptostreptococcus sp, 12 (8.96%) were Bacteroides sp.

TABLE - 6

PREDOMINANT ISOLATES OF SUPERFICIAL ABSCESS (n=71)

S.No.	Location of Abscess	No. of Cases	Isolates						
			Aerobic					Anaerobic	
			Staph. aureus	Strep. pyogenes	Escherichia coli	Klebsiella sp.	Proteus sp.	Peptostreptococcus	Bacteroides fragilis
1	Neck	4	4						
2	Breast	13	7					6	
3	Axilla	7	5					2	
4	Arm	2	2						
5	Hand	7	3		1	2		2	
6	Inguinal	2	1				1		1
7	Gluteal	1			1		1		
8	Perianal	10	1		7		2		3
9	Leg	4	3	1					
10	Feet	6	3	1	1			1	
11	Chestwall	3	3						
12	Ear	3	2					1	
13	Genital	2	2			1			
	Total	**64**	**36**	**2**	**10**	**3**	**4**	**12**	**4**

Out of 64 superficial abscess, 5 were aerobic isolates and 16 were anaerobic isolates. Of 55 aerobic isolates, 36 were Staphylococcus aureus,2 were Streptococcus pyogenes,10 were Escherichia coli, 3 were Klebsiella spp, 4 were Proteus spp. Of 16 anaerobic isolates, 12 were Peptostreptococcus spp, 4 were Bacterodes fragilis group.

TABLE - 7

PREDOMINANT ISOLATES OF DEEP ABSCESS (n=63)

S.No.	**Location of Abscess**	**No. of Cases**	**Isolates**						
			Aerobic					**Anaerobic**	
			Staph. aureus	**Strep. pyogenes**	**Escherichia coli**	**Klebsiella sp.**	**Proteus sp.**	**Peptostreptococcus**	**Bacteroides fragilis**
1	Neck	1						1	
2	Arm	3	2					1	
3	Hand	4	3					1	
4	Gluteal	10	4		4	2	1		2
6	Thigh	2	1		1		1		
7	Feet	1	1						
8	Bone &joints	5	3	1		2			
9	Abd. wall	7	2	1				2	2
10	Liver	7			3	2			3
11	Gall bladder	2			1	1			
12	Appendix	3			3				1
13	Lung	4	1	1		2			
14	Ear	4	2					2	
15	Genital	2			2	1			
	Total	**56**	**19**	**3**	**14**	**10**	**2**	**7**	**8**

Out of 56 deep abscess, 48 were aerobic isolates and 15 were anaerobic isolates. Of 48 aerobic isolates, 19 were Staphylococcus aureus, 3 were Streptococcus pyogenes,14 were Escherichia coli, 10 were Klebsiella spp, 2 were Proteus spp. Of 15 anaerobic isolates, 7 were Peptostreptococcus spp, 8were Bacterodes fragilis group

TABLE – 8

TOTAL NUMBER OF AEROBES AND ANAEROBES ISOLATED FROM THE ABSCESSES (n=134)

Organism	Total (%)
Aerobic	**103(76.87%)**
Gram positive cocci	**60(44.77%)**
Staphylococcus aureus	55(41.04%)
Streptococcus pyogenes	5(3.73%)
Gram negative bacilli	**43(32.09%)**
Escherichia coli	24(17.91%)
Klebsiella spp	13(9.7%)
Proteus spp	6(4.48%)
Anaerobic	**31(23.14%)**
Gram positive cocci	**19(14.18%)**
Peptostreptococcus spp	19(14.18%)
Gram negative bacilli	**12(8.96%)**
Bacteroides fragilis	12(8.96%)

Out of 134 isolates, 103 (76.87%) were aerobic isolates , which included 60 (44.77%) gram positive cocci and 43 (32.09%)gram negative bacilli .Total anaerobic isolates were 31(23.14%),which included 19 (14.18%) gram positive cocci and 12 (8.96%) gram negative bacilli

TABLE - 9

ANALYSIS OF ISOLATES OF ABSCESSES

Isolates	Number(%)
Total number of patients	**120**
Culture positive	120
Number of isolates	**134**
Organism rate per lesion	1.12
Only aerobes	89(74.17%)
Only anaerobes	25(20.83%)
Aerobes + anaerobes	6(5%)
Aerobe/Anaerobe ratio	3.32
Monomicrobial	**106(88.33%)**
Aerobes	81(76.42%)
Anaerobes	25(23.58%)
Polymicrobial	**14(11.67%)**
2 aerobes	**8(57.14%)**
Staph.aureus+Klebsiella	2
Esch.coli+ Klebsiella	2
Esch.coli+Proteus	4
1 aerobe+ 1 anaerobe	**6(42.86%)**
Staph.aureus+Bacteroides	2
Esch.coli+ Bacteroides	1
Esch.coli+Peptostreptococcus	3

Out of total 134 isolates, 89 (74.17%) were only aerobes , 25 (20.83%)were only anaerobes and 6 (5%) were both aerobes and anaerobes. Of which 106 (88.33%) were monomicrobial and 14 (11.67%) were polymicrobial.

TABLE – 10

ANTIBIOTIC SENSITIVITY PATTERN OF ENTEROBACTERIACEAE (n=43)

S.No.	Antibiotic	Sensitive	Percentage
1	Ampicillin	9	20.93%
2	Co-trimoxazole	11	25.58%
3	Gentamicin	24	55.81%
4	Amikacin	36	83.72%
5	Ciprofloxacin	32	74.42%
6	Cefotaxime	29	67.44%
7	Ceftazidime	29	67.44%
8	Imipenem	43	100%

Out of 43 enterobacteriaceae 9 (20.93%) were sensitive to ampicillin, 11 (25.58%) were sensitive to cotrimoxazole, 36 (83.72%) were sensitive to amikacin, 24 (55.81%) were sensitive to gentamicin, 32 (74.42%) were sensitive to ciprofloxacin, 29 (67.44%)were sensitive to cefotaxime and ceftazidime.

TABLE - 11

PERCENTAGE OF ESBLAND NON ESBL AMONG ENTEROBACTERIACEAE (n=43)

SusceptibilityPattern	No. of cases	Percentage
ESBL	14	32.56
Non ESBL	29	67.44
Total	43	100

Among 43 enterobacteriaceae, 29 (67.44%) were non ESBL and 14 (32.56%) were ESBL producers and all (100%) were sensitive to imipenem.

TABLE - 12
ANTIBIOTIC SENSITIVITY PATTERN OF STREPTOCOCCI (n=5)

S.No.	Antibiotic	Sensitive	Percentage%
1	Penicillin	5	100%
2	Ampicillin	4	80%
3	Co-trimoxazole	3	60%
4	Erythromycin	5	100%
5	Ciprofloxacin	5	100%

Out of 5 Beta hemolytic streptococci 3 (60%) were sensitive to cotrimoxazole, 4 (80%) were sensitive to ampicillin and all (100%) were sensitive to penicillin, erythromycin and ciprofloxacin

TABLE - 13
ANTIBIOTIC SUSCEPTIBILITY PATTERN OF STAPH. AUREUS (n=55)

S.No.	Antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
1	Penicillin	2(3.63%)	-	53(96.37%)
2	Ampicillin	7(12.72%)	-	48(87.28%)
3	Cefotaxime	29(52.73%)	4(7.27%)	22(40%)
4	Amikacin	40(72.73%)	7(12.73%)	8(14.54%)
5	Erythromycin	25(45.46%)	6(10.9%)	24(43.64%)
6	Ofloxacin	43(78.19%)	-	12(21.81%)
7	Oxacillin	37(67.27%)		18(32.73%)

Out of 55 strains, 2 (3.63%) were sensitive to penicillin, 7 (12.72%) were sensitive to ampicillin, 25 (45.46%)were sensitive to erythromycin, 29 (52.73%) were sensitive to cefotaxime, 37 (67.27%) were sensitive to oxacillin,40 (72.73%) were sensitive to amikacin and 43 (78.19%)were sensitive to ofloxacin

TABLE - 14
ANTIBIOTIC SUSCEPTIBILITY PATTERN OF MRSA (n=20)

S.No.	Antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
1	Penicillin	-	-	20(100%)
2	Ampicillin	3(15%)	-	17(85%)
3	Cefotaxime	2(10%)	2(10%)	16(80%)
4	Amikacin	12(60%)	3(15%)	5(25%)
5	Erythromycin	3(15%)	1(5%)	16(80%)
6	Ciprofloxacin	14(70%)	-	6(30%)
7	Vancomycin	20(100%)	-	-
8	Teicoplanin	20(100%)	-	-

Out of 20 MRSA , all (100%) were resistant to pencillin, 85% resistant to ampicillin, 80% resistant to cefotaxime and erythromycin, 60% sensitive to amikacin, 70% sensitive to ciprofloxacin and 100% sensitive to vancomycin and teicoplanin.

TABLE - 15
Results of Methicillin resistance to Staphylococcus aureus as determined by Oxacillin(1µg) Disc Diffusion method(n=55)

S.No.	Susceptibility Pattern	No. of Cases	Percentage
1	Sensitive	37	67.27%
2	Resistant	18	32.73%
	Total	**55**	**100**

Out of 55 strains 37 (67.27%) were found to be sensitive and 18 (32.73%)were found to be resistant to oxacillin as determined by Oxacillin (1µg) Disc Diffusion method.

TABLE – 16

RESULTS OF METHICILLIN RESISTANCE TO STAPHYLOCOCCUS AUREUS AS DETERMINED BY CEFOXITINX (30µG) DISC DIFFUSION METHOD(n=55)

S.No.	Susceptibility Pattern	No. of Cases	Percentage
1	Sensitive	35	63.64%
2	Resistant	20	36.36%
	Total	**55**	**100**

Out of 55 strains 35 (63.64%) were found to be sensitive and 20 (36.36%) were found to be resistant to cefoxitin as determined by Cefoxitin (30µg) Disc Diffusion method.

TABLE - 17

RESULTS OF METHICILLIN RESISTANCE TO STAPHYLOCOCCUS AUREUS AS DETERMINED BY OXACILLIN SCREEN AGAR (n=55)

S.No.	Growth on Oxaciilin screen agar	No. of Cases	Percentage
1	Present	20	36.36%
2	Absent	35	63.64%

Out of 55 strains, 20 (36.36%) were methicillin resistant and 35 (63.64%)were methicillin susceptible as detected by oxacillin screen agar.

TABLE 18

RESULTS OF MECA GENE DETECTED BY PCR (n=55)

S.No	mecA	Total no of cases	Percentage
1	Positive	20	36.36%
2	Negative	35	63.64%

PCR analysis of mecA gene detection showed 20 (36.36%) were positive for mecA gene and 35 (63.64%) were negative .

TABLE - 19

COMPARISON OF RESULTS OF PHENOTYPING AND GENOTYPING METHODS IN DETECTION OF MRSA (n=55)

TEST METHODS	DETECTED AS MRSA	SENSITIVITY%	SPECIFICITY%
Oxacillin Disc Diffusion	18	90	100
Cefoxitin Disc Diffusion	20	100	100
Oxacillin Screen Agar	20	100	100
PCR for mecA gene	20	100	100

Oxacillin disc diffusion method was 90% sensitive and 100% specific, where as Cefoxitin Disc Diffusion , Oxacillin Screen Agar and PCR for mecA gene were 100% specific and sensitive for detection of MRSA.

DISCUSSION

Abscesses, cellulitis and necrotizing bacterial infections are seen more commonly in developing countries because of the higher incidence of malnutrition with its resultant immunosupression..Abscesses are accumulation of pus in tissue and any organism isolated from them may be of significance.

The type and location of abscess will determine the specific management of abscesses. The management will therefore differ considerably, but there are overriding principles of management for all abscess and these will include: (1)drainage of pus (2) removal of necrotic tissue and foreign material(3)correction of predisposing cause(4) amtimicrobial cover. The choice of antimicrobial will initially be empirical,with the view of the known pyogenic bacteria of most type of abscesses and the result of culture and sensitivity will indicate the exact type required.[43]

In the present study pus samples from 120 cases of abscesses were taken between age group 1 to 80 years. Maximum cases were in age group 21 to 40 years.(39.17%)(Table 1).Out of 120 cases 76(63.33%) cases were male and 44(36.67%)cases were female. In all age group the sex distribution was predominantly male. In study conducted by Christian et al,60% isolates were male and 40% were female. [15]

Maximum cases of abscesses were recorded from General Surgery department (53.34%) followed by Orthopaedics department(28.33%) (Table 2) High case turn over in surgery department occurs due to following factors like severe trauma, burns, diabetes, cancer, chemotherapy, Aids, peripheral vascular disease etc.

In this study distribution of 120 samples as per site showed 53.33% were from superficial abscess and 46.67% were from deep abscess (Table 3). Similar study done by Itzhak Brook et al[28]. showed 676 (52.16%) cases were from superficial abscesses and 620 (47.84%) cases were from deep abscesses.

Microbiological characteristics of abscesses showed in this study showed out of 120 samples,74.17% had aerobic isolates only ,20.83% had anaerobic isolates only and 5% had mixed infections.(Table 4) Santosh Saini et al[50] in his study observed 87.5%, had only aerobic bacteria isolates, 5% had anaerobes only and mixed aerobic and anaerobic bacteria were present in 7.5%

Study on isolates of 64 superficial abscesses showed , predominant aerobes were: S aureus (36 isolates), and predominant anaerobes were anaerobic Gram-positive cocci (12 isolates) (Table 5& 6**)** Study by Itzhak Brook and Sydney M. Finegold et al[20] on specimens from 209 cutaneous abscesses cultured for aerobic and anaerobic microorganisms showed predominant aerobes recovered were: S aureus (89 isolates), and predominant anaerobes recovered were anaerobic Gram-positive cocci (79 isolates) similar to this study.

Another study by Meislin et al[41] and Ghoneim et al[23] in concluded that Staphylococcus aureus was present in half or more of axillary abscesses, finger and toe paronycia ,breast abscesses. ,abscesses of the trunk, extremities, hand, buttocks and inguinal regions.

Whitehead et al[65] in his study concluded perineal abscesses involving the vulvovaginal, inguinal, scrotal, perianal, and buttock regions, by contrast, typically yield cultures containing fecal flora, such as Enterobacteriaceae, Bacteroides spp, anaerobic gram positive cocci and alpha hemolytic or nonhemolytic streptococci.[22]

Study on 56 isolates of deep abscess in this study showed predominant aerobic isolates were Enterobacteriaceae and anaerobic isolates were Bacteroides spp (Table 5&7) Similar study by Itzhak Brook et al [28] ,observed that in deep abscesses ,predominant aerobic isolates were Enterobacteriaceae and group D streptococci and predominant anaerobic isolates were Bacteroides fragilis group and Peptostreptococcus spp

Bacteroides species are significant clinical pathogens and are found in most anaerobic infections, with an associated mortality of more than 19%. The bacteria maintain a complex and generally beneficial relationship with the host when retained in the gut, but when they escape this environment they can cause significant pathology, including bacteremia and abscess formation in multiple body sites.

Intra-abdominal sepsis is the most common infection caused by Bacteroides. After disruption of the intestinal wall, rupture of the diverticula, or other perforations due to a surgical wound, malignancies, or appendicitis, members of the normal flora infiltrate the normally sterile peritoneal cavity, and the resultant infections reflect the gut flora composition. During the early, acute stage of infection (approximately 20 h), the aerobes, such as E. coli, are the most active members of infection, establishing preliminary tissue destruction and reducing the oxidation-reduction potential of the oxygenated tissue. Once sufficient oxygen has been removed to allow the anaerobic Bacteroides species to replicate, these bacteria begin to predominate during the second, chronic stage of infection[25]

In this study 44.77% isolates were gram positive and 32.09% were gram negative bacilli. (Table 8). S Mohanty et al [53] in his study observed 45.96 % were gram positive cocci and 54.04% were gram negative bacilli.

Our study showed 23.14% anaerobes from pyogenic infection .In study conducted by Reena Set et al [47] on prevalence of anaerobic bacteria in pyogenic infections showed 18.7%

anaerobes from pyogenic infection .

Overall the predominant isolates of both superficial and deep abscesses in this study group was Staphylococcus aureus around 41.04% in both superficial and deep abscesses. Santosh Saini et al [50] in his study on abscesses showed 40% isolates of Staphylococcus aureus. In another study by R.M.Banda et al [46] showed that Staphylococcus aureus was the predominant isolates 51.8% from surgical bacterial infection, of which 37.55% were from abscess .

In this study ,out of 120 culture positive cases, 106 (88.33%) cases were monomicrobial and 14(11.67%) cases were polymicrobial, in which 8 (57.14%) cases had two aerobes. (Table 9) Study by Santosh Saini et al[50] showed out of 40 culture positive cases, 26 (65%)were monomicrobial and 14(35%) were polmicrobial of which 8 (57%) cases had two aerobes similar to this study

Surgical infections, such as abscesses, secondary peritonitis, necrotising fascitis and wounds with devitalized tissues are largely polymicrobial, and the role of both aerobic and anaerobic bacteria in the pathogenesis of these infections is well recogniszed. Microbial synergy may increase the net pathogenic effect and hence the severity of infection in several ways[7].

In the present study antibiotic susceptibility pattern of enterobactericeae showed out of 43 isolates, 29 (67.44%) were sensitive to third generation cephalosporins, cefotaxime and ceftazidine and 14(32.56%) were resistant to it. All isolates were sensitive to imipenem.(Table 10)

In the present study the occurence of ESBL producing enterobacteriaceae in abscesses was 32.56%. (Table 11). Shukla et al [54]reported prevalence of ESBL in enterobactericeae was

found to be 36.1% similar to our study. All the ESBL's in the study were sensitive to imepenem (100%) which correlated with the study by Baby Padmini S.[5]

ESBL producing organisms pose a major problem for clinical therapeutics. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options.[2] Initially restricted to hospital acquired infections, they have also been isolated from infections in outpatients. Major outbreaks involving ESBL strains have been reported from all over the world, thus making them emerging pathogens

Sensitivity pattern of 5 isolates of Streptococcus pyogenes showed 100% sensitivity to penicillin, erythromycin, ciprofloxacin. (Table12).S pyogenes is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders. Although humans can be asymptomatic nasopharyngeal or perineal carriers of S pyogenes, the organism should be considered abnormal if it is detected by culture. The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration.[32] Almost all Streptococcus pyogenes are susceptible to penicillin G, and most are susceptible to erythromycin which correlates with our study.

Antibiotic susceptibility pattern of 55 isolates of Staphylococcus aureus showed , 37 (67.27%) isolates were sensitive and 18 (32.73%) were resistant to oxacillin(Table 13)

In our study resistance pattern of MRSA isolates were as follows, penicillin 100%, ampicillin 85%, cephotaxime & erythromycin 80%,ciprofloxacin 30%,amikacin 25% and all strains were 100% sensitive to vancomycin.(Table 14)

More than 80% of MRSA were found to be resistant to majority of antibiotics tested like cephalexin, ciprofloxacin, penicillin, co-trimoxazole, gentamicin, erythromycin and tetracycline and 60.5% to amikacin, no strains resistant to vancomycinin study conducted by S Anupurba et al [55].

Study conducted by Vidhani et al [63] showed similar pattern of resistance of MRSA to beta lactams like penicillin and amoxycillin was 100% while 87.4% isolates were resistant to augmentin. Cefotaxime resistance was seen in 78.5% of isolates.

In this study, oxacillin disc diffusion method detected 18(32.73%) cases of MRSA from 55 isolates with sensitivity of 90% and specificity of 100%.(Table15&19). KB Anand et al [34] in his study showed 87.5% specificity and 100%sensitivity by oxacillin disc diffusion method.

Cefoxitin disc diffusion method detected 20 (36.36%)cases out of 55 isolates as MRSA which accounts for 100% sensitivity and specificity(Table16&19)) Cefoxitin is a potent inducer of the mecA regulatory system and an accurate surrogate marker for the detection of MRSA in routine susceptibility testing for disk diffusion [18]. It is suggested that no special medium or incubation temperature is required with cefoxitin as for oxacillin.

Cefoxitin disk diffusion testing is now recommended by CLSI as a preferred method of detection of oxacillin resistance in staphylococci; however, it is important to report the findings from cefoxitin disc diffusion test as indicative of either oxacillin susceptibility or resistance; cefoxitin report should not be reported

MRSA detection by oxacillin screen agar method isolated 20(36.36%) cases with 100% sensitivity and 100% specificity.(Table 17&19)) Chambers et al reported that the sensitivity of this method approaches 100% for the detection of methicillin resistant S. aureus and 95% for the coagulase-negative strains[14]

Detection of mecA gene by PCR was positive for 20 (36.36%) cases with 100% sensitivity and specificity.(Table 18&19) mecA encodes PBP 2a (also termed PBP 29), an inducible 76-kDa PBP that determines methicillin resistance. The heterogeneous nature of methicillin resistance is an inherent limitation to the accuracy of susceptibility testing mecA detection tests based on PCR or DNA hybridization will correctly identify even the most heterogeneous of strains and should be considered the gold standard [14]for methicillin resistance.

Isolation rate of MRSA in this study was 36.36% .K Rajaduraipandi et al [33] in his study showed that 33.6% of strains were obtained from pus among clinical isolates. Similar observation was made by Mehta[40], who in his study on control of MRSA in a tertiary care center, had reported an isolation rate of 33% from pus and wound swabs.

Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice Resistance was termed "intrinsic" because it was not due to destruction of the antibiotic by beta lactamase. Methicillin resistant Staphylococcus aureus (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens[38]. Infected and colonized patients provide the primary reservoir and transmission is mainly through hospital staff [39]. The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalisation in intensive care unit.[16] .With the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many stains of MRSA exhibit resistance to both beta -lactams and aminoglycosides[59]. Hence, a knowledge of prevalence of MRSA and their antimicrobial profile becomes necessary in the selection of appropriate empirical treatment of these infections

SUMMARY

1. Among 120 cases taken for study, maximum number of cases were in age group 21 – 40 years (39.17%)

2. Sex distribution showed male preponderance (71.3%) among the 120 isolates.

3. Majority of samples were from general surgery (53.34%) followed by Orthopedics department (28.33%)

4. Among 120 cases, 64(53.33%) cases were taken from superficial abscess and 56(46.67%) cases were taken deep abscess.

5. Out of 120 pus samples, 89 (74.17%) samples had only aerobic isolates 25 (20.83%) samples had only anaerobic isolates and 6 (5%)samples had both aerobes and anaerobes..Of which 106 (88.33%) were monomicrobial and 14 (11.67%) were polymicrobial.

6. Of total 134 isolates, 44.77% and 32.09% were gram positive and gram negative aerobic isolates respectively, 14.18% and 8.96% were gram positive and gram negative anaerobic isolates respectively.

7. Study on isolates of 64 superficial abscesses showed , predominant aerobes were: S aureus (36 isolates), and predominant anaerobes were anaerobic Gram- positive cocci. Study on 56 isolates of deep abscess in this study showed predominant aerobic isolates

were Enterobacteriaceae and anaerobic isolates were Bacteroides spp

8. Out 134 isolates, 103(76.87%) were aerobic isolates, of which 60(44.77%) were gram positive cocci and 43(32.09%) were gram negative bacilli and 31(23.14%) were anaerobic isolates, of which 19(14.18%) were gram positive cocci and 12(8.96%)were gram negative bacilli.

9. Of 43 enterobacteriaceae ,14(32.56%) were ESBL and 29(67.44%) were non ESBL producer.All isolates were 100% sensitive to imipenem.

10. Isolation of Streptococcus pyogenes 5(3.73%) from 60(44.77%) aerobic gram positive cocci showed 100% sensitivity to penicillin, erythromycin and ciprofloxacin.

11. Staphylococcus aureus were predominant isolates of abscesses constituting to about 55(41.04%) ot total 134 isolates.

12. Antibiotic sensitivity result of Staphylococcus aureus by disc diffusion method with oxacillin showed ,out of 55 strains ,37(67.28%) were detected as methicillin sensitive and 18(32.72%) were detected as methicillin resistant.

13. Out of 55 isolates of Staphylococcus aureus ,35(63.64%) isolates were detected methicillin sensitive and 20(36.36%)were detected as methicillin resistant by Cefoxitin disc diffusion and Oxacillin Screen Agar method.

14. PCR analysis of mecA gene detection showed 20(36.36%) were mecA gene positive

and 35(63.64%) were mecA gene negative.

15. All isolates of Staphylococcus aureus were 100% sensitive to vancomycin and teicoplanin.

CONCLUSION

Among 120 cases of superficial and deep abscesses studied for bacteriological profile showed 134 isolates, of which 88.33% were monomicrobial and 11.67% were polymicrobial. Of 76.87% aerobic isolates, 44.77% were gram positive cocci and 32.09% were gram negative bacilli. Of 23.14% anaerobic isolates , 14.18% were gram positive cocci and 8.96%were gram negative bacilli.

Of 134 isolates, Staphylococcus aureus was predominant isolate (41.04%) from both superficial and deep abscess, of which 36.36% were detected as Methicillin resistant .MRSA emerge to be significant problematic pathogen in the surgical settting with vancomycin probably the only reliable choice of this infection. The conventional phenotypic methods ,Cefoxitin disc diffusion method and Oxacillin screen agar had high degree of sensitivity and specificity, in concordance with 'gold standard' mecA gene detection by polymerase chain reaction.

Essential infection control practices should include hand washing by hospital personnel, basic cleaning of all surface levels (hand touch sites) , increased barrier precautions and isolation of patients colonized or infected with MRSA.. A multidisciplinary approach, coordinated participation of microbiologists, clinicians, nursing personnel, hospital infection control team is necessary for management of MRSA producing infection. Continuous monitoring of antimicrobial susceptibility pattern in individual settings together with judicious use is emphasized to minimize emergence of drug resistant bacteria.

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APPENDIX

1. Peptone Water

Peptone	10 g
Sodium chloride	5g
Distilled water	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4-7.5 and filter. Distribute as required and autoclave at 121ºC for 15 minutes.

2. Nutrient Agar

Ingredients	**Grams/litre**
Peptic digest of animal tissue	5
Sodium Chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	15.00

Final pH(at 25º C) 7.4 ±0.2.

Suspend 28 gms in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 minutes and pour into sterile petridishes.

3. Blood agar

Sterile sheep blood	5 ml
Nutrient agar	100ml

Autoclave the nutrient agar base at 121°C for 15 minutes. Cool to 45-50 ºC and add blood with sterile precautions and distribute in petri dishes.

4. Mac Conkey Agar

Ingredients	Grams/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 (25º C) 7.1 ± 0.2 .

Suspend 51.53 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 min. Mix well before pouring.

5. Mueller Hinton Agar

Ingredients	**Grams/litre**
Beef Infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00

Final pH at 25º C 7.3± 0.2.

Suspend 38 gms in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilze by autoclaving at 15lbs pressure (121ºC) for 15 minutes. Mix well before pouring. Pour 20-25ml of it into petridishes of 9cm diameter to give a thickness of 4 mm.

6. GPC were identified on the following media as mentioned below:

Media	Colony morphology	Gram stain	Probable organism
Blood Agar	Beta hemolytic white to golden	GPC in clusters	Staphylococcus species
Blood Agar	Beta hemolytic colonies	GPC in chains	Beta hemolytic streptococci

7. Differentiating characters of gram negative bacilli for their identification were as follows

Organisms	Motility	Catalase	Oxidase	Indole	Methyl Red	Voges Proskauer	Citrate	TSI	Glucose	Lactose	Sucrose	Maltose	Mannitol
Eshenchia coli	+	+	–	+	+	–	–	A/A withgas	+	+	–	+	+
Klebsiella Pneumoniae	–	+	–	–	–	+	+	A/Awith gas	+	+	+	+	+
Klebsiella oxytoca	–	+	–	+	–	+	+	A/A	+	+	+	+	+
Citrobacter freundii	+	+	–	–	+	–	+	K/Awith gas & H2S	+	±	+	+	+
Citrobacter koseri	+	+	–	–	+	–	+	K/A with gas	+	±	±	+	+
Proteus vulgaris	+	+	–	+	+	–	V	K/Awith gas & H2S	+	–	–	+	–
Proteus mirabilis	+	+	–	–	+	–	V	K/Awith gas & H2S	+	–	–	–	–

8. Robertson Cooked meat medium

Ingredients	Grams/litre
Brain heart solids	*98*
Proteose peptone	20
Dextrose	**2**
Sodium Chloride	5

Final pH at 25 ºC 7.2 ±0.2.

To each test tube add 1 part of the ingredients and 3 parts of distilled water, mix thoroughly and allow to stand for 15 minutes until all particles are thoroughly wetted. Sterilize by autoclaving at 15 lbs pressure (121º C) for 15 minutes.

9. Neomycin Blood Agar

Nutrient Agar	100 ml
Sterile sheep blood	7 ml
Neomycin 1% solution	0.25 ml

Sterilize nutrient agar. Cool to 45-50ºC. Add blood and neomycin solution. Mix thoroughly and pour 25 ml per plate.

10. Bacteroides Bile Esculin Agar

Ingredients	**Grams/litre**
Casein enzymic hydrolysate	15
Peptic digest of soyabean meal	5
Sodium Chloride	5
Ox gall	20
Esculin	1
Ferric ammonium citrate	0.5
Haemin	0.01
Vit K1	0.01
Agar	15

Final pH at 25ºC 7.0 ± 0.2.

Suspend 61.51 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121º C) for 15 minutes. Cool to 45-50 ºC and aseptically add rehydrate contents of Bacteroides selective supplement(growth factor1.8g/l and gentamicin0.1g/l). Mix will and pour into sterile petridishes.

11.Grouping of anaerobes using the antibiotic identification disc pattern

GROUP	VANCOMYCIN (5µG)	KANAMYCIN (1 MG)	COLISTIN (10µG)
Gram -positive	S	V	R
Gram -negative	R	V	V
Bacteroides fragilis group	R	R	R
Bacteroides ureolyticus&GNC	R	S	S
Prevotella sp	S	R	V
Porphyromonas sp	S	R	R
Fusobacterium	R	S	S

12. Mannitol salt agar

Ingredients	Grams/litre
Beef extract	0.1
Peptone	1
NaCl	7.5
Mannitol	1
Agar	1.5
Phenol red	0.0025
Distilled water	1000

The above ingredients were dissolved in 1000ml of dissolved in distilled water, mixed thoroughly. Heated with frequent agitation and pH adjusted to 7.6. Sterilised by autoclaving and poured in plates.

13. Oxacillin screen agar

Ingredients	
Mueller Hinton Agar	100gms
NaCl	4%
Distilled water	1000ml
Oxacillin	6µg/ml

Mueller Hinton Agar was dissolved in 1 litre of distilled water and NaCl -4gms/100ml was added to it. Mixed thoroughly.Sterilised byautoclaving. Cooled to about 45-50° C. Oxacillin solution 6µg/ml was added to it and poured in plates.

14. NCCLS Table-2 Volume 20; No 1: 2000 (Zone Diameter Interpretive Standards).

S.No		Drug	Disk content. µg	Resistant mm or less	Intermediate mm	Sensitive mm or more.
1.	GNB	Ampicillin	10	13	14-16	17
		Cotrimoxazole	25	10	11-15	16
		Ciprofloxacin	5	15	16-20	21
		Cefotaxime	30	14	15-22	23
		Ceftazidime	30	14	15-17	18
		Gentamicin	10	12	13-14	15
		Amikacin	30	14	15-16	17
		Imipenem	10	13	14-15	16
2.	Staphylococci	Erythromycin	15	13	14-22	23
		Penicillin	10 U	28	-	29
		Oxacillin	1	10	11-12	13
		(CONS)		17	-	18
		Cotrimoxazole	25	10	11-15	16
		Cefotaxime	30	14	15-22	23
		Ciprofloxacin	5	15	16-20	21
		Gentamicin	10	12	13-14	15
		Amikacin	30	14	15-16	17
		Vancomycin	30	-	-	15
3.	Streptococci	Erythromycin	15	15	16-20	21
		Penicillin	10 U	19	20-27	28
		Cephalexin	30	14	15-17	18
		Cotrimoxazole	25	10	11-15	16

Controls S.aureus ATCC 25923, E. coli ATCC 25922 and P. aeruginosa ATCC 27853 was put up with each batch

15. DNA EXTRACTION
Reagents required:

a)TE buffer:

Chemicals	Stock conc	Final conc	pH
Tris HCl	10mM	1 M	8
EDTA	1 mM	0.5 M	8

b)TAE buffer- 10 X

(242 gms of Tris base

57.1 ml of glacial acetic acid

100 ml of 0.5 EDTA)

c)Sodium Dodecoyl sulphate

d)EDTA

e) 3 M Sodium acetate

f)Phenol : Chloroform (1: 1)

g) 70% and 95% Ethanol

h)Lysozyme 3 mg/ml

i)Proteinase K

PROFORMA

1.Patient's name: 2. Age/Sex:

3.Ip/Op no: 4.Occupation:

5.Address: 6.Ward:

7.Patient's consent:

8.Chief complaints:

Swelling – Onset , Duration, Progression

Associated symptoms: Pain/Discharge/Ulcer

9.Past history:

10.Personal history:

11.General Examination:

Nutrition,Anaemia,Lymphadenopathy,Edema

12.Local Examination:

Swelling- size,shape,margin,consistency,base

Discharge-colour,consistency,odour,granules,gas/crepitus

13.Diagnosis:

14.Investigations:

15.Treatment & Management:

KEY TO MASTER CHART

M	:	MALE
F	:	FEMALE
IP	:	INPATIENT
OP	:	OUTPATIENT
S	:	SUPERFICIAL
D	:	DEEP
ABD.WALL	:	ABDOMINAL WALL
STAPH. AUREUS	:	STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS	:	STREPTOCOCCUS PYOGENES
KLEBSIELLA	:	KLEBSIELLA PNEUMONIAE
PEPTOSTREP	:	PEPTOSTREPTOCOCCUS SPECIES
BACTEROIDES	:	BACTEROIDES FRAGILIS
MSSA	:	METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS
MRSA	:	METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS
R	:	RESISTANT
S	:	SENSITIVE