Prospective study of isolates in Pyogenic Samples

FULL TERM REPORT

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Degree of

MASTER OF SCIENCE
(CLINICAL MICROBIOLOGY)

By

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CERTIFICATE

This is to certify that the **Full Term Internship Training** was carried out by **Mr. Gautam Devgan** under my direct supervision. This is to further certify that this report embodies the original work carried out by the candidate himself/herself and has not been submitted elsewhere in any form or for any other degree/diploma.

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DECLARATION

I hereby declare that work embodied in this Full Term Internship Training report was carried out by me under the direct supervision of **Dr. Anania Arjuna,** Assistant Professor, Dept. of Paramedical Sciences, Lovely Professional University (Phagwara, Punjab). This work has not been submitted in part or in full in any other university for any degree or diploma.

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1. ABSTRACT

The advancement of specific automated systems in microbiology lab has increased the prognosis of treatment for various microbial diseases that were uncured during past. Various automated systems like VITEK 2, microscan make investigation criteria in simple and easy way such that microorganism specific for there biochemical tests are observed under computer operated device which makes the reporting quick and unbiased. In our medical center, we have diagnosed many patients with pyogenic infection. Our aim was to study the isolates that were isolated from consecutive pyogenic samples coming in the hospital. We performed a prospective review of the records of the 70 unselected patients coming with pyogenic infection. We proceed the samples manually along with automated systems. Initial staining and culturing was done on BA, CA, MA which were later observed twice after 24hrs and 48hrs. There was significant change in positivity of sample in case of difference in age and gender. According to the observations carried out by us that percentage of positivity increase with increase of age and with every male gender. Staphylococcus aureus was the most commonly identified organism in study of consecutive pyogenic samples. Various anti toxins, enzymes, anti immune strategies of this organism were studied that are helpful for this bug to cause disease. The evaluations also included an assessment of risk factors, antimicrobial therapy, manual diagnosis methods. The transmission of infections may be limited by universal infection-control measures, patient education, screening and decolonization of asymptomatic SA carriers in both health-care and community settings.

2. Introduction

The term pyogenic drives from the word pus or abscess.Any infection that produce pus in the tissue or organ is known as pyogenic infection. For infection to takes place we require 3 set of criteria. 1) Micro-organism that is capable of invading inside tissue and can do lysis of tissue or organ. 2) Suseptical host in which micro organism may reproduce and fullfil all its requirements of nutrition. 3) Route of exit from which organism may comes out of body to transmit into other host. (Suzanne.j.Templerpp.9-16,26)

Pyogenic infections are generelly non fatal if present in case of superficial surfaces of skin and dermis.Fewer cases like liver abscess, brain abscess are can lead to death of the patient if not being properly treated.(Johnathan Karperlowsky Ch. 47 pg 305). These kind of infections are prominently cause by Gram positive organisms mostly *Staphylococcus* spps. Now a days many Gram negative bacteria also lead to pyogenic infection. Data from National Nosocomial Infections Surveilllance system suggests that isolation of Staphylococcus aureus has been increased from 35.9% to 64.4% from pyogenic samples.(NNISS, 2007)

These kind of infections are mostly treated well by various antibiotic regimens. Before introduction of penicillin for the microbial treatment, the mortality rate of individuals caused by *S. aureus* infection was about 80%.((Deurenberg and Stobberingh, 2008). In a general population, the mean carriage rate of pyogenic infection is 37% but some subpopulations show significantly higher carriage rates e.g. patients with insulin-dependent diabetes mellitus, patients in dialysis, intravenous drug users, individuals with human immunodeficiency virus.(Regional Health Forum – Volume 15, Number 1, 2011)

3. Signs and Symptoms

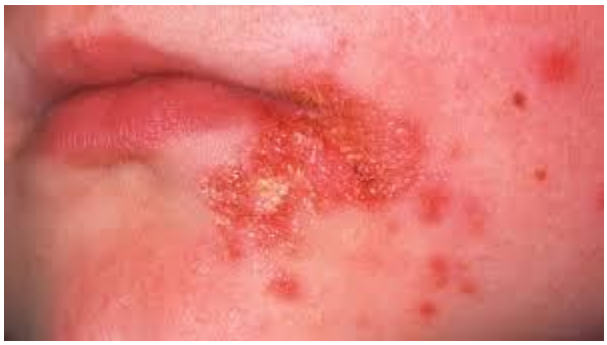
3.1 Superficial Infections

Impetigo

Impetigo is a localized skin and soft tissue infection commonly seen in pre-school-aged young childern and in patients from economically poor areas. It is more prevalent in tropical regions and occurs more frequently in the high temperature in the northern hemisphere. The spread of infection is due to person-to-person contact as well as via fomites (eg, towels, gym equipment). Clinical infection becomes apparent approximately 10 days after colonization with the implicated bacteria.(maxamo.o.britoMD,PP.9-16,26)

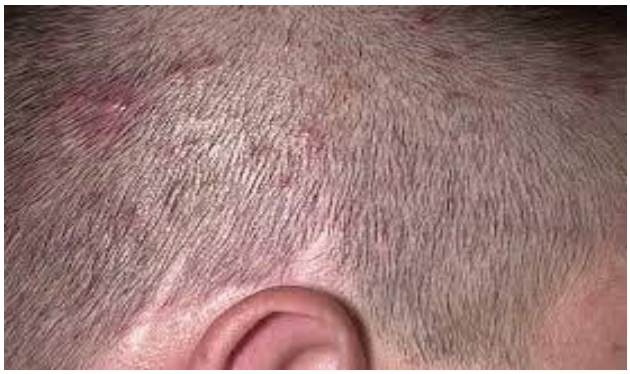
There are 2 forms of impetigo: bullous and nonbullous. Nonbullous impetigo is the most common form and is caused by *Streptococcus pyogenes* alone or as part of a mixed infection with *Staphylococcus aureus*. The common characteristic lesions of impetigo are thin-walled vesicles that rupture, leaving yellow crusts on the face and both extremities. Lymphadenopathy is a common feature of this infection. Streptococcal impetigo is usually caused by *Streptococcus pyogenes* strains.

These strains are different from those that cause tonsillitis. However, these skin strains may eventually colonize the nasal-pharyngeal cavity then leading to upper respiratory infections. Bullous impetigo is always associated with *Staphylococcus aureus*. This presentation is often localized to the trunk, and the bullae are tend to be larger and not prone to rupture.



Folliculitis

Folliculitis has many etiologies, which include commonly infectious, eosinophilic, and drug-related causes. The most common bacterial causes of folliculitis include *Staphylococcus aureus* along with *Streptococcus pyogenes*, *Pseudomonas* species, and *Proteus* species. Infection typically follows follicle damage on skin. The presentation of folliculitis depends on its severity, which ranges from superficial inflammation of an individual hair follicle to a deeper infection of the follicle (known as a furuncle) to clusters of coalescing abscesses found deeper in the subcutaneous tissues(carbuncles) that are more severe. Folliculitis is generally diagnosed clinically. Signs and symptoms of the various types of folliculitis include pruritus, papules, and pustule formation surrounding the hair follicle. Systemic signs such as fever and malaise are sometimes present and may be indicative of bacteremia in fewer cases.

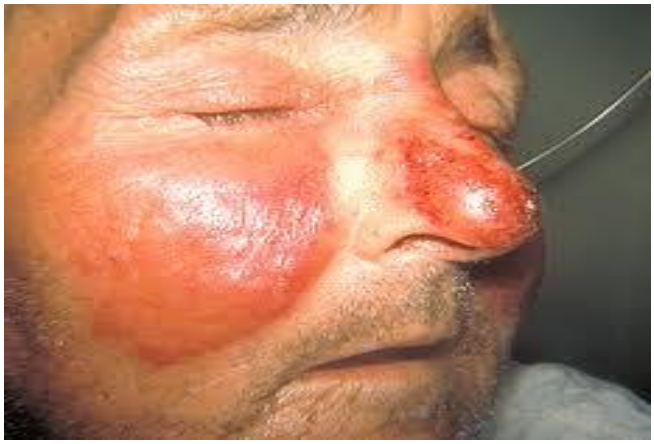


Hidradenitis Suppurativa

Hidradenitis suppurativa is caused by an obstruction in the draining ducts of the apocrine glands of the axillae and genitalia, resulting in a tender nodular dilatation of the glands both in male and female. The pooled secretions often become secondarily infected with gram-positive and gram-negative bacteria in few cases.
(eg, staphylococci, various streptococci including *Staphylococcus anginosus*, *Bacteroides* species, *Escherichia coli*, *Pseudomonas* species). Risks for developing hidradenitis suppurativa include hormonal factors and obesity. For a significant number of patients, this condition represents a chronic and difficult-to-treat problem that commonly requires multiple surgical drainages along with antibiotics. The repeated bouts of infection may cause scarring and sinus tract formation of the affected area, which complicates the long-term management of the disease and causes significant frustration and irritation to the patient.

Erysipelas

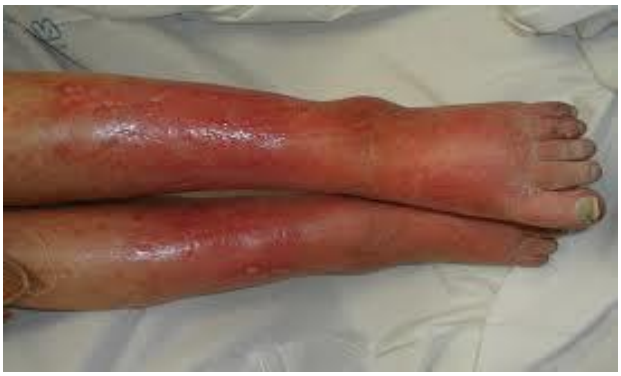
Streotococcus pyogens is the most common cause of erysipelas. This infection is characterized by acute onset of skin erythema associated with fever and lymphangitis as systemic symptoms. The classic skin lesion is raised with well-demarcated, erythematous borders and is caused by prominent lymphatic compromise of the affected area. Erysipelas most often develops on the lower extremities in both sexes. *Streptococcus pyogenes* colonization of the skin or recent oropharyngeal infection, dermatophyte infection between the toes or of the toenails (ie, tinea pedis), chronic venous stasis, and preexisting leg ulcers are all predisposing factors for developing erysipelas. Because of the superficial nature of the disease, lymphatic spread and subsequent bacteremia are rarely found, making blood culture collection unnecessary in suspected cases.



Erysiples on face of person

Cellulitis

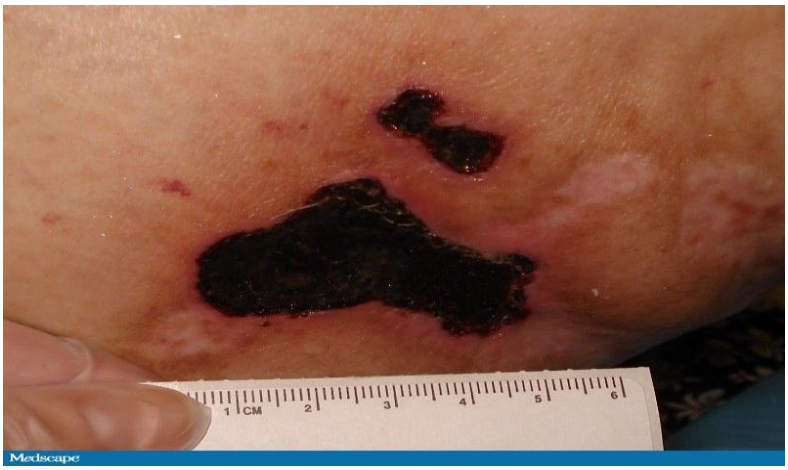
Cellulitis is a spreading infection of the epidermis and subcutaneous tissues . Staphylococcal and streptococcal species are the most frequent isolates recovered and are also the most common organisms implicated in recurrent cellulitis. Common physical findings in cellulitis include erythema, edema, warmth, and tenderness of the affected area. Patients may also experience fever, tender lymphadenopathy, and abscess formation, especially if *Staphylococcus aureus* is implicated as the causative agent. Unlike erysipelas, the involved area is poorly demarcated. In a study of US veterans, smoking, homelessness, obesity, venous stasis, and untreated tinea pedis were associated with an increased risk of developing recurrent infection. McNamara et al found that concomitant dermatitis, a history of cancer, and a first episode of cellulitis located over the tibial region were important risk factors for developing subsequent infections. Lymphedema has also been identified as a major risk factor for recurrent cellulitis. This abnormality is usually seen after surgical procedures that damage the local lymphatic drainage. These patients may have abnormal lymphatic elimination of bacteria and higher than normal bacterial counts on the surface.



Necrotizing Infections

Necrotizing fasciitis may be caused by a single organism (eg, *Streptococcus pyogenes*, *Staphylococcus aureus*) but is more commonly polymicrobial (mixed aerobic and anaerobic species). *Vibrio* species, which are associated with seawater exposure, can cause very severe infections, particularly in patients with chronic liver disease or diabetes. Other predisposing factors for necrotizing complications include varicella infection, injection drug use, penetrating injuries, burns, childbirth, recent surgery, and muscle strain. The most commonly affected sites are the extremities. Extremes of age and intravenous drug use portend a poorer prognosis.

Necrotizing infections typically progress more rapidly (within 24–48 hr) than more superficial cellulitic processes and have more devastating consequences, namely the destruction of fat, fascia, and underlying muscle. A recent study noted exceptions to this rapid course, finding that symptoms could be present for an average of 6 days prior to admission. Initial signs and symptoms are diffuse swelling of the affected area without well-demarcated borders and pain out of proportion to physical findings, followed by bullae and blisters due to tissue ischemia from locally thrombosed blood vessels. The skin turns violaceous or ecchymotic and becomes gangrenous if left untreated. The patient may develop anesthesia, as the superficial nerves in the affected area infarct. Necrosis and ischemia block antibiotic delivery to the affected area. Necrotizing fasciitis also should be suspected in the patient who appears toxic, fails to respond to antibiotics, and presents with skin necrosis. A “finger test” can be performed in cases where necrotizing fasciitis is suspected. An incision is made on the skin down to the deep fascia and a finger is used to dissect through the tissue planes. If minimal resistance is encountered or if a murky, foul-smelling fluid emanates, the test is considered positive.



Accidental wounds

Sometimes there is a accumulation of pus in the wound cause by accidental reason. For eg. Accidental cut in hands and foot while farming, while using knife in daily routine household things, road accidents, surgical cuts or injury etc.

3.2 Deep infections

Infections that spread into the internal vital organs via blood or lymph. Mostly involved organs are brain, liver, lungs.

Brain Abscess

The incidence of brain abscess depends on geographic location and living standards within a given region. The incidence is higher in underdeveloped countries where living conditions remain poor.(Ersin ErdoganMD,Nerosung,Focus24).
A brain abscess is initiated when microorganisms are introduced into cerebral tissue. Most infective agents gain access to the central nervous system either directly or via hematogenous spread. Although the source of infection is frequently apparent, the definitive cause remains obscure in 10-37 percent of patient. Approximately 80 percent of patient with a brain abscess have a known predisposing factor, while the remaining 20 percent are cryptogenic . Brain abscess is a focal suppurative process of the brain parenchyma caused by a spectrum of microbes. The finding of sterile brain abscess was a serious diagnostic dilemma to the neurosurgeon until Ingham et al (1977) introduced the routine anaerobic culture technique with anaerobic organisms as causative factors. In the pre-antibiotic era, bacteriological analysis revealed that S. aureus was causative organism in 25 to 30 percent of cases, *Streptococci* in 30 percent, coliform in 12 percent and no growth in over 50 percent .The most common anaerobic isolates from brain abscess include the *Bacteroid spp*.(S.K.das,divyaparkash,world.j.med2013)

Liver Abscess

A liver abscess is a pus-filled cavity within the liver, usually caused by a biliary tract source; occasionally, multiple cavities are seen. Origin may be pyogenic, amebic, or (rarely, and usually in severely immunocompromised patients) fungal. Clinical presentation is with fever and abdominal pain but is frequently nonspecific, without localized right upper quadrant symptoms. Computed tomography (CT), both with and without intravenous and oral contrast, and ultrasound are the imaging studies of choice. Treatment involves antimicrobial therapy with or without percutaneous or surgical drainage. Liver abscess is almost uniformly fatal if left untreated. Timely treatment reduces mortality to 5% to 30%.(M.P Sharma;Indian.j.pediatr2006)

The most common source of liver abscess is the biliary tree in patients with cholecystitis, choledocholithiasis, or cholangitis. Less common sources include other intra-abdominal processes, such as appendicitis or diverticulitis, and hematogenous spread from sources such as an infected heart valve or the oral cavity. Amebic liver abscess should be considered in endemic areas or patients who have been to the tropics.(Joseph Rahman;2004)

Lung Abscess

A lung abscess is a cavity in the lung parenchyma that contains purulent material resulting from pulmonary infection. A pulmonary abscess develops when a localised infection within the parenchyma becomes necrotic, with subsequent cavitation. Several mechanisms exist for this process.Many pathological findings leads to lung abscess for eg. Person with immuno compromised stage, person with nutritional deficiency, smoking habbits, obstructive disorders, choronic pyogenic infections in oral cavity. (johnathan karpelowsky;pg-305)

Both lung abscess and necrotizing pneumonia are manifestations of a similar pathologic process. Failure to recognize and treat lung abscess is associated with poor clinical outcome. Sample is mostly taken by aspiration technique and sputum.

Acute pyogenic discitis

Pyogenic spondylodiscitis, infection occurs primarily in the anterior corner of the vertebral body, and the infected lesion gradually extends to the adjacent vertebra via an intervertebral disc. However, we often encounter cases in which infectious changes are seen only in an intervertebral disc space without bone destruction or spur formation in the anterior corner of the vertebral body. Although an intervertebral disc is avascular in adults, such cases seem to be similar to "discitis" in infants. In these cases, the primary focus of the infection may not be an anterior corner of the vertebral body but in an intervertebral disc itself. Various factors, such as intervertebral disc degeneration, may be involved in the occurrence of hematogenous infection directly to an intervertebral disc in adults. However, the mechanisms are unclear.(Masamitsu Tanaka;Japan.2010)

Odontogenic Abscess

An odontogenic abscess is also known as dental abscess in common people language. It leads formation of pus affecting teeth or gums and sometimes surrounding alveolar bone. Dental abscess can cause throbbing pain and cause by bacterial infection. Its initiats from simple caries and then infects the soft tissue of tooth and further proceeds towards surrounding area including bone. The acute dental abscess is frequently underestimated in terms of its morbidity and mortality. The risk of potential serious consequences arising from the spread of a dental abscess is still relevant today with many hospital admissions for dental sepsis. The acute dental abscess is usually polymicrobial comprising facultative anaerobes, such as viridans group streptococci and

the Streptococcus anginosus group, with predominantly strict anaerobes, such as anaerobic cocci, Prevotella and Fusobacterium species.(D.Robertson,2009,58,155-1620)

4. Etiology

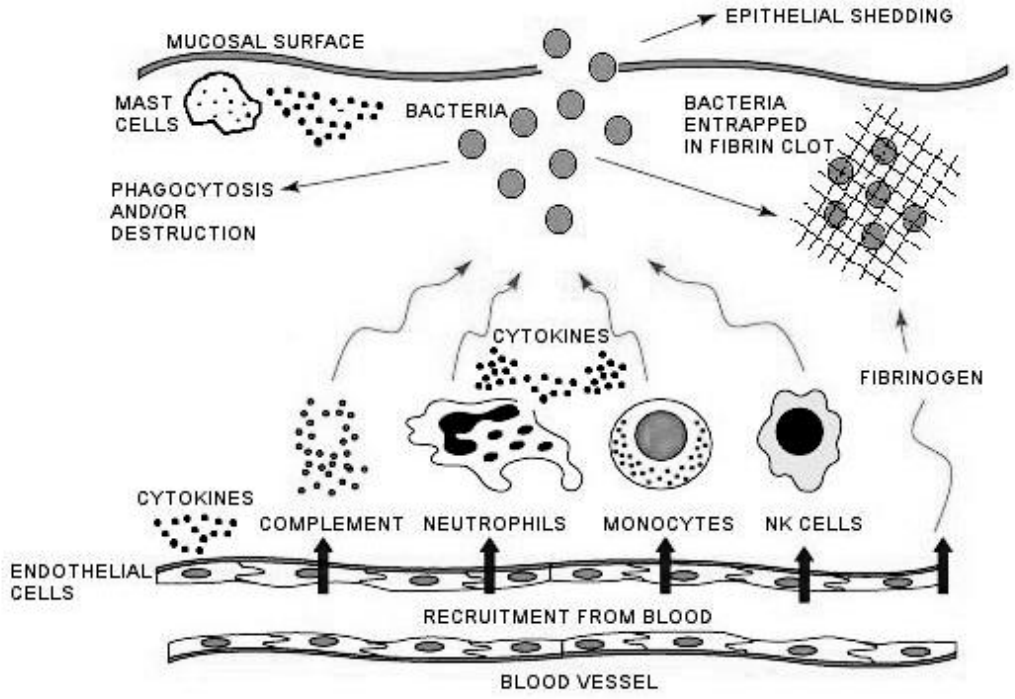
1]. Hand involvement is documented most commonly in manual workers and housewives, who, by nature of their work, frequently su¡er small abrasions or pricks [2]. Injections, whether of therapeutic or illicit drugs, are among the most important causes of superficial abscesses [3].Drug abuse is a prime cause of injection-induced abscesses in developed countries.
[4]. Abscesses are known to complicate insulin injections in diabetic patients and this has led to the use of disposable syringes for insulin administration [5]. In developing countries, abscesses are more frequently secondary to injection involving non-sterile techniques including the use of contaminated needles. Most of these are gluteal abscesses due to intra-muscular injection.
[6] Most of the gluteal abscesses are due to chloroquine injection.[7] Parasitic infections in the gastrointestinal organs may lead to abscess formation. [8] Protein calorie malnutrition. [9] Children which are deficient of compliment C1. [10] Chronic Grannulomatous Disease (CGD*)* is a rare inherited primary immunodeficiency where phagocytes cannot destroy catalase -positive bacteria and Fungi. Defect in phagocytic cells' respiratory bursts lead to life threatening infections including liver abscesses.(Srif Eldin,Ibrahim,sudan2009)

Hyper Immunoglobulin E syndrome is associated with recurrent abscesses involving several organs including liver. (M.P Sharma;Indian.j.pediatr2006)

5. Pathophysiology

Four important events occur during an inflammatory response to promote this aim; vasodilation, activation of endothelial cells, increased vascular permeability and production of chemotactic factors.
Vasodilation of the blood vessels increases the blood flow at the inflammatory site, increasing the supply of cells and other factors to the area. Activation of endothelial cells leads to increased expression of cell adhesion molecules, promoting the migration of leucocytes from blood to tissue. Increased vascular permeability makes it easier for cells and proteins to pass through the blood vessel wall and enter the tissue. Chemotactic factors are produced that attract cells into the tissue from the blood stream.(Chavakis,T,Preissner;2007)
During inflammation, mast cells release chemical factors such as histamine, bradykinin, serotonin, leukotrienes, and prostaglandins. These factors are responsible for sensitizing pain receptors, cause prolonged vasodilation of the blood vessels, and attract phagocytes, especially

neutrophils. Neutrophils will then trigger other parts of the immune system by releasing factors that recruit other leukocytes. Cytokines are produced by macrophages and other cells of the innate immune system and mediate the inflammatory response.



6. Risk Factors

Risk factors for the development of pyogenic infections include compromise of the epidermis as well as poor personal hygiene, crowding, comorbidities, and close contact with a person with an pyogenic infection. Breakdown of the epidermis serves as the entry point for infectious organisms and may be caused by ulceration, trauma, peripheral vascular disease, or preexisting skin conditions that allow bacteria to gain access to deeper tissues.(Laupland,K.B.,Church,Infect Dis 187). Skin conditions that can predispose to skin and soft tissue infections include eczema and psoriasis (which cause small fissures on the skin) and superficial fungal infections typified by tinea pedis and onychomycosis (which can cause changes in the affected skin that may lead to superimposed infection with bacteria). Venous stasis and lymphedema also can predispose patients to further deep infections. Patients with lymphedema have defective mechanisms for filtering bacteria and therefore tend to have higher microbial counts. Surgical procedures such as vein harvesting and mastectomy
with lymph node removal are also risk factors. Patients with bad oral hygiene is the most common risk factor for internal organ pyogenic infections because oral cavity provide passage

for bacteria to pass into internal organs. Good oral hygiene overcome most of the risk factors for deep pyogenic infections.(McCaigLF,McDonald,2006)

7. Diagnosis

Microscopy and culturing are the most simplest method that are carried out for the investigation of the micro organism. Samples are collected first then being cultured in the media. Obesrvation is done after 48hrs duration. Growth is examined carefully because every organism growth pattern is different from each other. Staining techniques also give revolution in the diagnosis criteria. Now adays computer operated automation system makes the isolation of microorganism quite easier then before.(olofsson,M.,Lindgren,P.E.,Scand Infect Dis44).

Most skin and soft tissue infections are diagnosed clinically. The cardinal signs of an SSTI include erythema, edema, tenderness to palpation, and increased warmth. Signs such as fluctuance, crepitus, induration, blisters, or bullae may help the clinician determine the depth of infection or the presence of an abscess. Symptoms such as fever, chills, and hypotension may be present in deeper infections.(Suzanne.J.Templer,pp.9-16,26)

A careful travel and environmental exposure history should be elucidated, as certain pathogens are associated with specific geographic locales. Examples include *Pseudomonas aeruginosa* acquired from hot tubs, *Vibrio vulnificus* and *Mycobacterium marinum* from water exposures, and *Pasteurella multocida* and *Capnocytophaga canimorsus* from animal bites. A careful history should also determine whether the patient has been recently hospitalized, as this may place the patient at risk for multidrug-resistant organisms (ie, HA-MRSA). When examining a patient with a skin infection, it is important to consider necrotizing soft tissue complications. Rapid spread, induration and crepitus of the affected tissues, fever, hypotension, and pain out of proportion to physical findings suggest necrotizing fasciitis, which should prompt surgical evaluation. Laboratory findings that may be seen in patients with necrotizing infections include leukocytosis greater than 15,000 cells/µL, haemoglobin below 11 g/dL, elevated C-reactive protein, new-onset renal failure, and hyponatremia.(Suzanne.J.Templer,pp.9-16,26).

Obtaining antibody serology for suspected streptococcal infections is generally not helpful in diagnosing superficial SSTIs, as this is a localized process and systemic antibodies are not produced. Cultures of secretions draining from abscesses and other skin lesions (eg, furuncles, carbuncles) may assist in determining the causative organism. Although culture of a bullae or purulent drainage may yield the offending organisms, empiric therapy is usually initiated before the culture results become available. Additionally, ordering cultures may not be cost effective. (Armstrong-Esther,1976,221-227)

Imaging studies should be ordered when deeper infections are suspected. Plain films may be helpful in confirming the presence of air in the tissues, and ultrasound may be used to exclude subcutaneous abscess formation and deeper infections. Computed tomography scans
and magnetic resonance imaging (MRI) may show air in the tissues or enhancement with intravenous contrast, but these signs are not specific to necrotizing SSTIs. Early surgical evaluation is required when possible signs of necrotizing infections appear on imaging. This step

allows the appropriate parties to become involved early in a patient's care. MRI can help determine the depth of infection by showing increased thickness and/or enhancement of the fascia; nonetheless, other medical conditions such as polymyositis also cause enhancement of the fascia and may be confused with infectious fasciitis. MRI has been noted, in some instances, to overestimate the depth of the infection.(Armstrong-Esther,1976,221-227)

8. TREATMENT

There are 3 treatment options for brain abscesses: 1) medical- involve treatment using antibiotics; 2) aspiration (freehand, stereotactically or endoscopically guided)- involve removal of abscess from lesion using various techniques or 3) total excision- involve complete removal of the tissue from body.(Raginee Chaudhary,Sasmita,2010(247-250)

8.1 Medical Treatment

8.1.1 General

In general, most skin and soft tissue infections can be managed on an outpatient basis, although patients with evidence of rapidly progressive infection, high fevers, or other signs of systemic inflammatory response should be monitored in the hospital setting. Superficial infections typically do not require systemic treatment and usually respond to topical agents. Impetigo is topically managed with mupirocin and fusidic acid.

Mild folliculitis may be treated with heat packs. In the case of furuncles and carbuncles, incision and drainage of abscesses is required. These procedures followed by application of heat packs are often all that is needed to resolve the infection, especially those caused by CA-MRSA. In some cases, the infection improves even when the initial oral antibiotic choice is faulty, further demonstrating that some superficial infections may resolve on their own. The reasons for this are unclear but may involve the anti-inflammatory effect of the medications.

Oral antibiotic drugs, however,may be given to patients with folliculitis following incision and drainage of an abscess when fever or extensive cellulitis is present. Superimposed infection with fungal organisms such as dermatophytes or *Pityrosporum* species also can occur in folliculitis, requiring combination antimicrobial therapy. Culture and sensitivity testing of pustular lesions should be performed to help guide oral antibiotic therapy. .(Raginee Chaudhary,Sasmita,2010(247-250)

8.1.2 Systemic Antibiotic Therapy

In addition to procedures such as incision and drainage of abscesses, antibiotic therapy is often required to eradicate SSTIs. Systemic antibiotic agents that provide coverage for both *Staphylococcus aureus* and *Streptococcus pyogenes* are most commonly used as empiric therapy for both uncomplicated and complicated deeper infections. Antibiotic resistance is a concern, given that many SSTIs are caused by MRSA and multidrug resistance is common with both CA-MRSA and HA-MRSA infections. HA-MRSA is generally susceptible to vancomycin, linezolid, and trimethoprim-sulfamethoxazole. In contrast, CA-MRSA is usually sensitive to these antibiotics (trimethoprim-sulfamethoxazole susceptibility depending on the location) as well as a broader range of oral antimicrobial agents such as clindamycin, quinolones, and tetracycline drugs.(SantniM,TolstoyISSN:2319-3832)

Clindamycin. Clindamycin is an important adjunct to therapy for SSTIs because of its ability to suppress bacterial toxin production, including streptococcal pyrogenic exotoxin A, PVL, and staphylococcal enterotoxin B

Linezolid. Much like clindamycin, linezolid suppresses bacterial toxin production and has a long postantibiotic effect. It is approved for the treatment of uncomplicated and complicated SSTI caused by *Staphylococcus aureus* and streptococci based on data from clinical trials comparing it to the β-lactam antibiotic drugs.

Fluoroquinolones. In general, *Streptococcus pyogenes* infection is susceptible to fluoroquinolones.

Corticosteroids. These drugs can also be use in case of emergency situations like in brain abscess although these have few side effects of causing vasogenic edema.

A combination of anti-staphylococcal drug like cloxacillin, an anti-anerobic and anti-amebic drug like **metronidazole** and an **aminoglycoside** or **cephalosporin** for gram negative bacilli is a good initial choice. Therapeutic drainage is not a must in all cases of pyogenic liver abscesses. However experiences with most series suggest that 80-90% pyogenic liver abscesses require some form of drainage. Medical antibiotic cover is additionally required for a period of 3-4 weeks.

95% of amebic abscesses do well on medical therapy alone and require therapy with nitroimidazoles for a total duration of ten days only. Metronidazole is the drug of choice. Oral dose is 30-50mg/kg/d, and i.v. dose is 7.5mg/Kg/dose 6^{th} hourly.(Chambers.,H.F.,Deleo;2009).

8.2 *Surgical*

In case of deep invasive infection surgical treatment may be required. It involves the surgical removal of the dead or necrosed tisse of body. It not being treated this may lead to metastatic spread of infection in whole of the body and vital organs.(Srif Eldin, A.J.Smith,Sudan,2009)

9. Review of literature

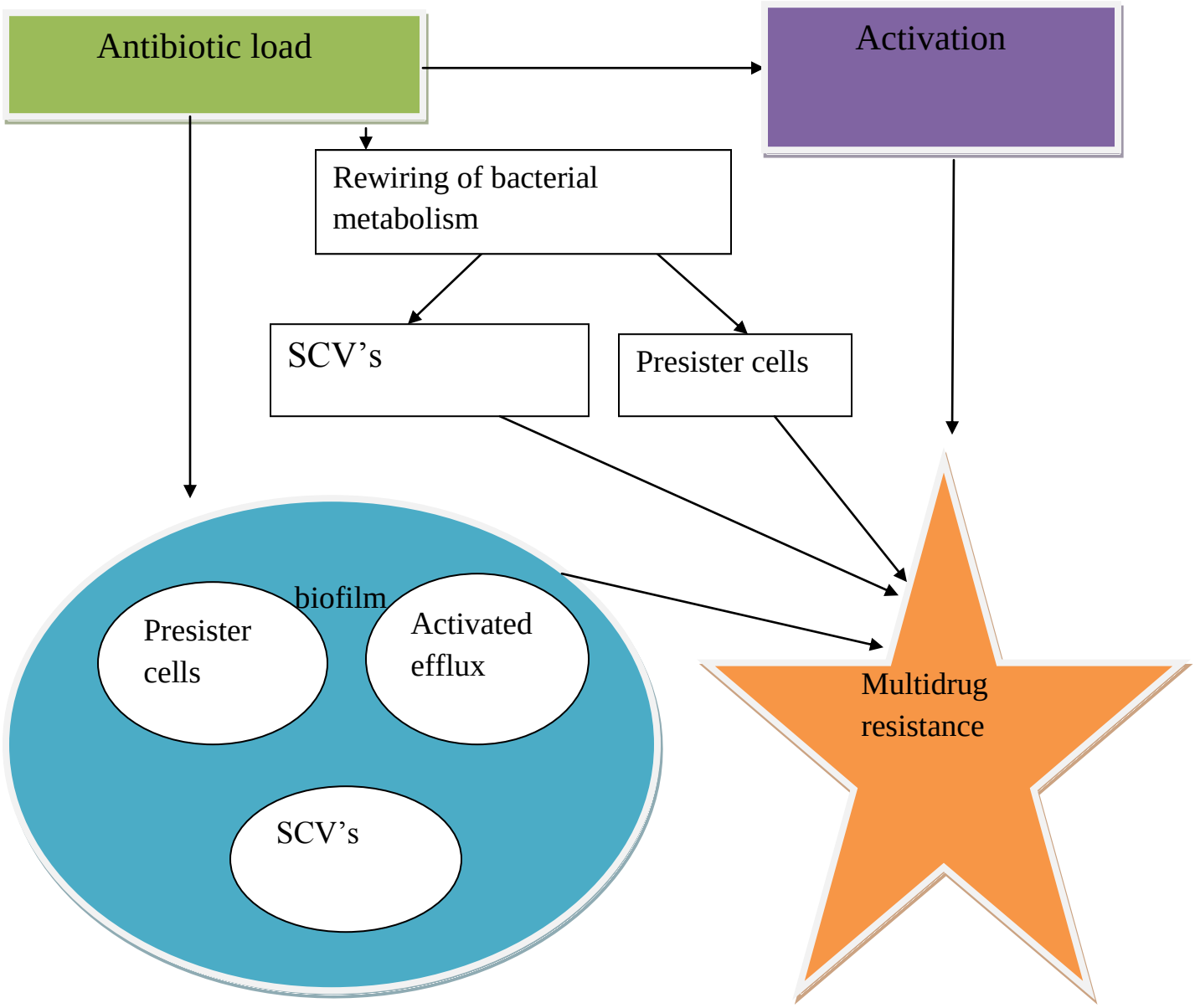
Pyogenic infections are usually caused by various micro organisms but one of the Gram positive cocci is the most commonly identified organism in pus samples that is Staphylococcus aureus. According to previous studies it is also named as most common cause for abscess formation. Although few of the deep invasive infections may cause by several Gram negative organisms too but staphylococcus spp. are the common.(HershAL,ChambersHf,2006;12:1715-23)

Staphylococcus aureus is a human commensal colonizing about 30 per cent of the population. Besides, it is a frequent cause of infections such as skin, wound and deep tissue infections and also more life-threatening conditions such as pneumonia, endocarditis and septicaemia. *S. aureus* may also cause different toxicoses. Moreover, this bacterium is one of the most common causes of nosocomial infections worldwide and an increase in antibiotic resistance, especially against methicillin, is seen. This underlines the importance to prevent and control outbreaks of *S. aureus*. The aims of this thesis were to increase the knowledge of *S. aureus* virulence and pathogenesis as well as to understand pattern of colonization and transmission.(Raginee Chaudhary,Savitri Sharma,2010). It is a highly versatile and adaptable pathogen, causing a range of infections of varying severity affecting the skin, soft tissue, respiratory system, bone, joints and endovascular tissues. The organism also exists as a commensal, colonizing the anterior nares of about one third of the healthy human population. Asymptomatic nasal carriers are at a high risk of subsequent *S. aureus* infection and are presumed to be an important source of strains that spread and cause infection in contacts. In addition, *S. aureus* represents a prototype for drug resistance, especially to β-lactam antibiotics.(Chambers,H.F,Deleo,F.R.MICROBIOL7,2009).
Although this bug has been naturally susceptible to almost every antibiotic developed so far, it frequently gains resistance by gene mutations and horizontal gene transfer, that protect the bug under antibiotic selection pressure, and has been implicated in episodes of epidemic and pandemic proportions.

9.1 Antibiotic resistance

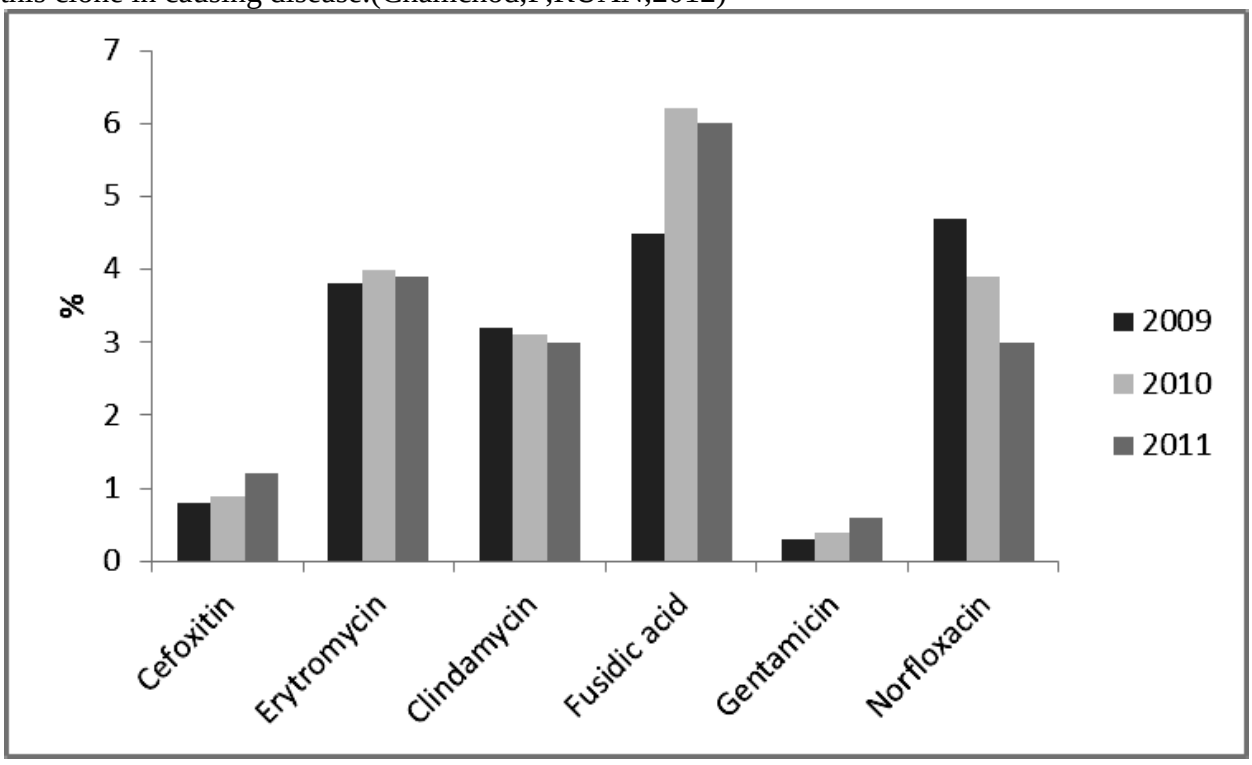
Antibiotics that are used against *Staphylococcus* spp. basically target cell wall synthesis, protein synthesis, nucleic acid synthesis and other metabolic pathways. The selection pressure applied by the antibiotics that are used in clinical and agricultural settings has promoted the evolution and spread of genes that confer resistance. Resistance to various antibiotics can be either internal or acquired by horizontal gene transfer via various mobile genetic elements like plasmids, transposons, integrons, etc. Internal mechanisms include mutational modification of gene targets, over expression of various efflux pumps; whereas acquired resistance involves enzymatic inactivation of the drug and bypassing of the target. Exposure to antibiotics may lead to the formation of persister cells, small colony variants(SCVs), biofilms and over-expression of efflux. Persisters are dormant, multidrug tolerant variants of regular cells that are formed through a combination of stochastic and deterministic events in microbial populations. Persisters over express genes such as chromosomal toxin-antitoxin modules that shut down their cellular functions, therefore, antibiotictarget inducing dormant cell to become tolerant to the lethal action

of antibiotics. Another major problem posed by persister cells is they hide at various niches evading the host immune system, such as central nervous system (*Treponema pallidum*), macrophages or granulomas (*Mycobacterium tuberculosis*), stomach (*Helicobacter pylori*), gallbladder (*Salmonella typhi*) etc.(Pallab Ray,VIkas,Reg health forum,2011)



Sub-inhibitory concentrations of antibiotics lead to formation of persister cells, small colony variants, biofilms and overexpression of efflux pumps

At first, penicillin was used to treat *S. aureus* infections. Soon afterwards, resistance emerged when strains acquired a genetic element coding for β-lactamase production, and today over 80 % of all *S. aureus* strains are resistant to penicillins. The next drug to be introduced for treating infections with *S. aureus* was the semisynthetic, penicillinase-resistant penicillin named oxacillin or methicillin, but shortly after its introduction the first isolate with resistance was detected.
With the emergence of resistance to the penicillinase-resistant penicillins, the glucopeptide agent vancomycin became the treatment of choice for infections with MRSA, and in 1996 the first isolate with intermediate vancomycin resistance was detected in Japan. So far, this has not emerged to be a major concern, but the resistance has been detected in different parts of the world and needs to be monitored. Although resistance to methicillin is considered the most important for *S. aureus*, other types of resistance exist. For example, a fusidic acid-resistant impetigo clone has caused infections around Europe. The antibiotic fusidic acid is used to treat superficial skin infections caused by *S. aureus*, which include impetigo and atopic dermatitis, and the substance has been in use since the early 1960s. Despite this, the resistance remained low until the 1990s.(Chambers,H.F,Deleo,F.R.MICROBIOL7,2009). Through the last decade an increase in prevalence of fusidic acid-resistant *S. aureus* has been seen in northern Europe, and this resistance has been primarily associated with strains causing impetigo bullosa.
The resistance is a consequence of the recruitment of the *fusB* gene. Since fusidic acid is the primary treatment for impetigo in many countries, this is likely to be the reason for the success of this clone in causing disease.(Chamchod,F,RUAN,2012)



Percentage of staphylococcus resistance for various antibiotics from 2009-2011

9.2 Bio film formation

Biofilmformationisthoughttoresultfromtheconcertedactionofprimaryattachmenttoaspecificsurface and accumulation in multilayered cell clusters. Bacteria in biofilms can tolerate ten to thousand fold higher levels of antibiotics than the genetically equivalent planktonic bacteria. Staphylococcal biofilms cause biomaterial-associated infections which do not respond to antimicrobial treatment often requiring removal of the same leading to substantial morbidity and mortality. It has also been observed that biofilms harbour persister cells and small colony variants. Whereas planktonic persisters are eliminated by the immune system *in vivo*, persisters in biofilms serve as a shield evading the immune response. According to Levin and Rozen, a reservoir of such shielded persisters is a potential source for the emergence of heritable antibiotic resistance.(Pallab Ray,VIkas,Reg health forum,2011)

9.3 Retrospective-Prospective Studies

Staphylococcus aureus belongs to the family *Micrococcaceae* and is part of the genus *Staphylococcus*, which contains more than 30 species such as *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus*. Among the staphylococcal species, *S. aureus* is by far the most virulent and pathogenic for humans. *S. aureus* is a 1 μm, Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci.
S. aureus was discovered in 1880 by the surgeon Sir Alexander Ogston. He observed grape-like clusters of bacteria when examining a purulent discharge from patients with post-operative wounds during microscopy. He named them staphylé, the Greek expression for a bunch of grapes. In 1884, Rosenbach succeeded in isolating yellow bacterial colonies from abscesses and named them *Staphylococcus aureus*, "aureus" from the Latin word for golden.(Lisa Stark:ISBN 978-91:2000)

S. aureus has the ability to adapt to different environments and it may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols. Colonization with *S. aureus* is an important risk factor for subsequent *S. aureus* infection. *S. aureus* causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicemia. This bacterium is also one of the most common species in nosocomial infections. However, little is known about the virulence factors behind all these conditions. In addition, *S. aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins.

9.4 Prevelance

In India

One of the study done in India in recent years give information about the epidemiology of MRSA infection. 1100 cases has been studied. Of 1100 MRSA infections, 131 (12%) were community-associated and 937 (85%) were health care–associated; 32 (3%) could not be classified due to lack of information. Skin and soft tissue infections were more common among community-associated cases (75%) than among health care–associated cases (37%) (odds ratio [OR], 4.25; 95% confidence interval [CI], 2.97-5.90). Although community-associated MRSA isolates were more likely to be susceptible to 4 antimicrobial classes (adjusted OR, 2.44; 95% CI, 1.35-3.86), most community-associated infections were initially treated with antimicrobials to which the isolate was nonsusceptible.(Harleen Kaur,Shashank,JKIMSU,Vol.1;2012)

In Europe

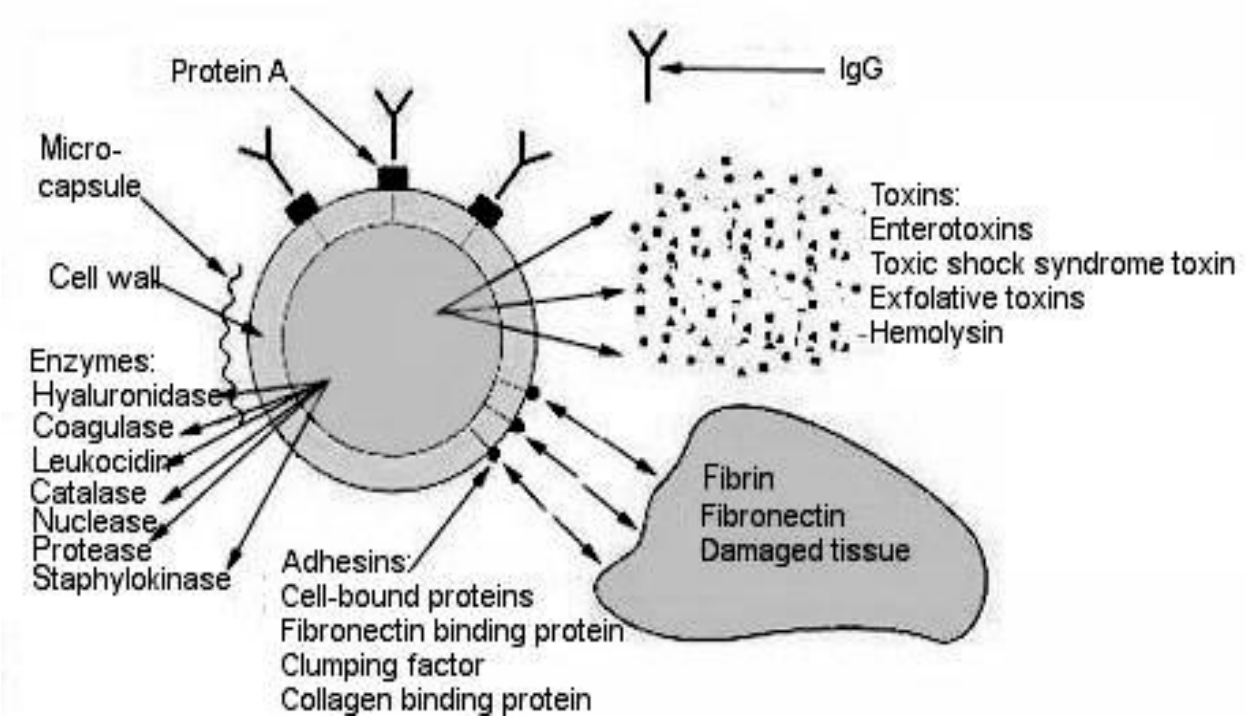
Similarly differences in prevalence between various countries have also been noted. In a recent European study, great variation in the nasal carriage rates was found, the lowest in Hungary (12 %) and the highest (29 %) in Sweden. In a Norwegian study, the same rate (29 %) in Norway as in the general Swedish population has been reported.(W.C,Valkenburg,H.A,lond65,567-573)

In Canada

In one study from Canada, the reported incidence of invasive *S. Aureus* infections was 28.4 cases /100 000 individuals and infections were more common in persons over 65 years and in males. In the United States, 0.8 % of all hospital inpatients were diagnosed with an *S. Aureus* infection and these patients had significantly longer stay in hospital, paid higher costs and had a higher risk of death than inpatients without *S. aureus* infection. In Europe the level of bacteraemia caused by methicillinsensitive *S. aureus* (MSSA) consistently increased between 2002-2008.(NoskinG.A,RubinR.J.Arch Intern Med 165).

9.5 Virulence factors and strategies

Various virulence factors contribute to the ability of *S. aureus* to cause infection; enzymes (Table 1), toxins (Table 2), adhesion proteins, cell-surface proteins, factors that help the bacteria to evade the innate immune defense, and antibiotic resistance mediate survival of the bacteria and tissue invasion at the site of infection. Moreover, certain toxins cause specific disease entities.(Chavakis,T,PREISSNER,K.T94,278-285).



A selection of *Staphylococcus aureus* virulence factors

9.6 Statergies used by Staphylococcus to survive in human body

The survival of *S. aureus* in the host is important for pathogenesis. The bacteria may be protected by a polysaccharide capsule that inhibits opsonization by complement and thereby escapes phagocytosis. It may also secrete cytolytic toxins and tissue-cleaving enzymes. Moreover, *S. aureus* may expresse a multitude of adhesion factors that mediate interactions with host cells and extracellular matrix (ECM), allowing efficient colonization. *S. Aureus* has developed strategies against the antimicrobial peptides, the complement system, and the recruitment and actions of phagocytes all of which are strategies against the innate immune response of the host.(Chavakis,T,PREISSNER,K.T94,278-285).

9.7 Enzymes that contributes for virulence

Ref. Kato,F,Kadomoto,N.,Iwamoto,Infect Immun79,1660-1670)

Virulence Factor	Enzymatic function	Effect as virulence factor in host
Catalase	Deactivates free hydrogen peroxide	Has been shown to be essential for nasal colonization
Coagulase	Binds to protrombin and thereby becomes enzymatically active	Catalyzes the conversion of fibrinogen to fibrin Coating the bacteria with fibrin and makes them Resistant to opsonization and phagocytosis
Hyaluronidase	Degrades hyaluronic acid in connective tissue Hydrolyzes the intracellular matrix of acid mucopolysaccharides in tissue and, thus may act to spread the organisms to adjacent areas in tissues	May convert local tissue into nutrients required for bacterial growth
Nuclease	Exonuclease and endonuclease activity	Contributes to evasion of neutrophil extracellular traps May degrade host tissue into nutrients required for bacterial growth

Protease	Degrades human fibronectin, fibrinogen and kininogen Cleves human α1-protease inhibitor, the heavy chain of all human immunoglobulin classes and elastin	May contribute to the ability of *S. aureus* to disseminate in host Aids in tissue invasion
Staphylokinase	Plasminogen activator that converts plasminogen to a serine protease, plasmin More than 67% of *S. aureus* strains express the gene for staphylokinase	Neutralizes the bactericidal effect by forming complex with α-defensin. May cleave complement factor C3 Controls fibrinolysis The bacteria exploit the proteolytic activity of plasmin to degrade components of ECM as well as fibrinogen for dissemination in the host

9.8 Selection of exotoxins of S. Aureus

Ref. Kato,F,Kadomoto,N.,Iwamoto,Infect Immun79,1660-1670)

Virulence Factor	Function	Virulence effect on host
Exfoliative Toxins	Glutamate-specific serine proteases that digest desmoglein 1, a keratinocyte cell-cell adhesion molecule. Exfoliative toxins (ETs) act as "molecular scissors" facilitating bacterial skin invasion Prevalence of *eta* and/or *etb* range from 0.5-3 % in MSSA but 10 % of MRSA strains have been found to be *eta* positive	The ETA and ETB are the two most important isoforms and they are associated with staphylococcal bullous impertigo and staphylococcal scalded skin syndrome ETA ETB ETC (not associated with human disease) and ETD Mediate superantigen activity
Hemolysins	Pore forming toxin with cytolytic effect on erythrocytes and monocytes (α–toxin) Cytolytic activity on cytokine containing cells (β –hemolysin also known as sphingomyelinase C) Neutrophil and monocyte binding (δ –hemolysin)	The vast majority of the hemolysins are hemolytic α-toxin has pro-inflammatory properties on host
Leukocidines	A bi-component pore-forming leukotoxin. Consists of one class S protein and one class F protein. The subunits form a ring with a central pore, through which cell contents leak Different members of the group are γ-hemolysin (hlg), Panton-Valentine leukocidin (PVL) and Leukocidins D, E, M (LukD, LukE, LukM)	Kills leukocytes PVL stimulates and lyses neutrophils and macrophages γ-toxin is hemolytic

Staphylococcal Enterotoxins	Gastroenteric toxicity; immunomodulation via superantigen activity	Causes food poisoning At least 20 serologically different staphylococcal superantigens have been described, including SEs A to V
Toxic shock syndrome toxin	Toxic for endothelium, direct and cytokinemediated Mediate superantigen activity	The toxin causes the rare condition ‘toxic shock syndrome’ (TSS) These infections are characterized by a rapid onset with high fever, rash, vomiting, diarrhea and multiorgan failure

10. Material and Methods

10.1 Collection and Transport of specimen

Collection:

There are different methods to collect pus on the basis of the diagnostic requirement. However, the most useful method is swab method. This was the initial step of diagnosis criteria.

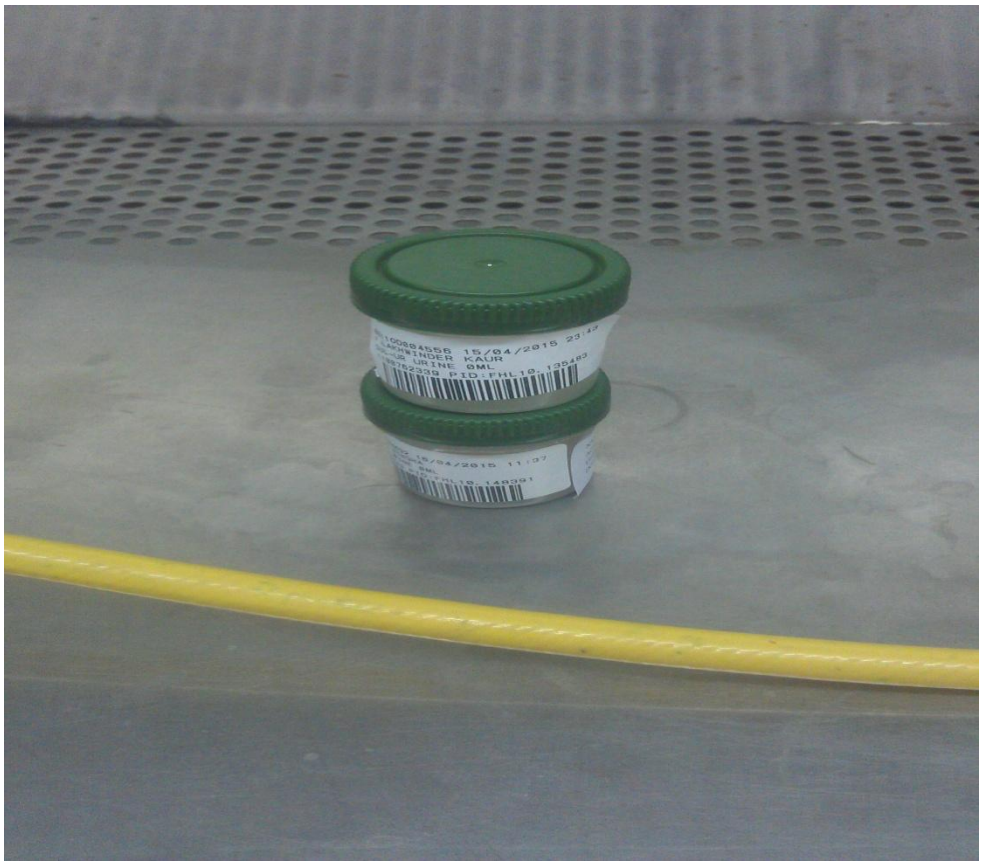
1. Swab method

We use cotton swab to collect the pus sample from the site. Sterile cotton swab taken first and then it is gently rubbed on the site. Swab is directly cultured on to media which is later incubated for 48hrs. Direct smear or staining is also done to check the Gram positive or negative character of causative organism.

Cotton swab used to collect sample

2. Aspiration

In case of deep pyogenic infection when there is no site for pus to ooze out of the tissue, then aspiration technique is used. Initially sterilization of site is done by alcohol. Puncture is done in the tissue with the help of fine bore needle. Aspiration is done slowly and sample is taken out. Then, the sample is drawn into the universal container with the help of clean needle and syringe.



Sample is carried in this container

Transport:

The sample must be transported at room temperature within half an hour. Or it should be refrigerated at 4 degree Celsius for up to four hours. The sample should not be proceeding after 4 hours for bacterial culture. Because there may be multiplication of contaminating bacteria can occur and give a false positive result.

10.2 Procedure/Method:

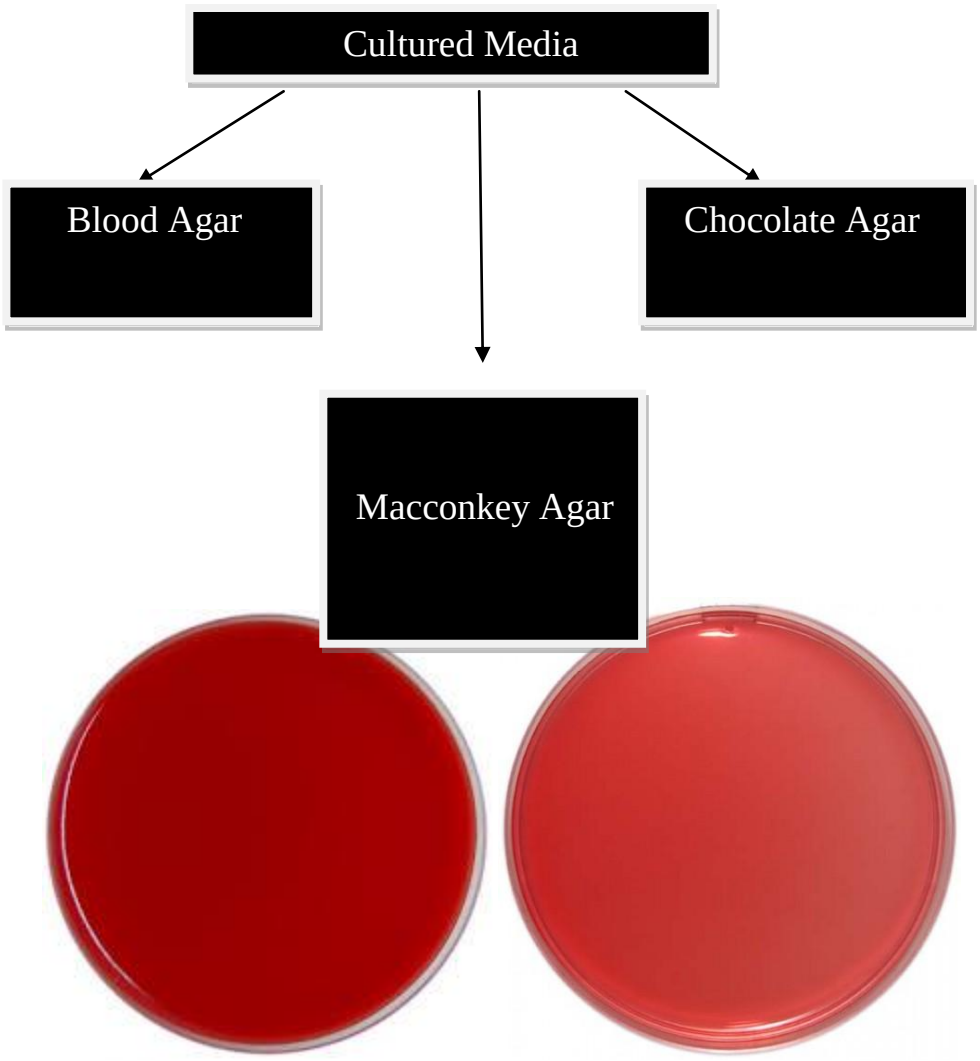
The following are the different approaches to diagnosis of pyogenic infection in the laboratory:

1. **Culture technique**:

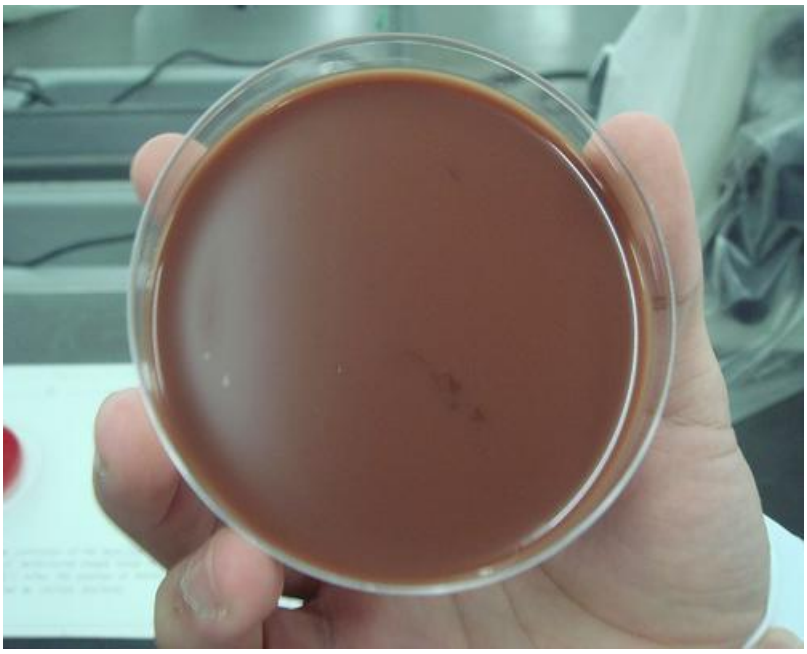
Principle- Pus is generally composed of causative micro organism, dead and decay tissue elements ,huge amount of unsurvived neutrophills and other immunogenic cells. If we provide it same environment and conditions just like in human body, the causative organism will grow on medium within 48hrs.

Procedure-

1. 100 samples of pus were collected randomly from the patients of Escort Hospital.
2. Samples were collected in sterile and screw capped containers in case of deep infections.
3. In case of superficial infection cotton swab were used for collection.
4. We use three type of culture media.
   (a) In case of swab, sample is directly rubbed over the media by streaking method.
   (b) In case of sample in container, sample is inoculated on media with the help of plastic loop having calibration of 0.5mm diameter.



Blood agar **Macconkey Agar**



Chocolate Agar

5. Plates are incubated at 37 degree C for 24 hours and colony formation units were counted for the presence of bacteria.

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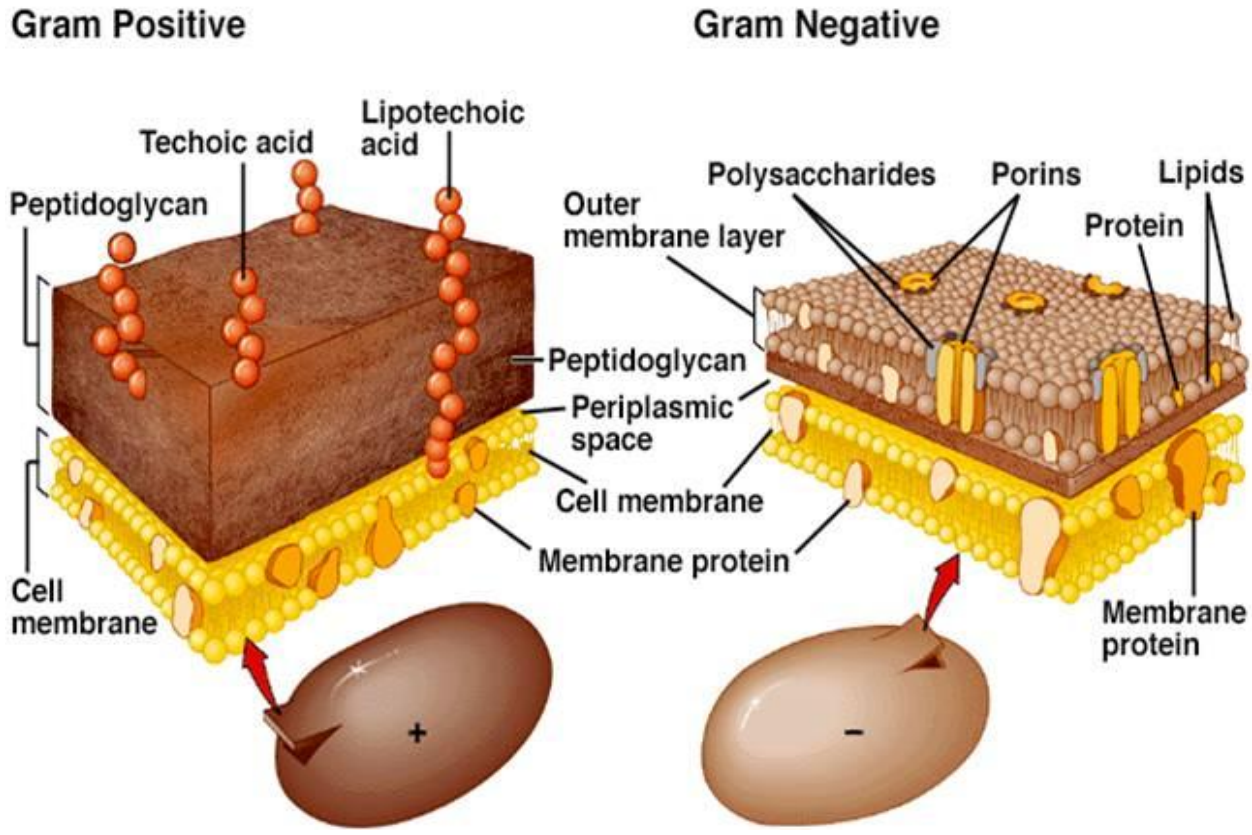
Incubator

2. Gram Stain

After growth appeared on culture media we proceed toward Gram Stain. Colony is picked from growth and we proceed for Gram stain. It is based on various theories which are discussing as follows-

1. Gram-positive bacteria have a thick peptidoglycan layer showing below in figure and these cells have more acidic protoplasm. So they will retain the primary dye and appear blue in color.
2. On the other hand, Gram negative bacteria contain lipid layers showing below in figure and these lipid layers will make the primary dye to permeable and will take the counterstain. These will appear pink in color (Ananthanarayan et al., pg 13).



Difference between Gram Positive and Gram Negative Bacteria's cell Membrane

Procedure:

Part 1: Preparation of the glass microscopic slide

Grease or oil free slides are essential for the preparation of microbial smears. We use water and soap for washing and removing oil and grease or oil on the slides. Wipe the slides with spirit or alcohol. After cleaning, dry the slides and place them on laboratory towels until ready for use.

Part 2: Labeling of the slides

Drawing a circle on the underside of the slide using a glassware-marking pen may be helpful to clearly designate the area in which you will prepare the smear. We may also label the slide with the initials of the name of the organism on the edge of the slide. Care should be taken that the label should not be in contact with the staining reagents.

Part 3: Preparation of the smear

- **Bacterial suspensions in broth:** With a sterile cooled loop, place a loopful of the broth culture on the slide. Spread by means of circular motion of the inoculating loop to about one centimeter in diameter. Excessive spreading may result in disruption of cellular arrangement. A satisfactory smear will allow examination of the typical cellular arrangement and isolated cells.
- **Bacterial plate cultures:** With a sterile cooled loop, place a drop of sterile water or saline solution on the slide. Sterilize and cool the loop again and pick up a very small sample of a bacterial colony and gently stir into the drop of water/saline on the slide to create an emulsion.
- **Swab Samples:** Roll the swab over the cleaned surface of a glass slide.

Please note: It is very important to prevent preparing thick, dense smears which contain an excess of the bacterial sample. A very thick smear diminishes the amount of light that can pass through, thus making it difficult to visualize the morphology of single cells. Smears typically require only a small amount of bacterial culture. An effective smear appears as a thin whitish layer or film after heat-fixing.

Part 4: Heat Fixing:

Heat fixing kills the bacteria in the smear, firmly adheres the smear to the slide, and allows the sample to more readily take up stains.

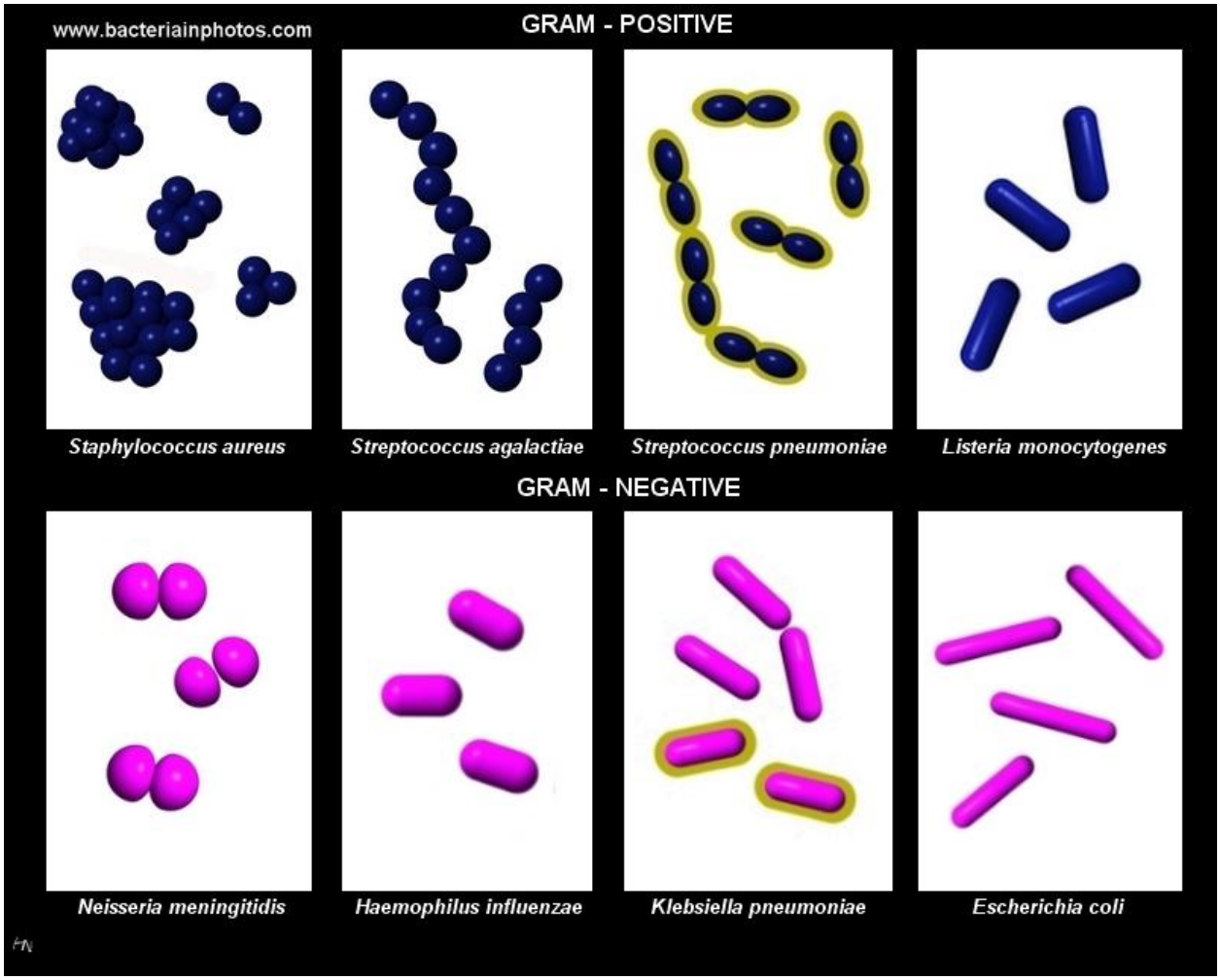
- Allow the smear to air dry.
- After the smear has air-dried, hold the slide at one end and pass the entire slide through the flame of a Bunsen burner two to three times with the smear-side up.

Now the smear is ready to be stained.

Please Note: Take care to prevent overheating the slide because proteins in the specimen can coagulate causing cellular morphology to appear distorted.

Part 5: Gram Stain Procedure

1. Place slide with heat fixed smear on staining tray.
2. Gently flood smear with crystal violet and let stand for 1 minute.
3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram's iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
6. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
7. Immediately rinse with water.
8. Gently flood with safranin to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.
11. View the smear using a light-microscope under oil-immersion.



3. Antibiotic Susceptibility Testing

When microorganism is grown on the culture media then next procedure is to check out the antibiotic susceptibility. This procedure helps us to observe that to which antibiotic the organism that is grown is resistant and susceptible. It will help physician to treat the infection in proper way. With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the

average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary.(Hemraj,Diksha,ISSN-2232,2013)

AST tells us about which of the antibiotic is susceptible and resistant to micro organism. This approach will help the physician to treat the infection more efficiently.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

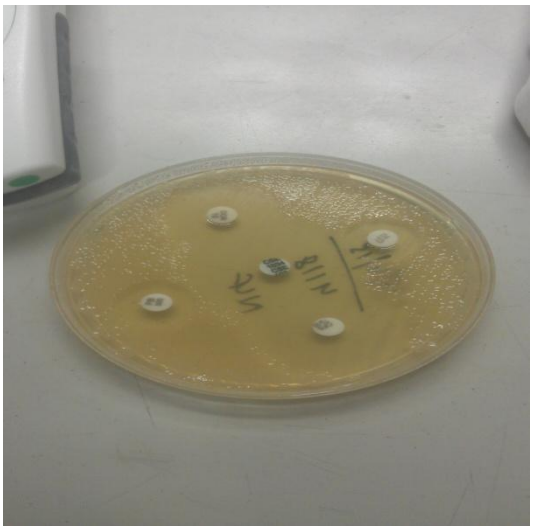
Diffusion	**Dilution**	**Diffusion&Dilution**
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)Agar Dilution	

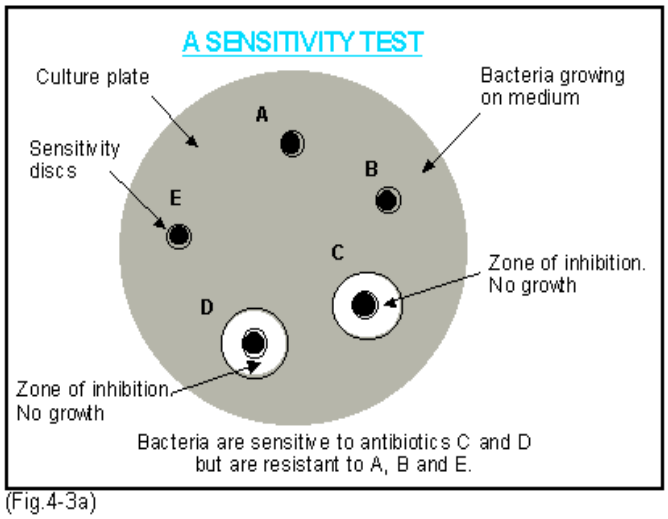
In case of manual AST, only disc duffusion methods are performed in Escorts Hospital Amritsar.

Kirby- Bauer method:

This method is a common method and the procedure is discussing in the following ways-

1. This test is done on the Mueller-Hinton agar.
2. A suitable dilution of pure culture will be prepared by taking 4-5 colonies in the normal saline.
3. Then the suspension of the test bacterium will be inoculated on the Mueller-Hinton agar plates as a lawn by using sterile cotton swabs.(Lawn Culture Method)
4. Let the plates dry for 30 minutes at 37 degree C.
5. Following that an antibiotics discs will be applied on the surface with the help of sterile forceps.
6. Keep the plates for overnight for incubation at 37 degree C.
7. The degree of sensitivity is determined by measuring the zones of inhibition of growth around the discs as showing in the following figure.
8. The growth can be only inhibited where the bacterium has shown the susceptibility to the antibiotics and it will show resistant around no inhibition (Ananthanarayan et al., pg 635).

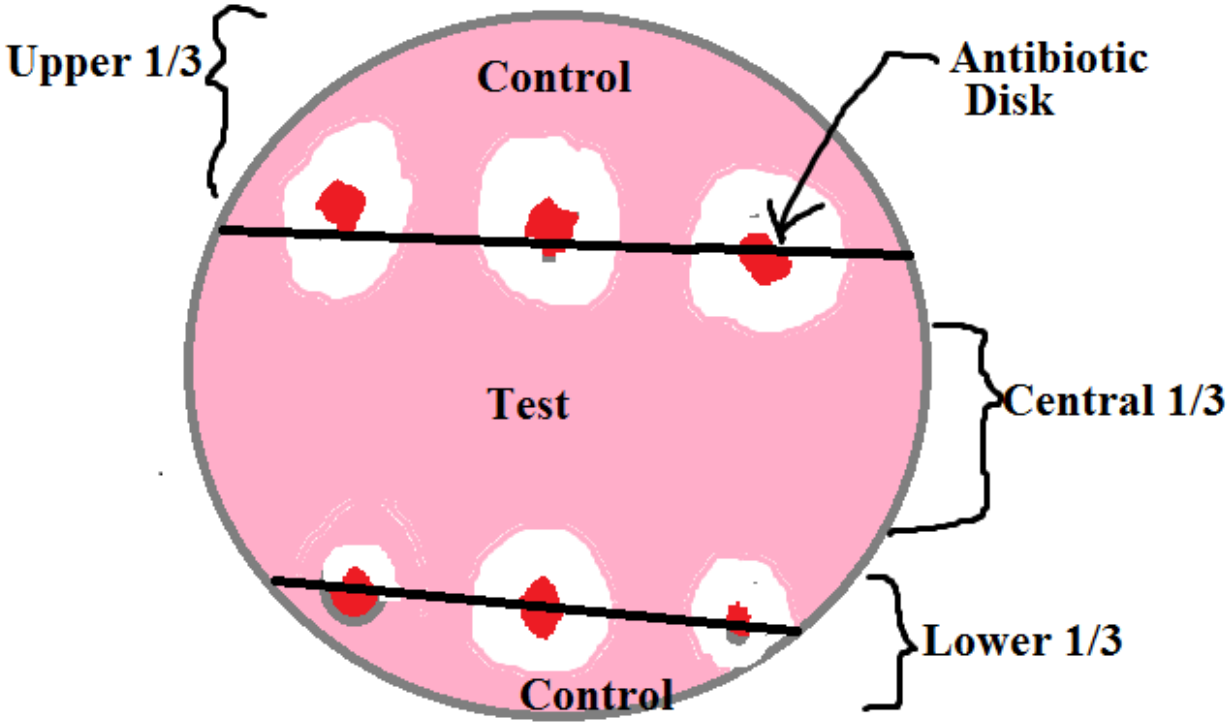




(Fig.4-3a)

Strokes disk diffusion method

In stroke disk diffusion method the plate is divided in three parts. The test organism is inoculated at central one third and control on upper and lower third of the plate. However in the modified strokes disk diffusion method, the test organism is inoculated in upper and lower third and control on the central one third.(Hemraj,Diksha,ISSN-2232,2013)[31]



4. VITEK 2 Compact System

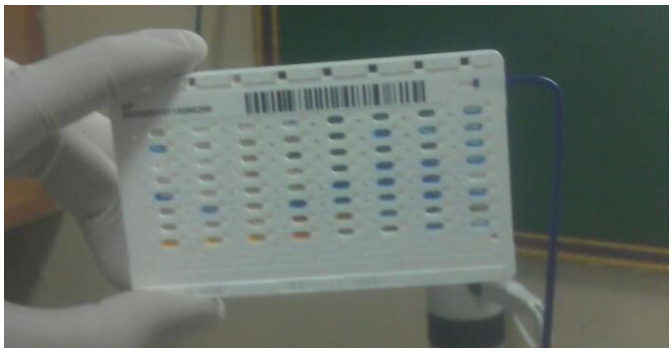
Along with manual sensitivity testing we use advance computer operated device to identify the oraganism on the basis of AST and biochemical tests.

Principle-

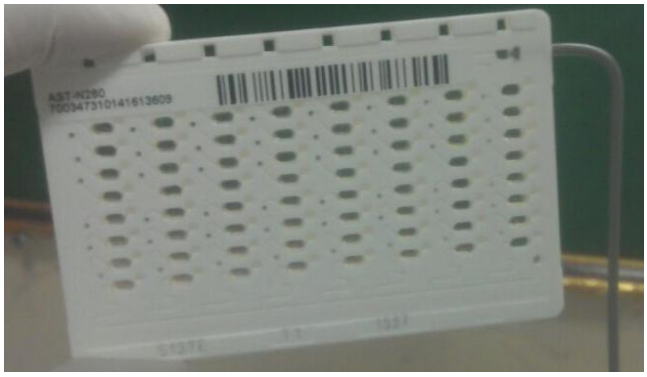
The VITEK 2 is an automated microbiology system utilizing growth- based technology. The system is based on turbidity and colorimetric reagent cards that are incubated and interpreted results (Sukhjinder, Scientific Officer). There are substrates on the tray which utilizes the enzymes and result in changing the color on reaction. Turbidity is based on the identification on the basis of susceptibility and the colorimetric is based on biochemical to detect the spore-forming Gram- positive bacilli.

Reagent Cards

There are 64 wells in reagent card and each well contains an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis and growth in the presence of inhibitory substances. The card has an optically clear film on both sides that maintains the level of oxygen transmission and prevents the contact with the organism-substrate admixtures. Each card has different bar codes that contain information on product type, lot number, expiration date, and a unique identifier (Pincus, bioMerieux, Inc).[34]



VITEK 2 GP COLORIMETRIC Identification Card.



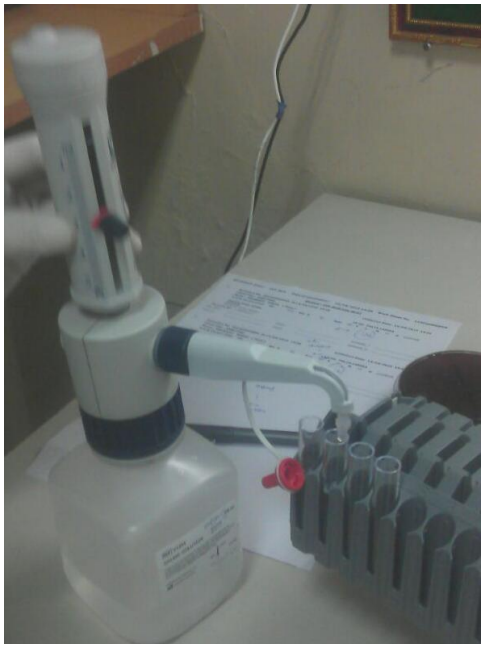
VITEK 2 N280 Turbidity Identification Card.

There are different panels available for the identification of different organism as follows:

1. GN- Gram- negative fermenting and non-fermenting bacilli
2. GP- Gram-positive cocci and non-spore-forming bacilli
3. YST- Yeasts and Yeast- like organisms
4. BCL- Gram-positive spore- forming bacilli
5. N280- Lactose fermenter
6. N281- Non Lactose fermenter
7. P628- *Staphylococcus* and *Enterococcus*
8. ST01- *Streptococcus*

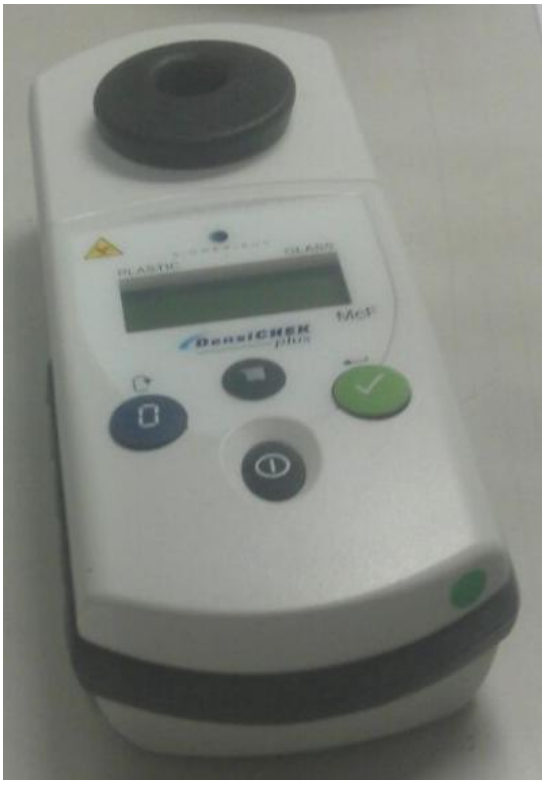
Procedure

1. A flexiloop is used to pick 4-5 isolated colonies and then emulsified in 3.0 ml of normal saline (pH 5.0-7.2) showing in the following figure-



Dispensette that contains normal saline.

2. All the test tubes (polystyrene) should be arranged in Cassette, a special rack to hold the test tubes.
3. The turbidity is adjusted accordingly (Table 1) and can be measured with the help of a turbidity meter known as the DensiChek as shown in the figure-



DensiChek used for measuring the turbidities.

Table 1: Suspension Turbidities Used for Card Inoculation.

Product	*Turbidity Range*
GN	*0.53-0.63*
GP	*0.53-0.63*
YST	*2.0-2.20*

4. Followed this, identification cards are going to be inoculated with microorganism suspensions as shown below-

cards inoculation for identifying the microorganism.

5. Then load the cards and suspension tubes into the Automated Transport system.
6. Take the reading following the day. A VITEK 2 Compact takes about 8-12 hours to indentify the organism and the susceptibility test (Sukhjinder, SRL)

5. Biochemical tests :

Last procedure to identify the microorganism manually is to perform biochemical tests. We proceed for biochemicals that were given under lab SOP's in the terminating step. Every organism has its definite biochemical test by which it is identified.

Biochemical tests for Gram positive bacteria:

Catalase Test

This test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result.

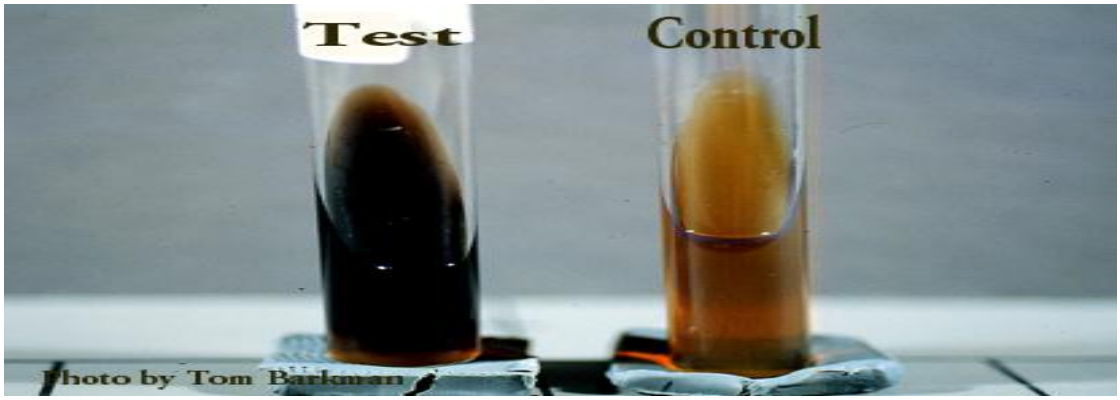
Catalase test

Coagulase test

Coagulase is an enzyme that clots blood plasma. This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus.* Coagulase is a virulence factor of *S. aureus.* The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis.

Bile Esculin Agar

This is a medium that is both selective and differential. It tests the ability of organisms to hydrolyze esculin in the presence of bile. It is commonly used to identify members of the genus *Enterococcus* (*E faecalis* and *E. faecium*).



Biochemical for Gram negative bacteria:

Oxidase Test:

Used to demonstrate the ability of a bacterium to produce the enzyme cytochrome- c oxidase, capable of reducing oxygen. Only Aerobic bacteria have this enzyme. This test will distinguish Aerobic vs. Anaerobic metabolism. A positive test will show a color change to blue, then to dark purple or black, within 10 to 30 seconds.

Urease test:

This demonstrates the ability of a bacterium to produce the enzyme urease, capable of hydrolyzing urea. Phenol red indicator is added(fuchsia above pH 8.4) to show rise in pH due to accumulation of ammonia.

Pink for positive urease test

TSI (Triple sugar iron):

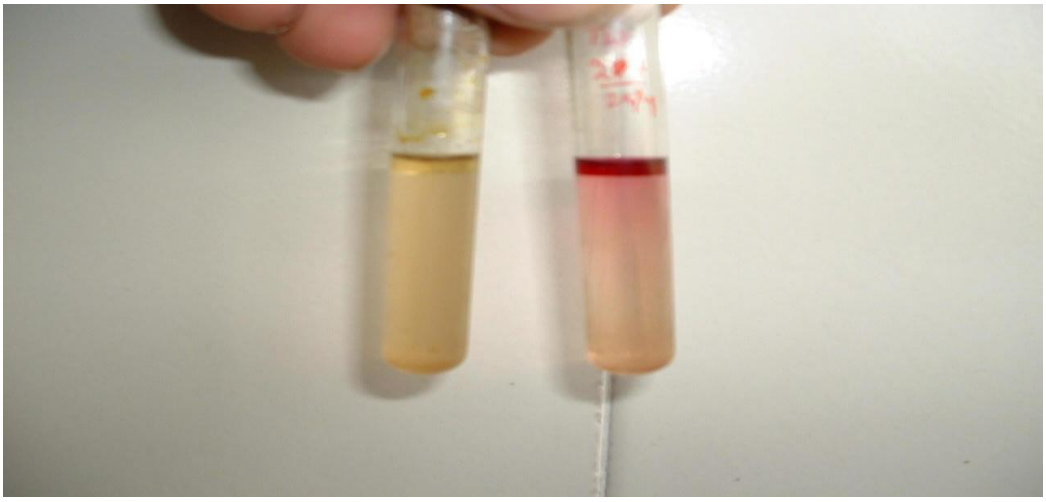
Capability of bacteria to ferment the sugars. Three set of sugars are present in the test tube i.e glucose, lactose, sucrose. Change in colour is observed in the tube along with production of H_2S gas.

IMViC:

A battery of biochemical tests known as IMViC are used in the clinical lab to distinguish between enteric microorganisms. The acronym IMViC stands for indole, methyl red, Voges-Proskauer and citrate.

Indole test ("I"):

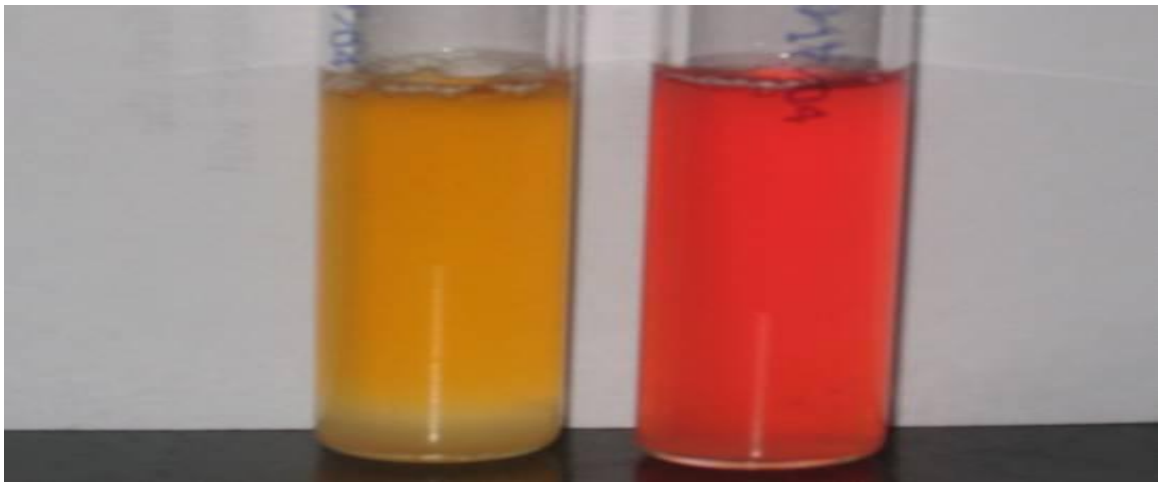
Used to demonstrate the ability of a bacterium to produce the enzyme tryptophanase. This enzyme acts on the amino acid to produce "indole"



Ring formation shows positive test

Methyl Red ("M") :

An indicator of low pH (red below pH of 4.4) – used to show the mixed acid fermentation ability of bacteria.

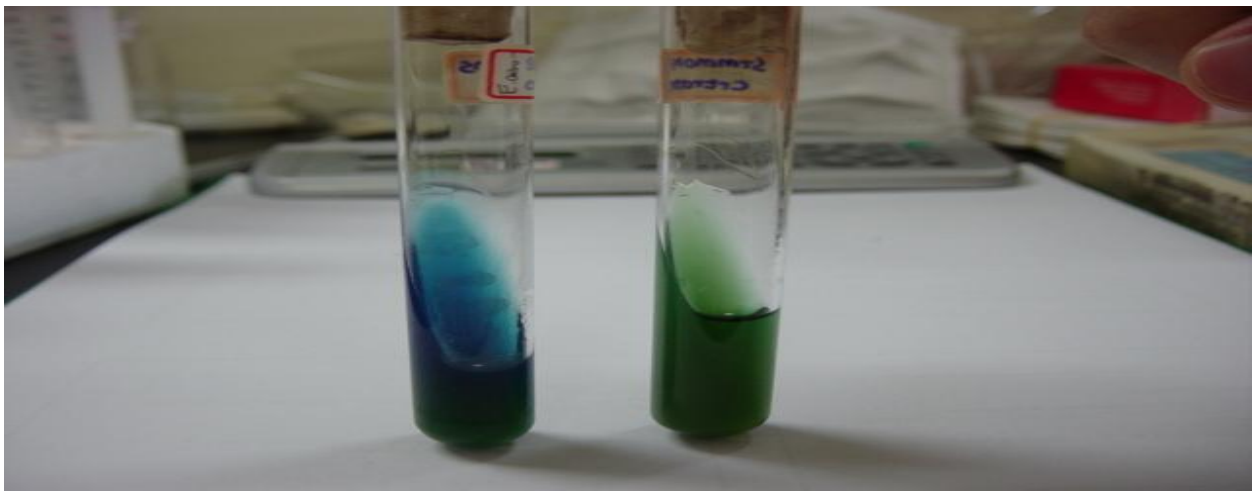


VP -Voges-Proskauer Test (“Vi”) :

used to show bacterial production of acetoin, also known as 2,3-butanediol.

Simmons citrate slant (“C”):

Simmons citrate agar tests for the ability of a gram-negative organism to import citrate for use as the sole carbon and energy source. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium.



Blue for positive test

Motility test:

To differentiate between motile and non motile organisms.

Phenylanaline test:

Phenylalanine deaminase medium tests the ability of an organism to produce the enzyme deaminase. This enzyme removes the amine group from the amino acid phenylalanine and releases the amine group as free ammonia. As a result of this reaction, phenylpyruvic acid is also produced.

11. Observations

In the training period of four months, about 63 samples are processed. Out of which 45 are found positive. Here is the observations that are carried out in training period. Theses observations are carried out manually and by one of the most advanced automated system in microbiology branch i.**e VITEK 2**. Detail of the isolations that were observed in pus samples is given below:

Acc. No./ID	Age/sex	Ward	Type of organism	No of sensitivity
0326	62/M	OT	Sterile	00
0677	71/M	IPD	S. aureus	01
0690	31/F	OT	E. faecalis	01
0685	52/F	CC	S. aureus	01
0995	73/M	ICU	A.baumanii	01
1255	50/M	OT	E. coli	01
1304	59/M	IPD	E. coli	01
1324	23/M	CC	S. aureus	01
1730	70/F	OT	K. pnemonie P. aeroginosa	02
2086	68/F	OT	E. coli E. facalis	02
2397	68/F	OT	E. coli	01
2449	68/F	ICU	Sterile	00
2813	58/M	OT	Sterile	00
3066	65/M	ICU	Sterile	00
3088	68/F	ICU	K. pnemonie A. baumanii	02
3089	65/F	ICU	A.baumanii Proteus mirabilis	02
3098	37/M	CC	A.baumanii P. aerogenosa	02
3251	63/M	IPD	E. coli	01
3907	75/M	CC	S. aureus	01
4349	23/F	CC	S. aureus	01
4880	58/M	OPD	Sterile	00
4992	60/F	OPD	S. aureus	01
5872	41/M	CC	S. aureus	01
6080	55/F	CC	Sterile	00
6092	55/F	ICU	Sterile	00
6818	73/M	OPD	S. aureus	01
6948	58/M	OPD	Sterile	00
6834	68/M	ICU	Sterile	00
7017	49/F	IPD	P. aeroginosa	01
7516	62/F	IPD	K. pnemonie P. aeroginosa	02
7634	70/F	OT	S. aureus	01

Acc. No/ ID	Age/sex	Ward	Type of organism	No. of sensitivity
0891	49/F	CC	P. aeroginosa	01
0943	62/F	IPD	K. pnemonie	02

			P. aeroginosa	
1359	65/F	CC	Sterile	00
2163	40/M	CC	S. aureus P. aeroginosa E. coli	03
2260	68/M	ICU	P. aeroginosa	01
2724	29/F	OPD	Sterile	00
2681	71/M	IPD	E. faecalis	01
2956	62/M	IPD	S.aureus	01
4422	21/M	CC	S.aureus	01
5048	62/F	OPD	S. aureus	01
5699	72/F	ICU	Sterile	00
5769	65/M	ICU	P. aeroginosa	01
6139	52/F	CC	E.coli S.aureus	02

Acc. No./ ID	Age/ sex	Ward	Type of organism	No. of sensitivity
0419	-	OT	K. pnemonie A. baumanii	02
0448	-	OPD	S. aureus	01
0677	-	IPD	Sterile	00
1358	-	ICU	Sterile	00
1438	-	OPD	Proteus	01
1413	-	OPD	S. aureus	01
2297	-	ICU	E. coli	01
2332	-	OT	K. pnemonie A. baumanii	02
2966	-	OT	Sterile	00
3333	-	CC	E. coli	01
3930	-	-	S. viridians	01
3961	-	CC	K. pnemonie	01
4471	-	OPD	S. aureus	01
5032	-	IPD	Proteus	01
5102	-	CC	S. aureus	01
5114	-	OPD	E.coli	01
5653	-	OT	S. aureus	01
6071	-	OPD	Sterile	00
6552	-	CC	Sterile	00

11.1 Colony Characters and biochemicals of commonly identified micro organisms :

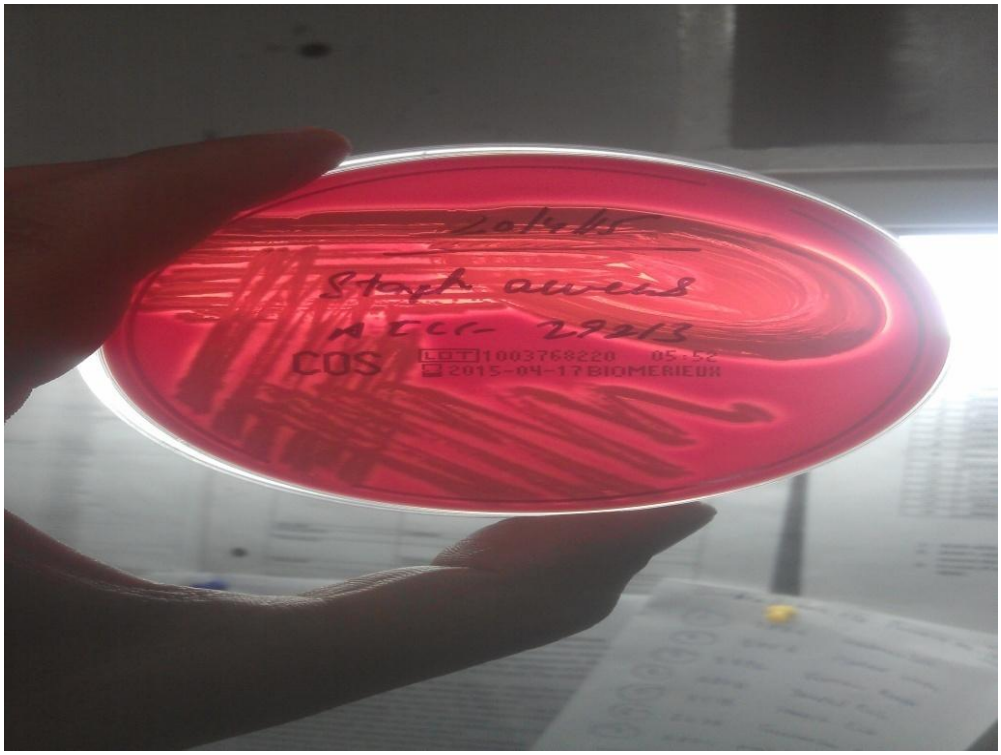
Staphylococcus aureus :

Colony characters:

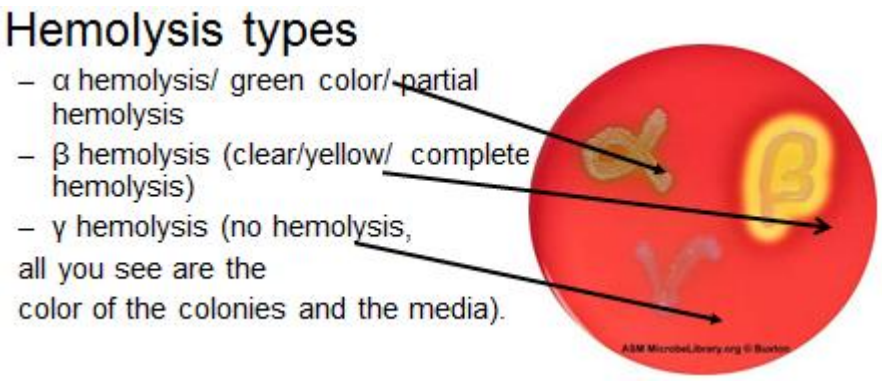
- Individual colonies on agar are round, convex, pin head and 1-4 mm in diameter with a sharp border and golden yellow or creamy in colour.
- On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of **clear beta-hemolysis**.

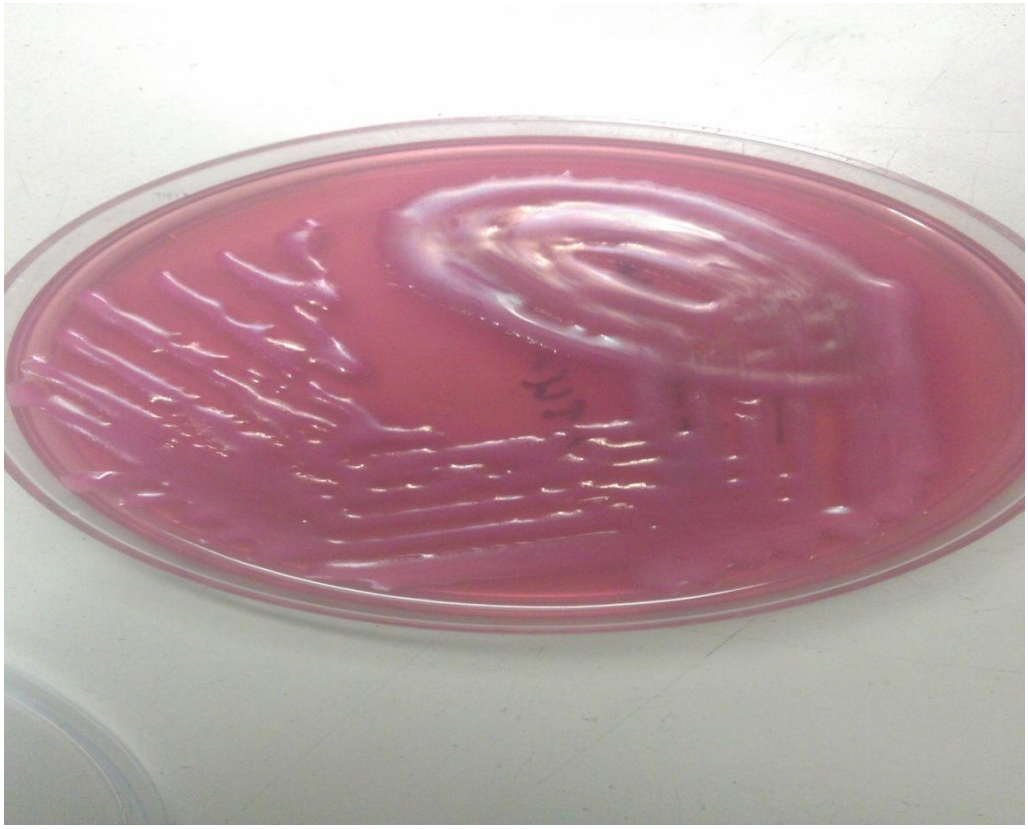
Biochemical tests:

- Catalase positive
- Coagulase positive



Clear haemolysis is seen on the blood agar produced by Staphylococcus aureus





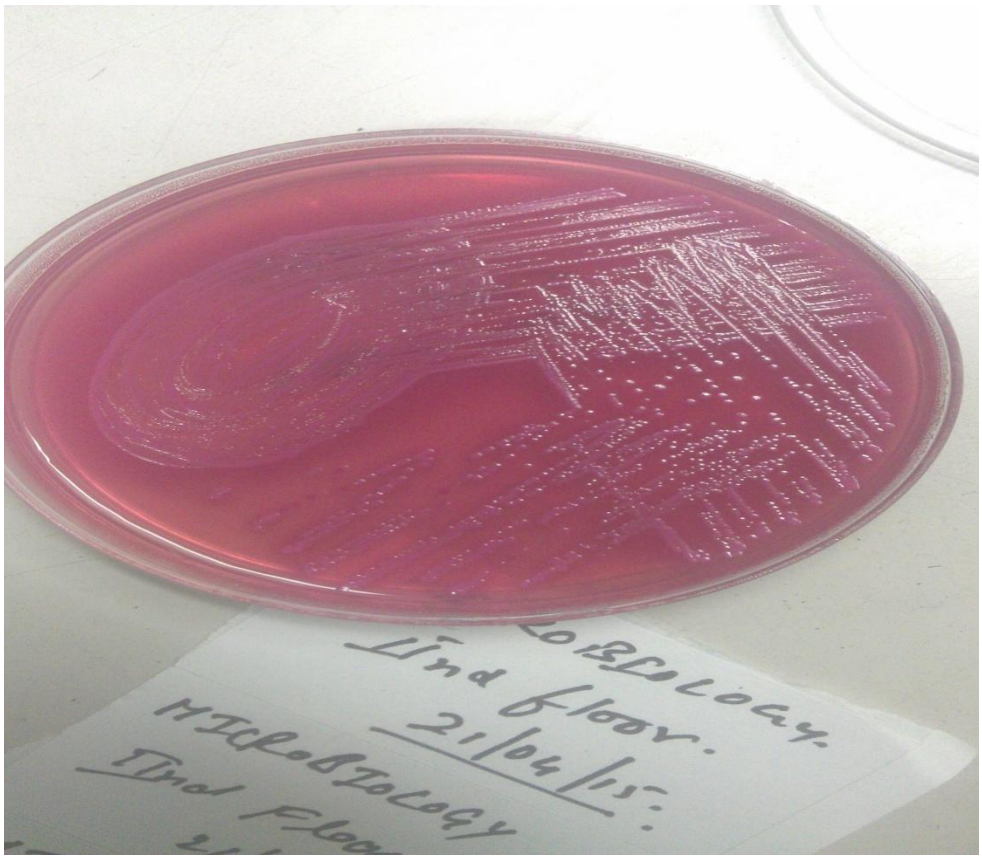
Klebsiella pnemonie :

Colony characters:

Lactose fermenting, pink coloured, mucoid colonies due to production of large number of capsular material.

Biochemical tests:

Indole	Negetive
Methyl red	Negative
VP	Positive
Citrate	Positive
Oxidase	Negative
Urease	Positive
TSI	A/A gas +ve



E. coli :

Colony characters:

Circular, small, punctiform, smooth, raised margins, pink coloured, lactose fermenting colonies.

Biochemical test:

Indole	Positive
Methyl red	Positive
VP	Negative
Citrate	Negative
Urease	Negative
TSI	A/A gas +ve

Pseudomonas :

Colony characters:

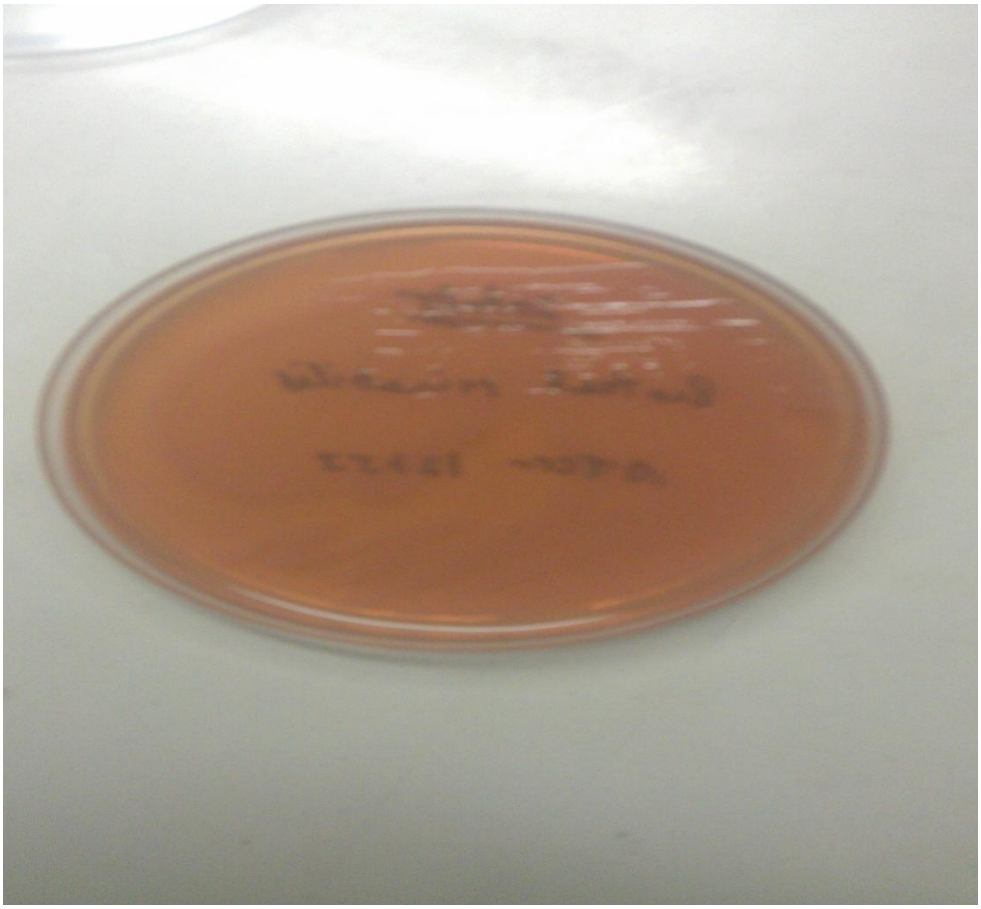
Small, rough colony, surface is wrinkled, translucent, pigmented, non lactose fermented

- Pyocyanin
  - *Bluish green phenazine pigment*
- Pyoverdin(fluorescin)
  - *It is a greenish yellow pigment*
- Pyorubin
  - *Reddish brown pigment*
- pyomelanin

- *Brown to black pigment*

Biochemical tests:

Indole	Negative
Methyl red	Negative
VP	Negative
Citrate	Positive
Urease	Negative
Oxidase	Positive
TSI	K/K gas –ve



Proteus :

Colony characters:

Small, opaque, non lactose fermenting, having typical swarming growth pattern due to presence of multiflagella and produce very distinct fishy smell

Biochemical tests:

Indole	Positive
Methyl red	Positive
VP	Negative
Citrate	Negative
Urease	Positive
Oxidase	Negative
TSI	A/A gas +ve

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12. Results :

Parameters	January	February	March
Total no. of samples	31	12	20
Sterile samples	09	03	06
Positive samples	22	09	14
Staphylococcus	10	05	05
Klebsiella	03	01	02
E. coli	05	01	03
Pseudomonas	04	05	00
Enterococcus	02	01	00

Total number of samples	63
Total number of positive samples	45
Number of most commonly identified bacteria	20

Total number of positive samples taken from inside the hospital	26
Total number of positive samples coming from outside the hospital	19

Total number of patients above age 50	< 30
Total number of patients above below age 50	>15

13. Discussion:

Staphylococcus aureus has emerged as the most common identifiable cause of skin and soft – tissue infections including deep lesions in several areas of Punjab. As around 50..0% patient with pyogenic infections associated with Staph. in this study received empirical therapy. Rapid and automated technology for isolation of bacteria has become a routine practice in resource limited settings for initial screening.

The objective of this study was to gain prospective knowledge about the pyogenic infections, its causative agents, pathogenesis, treatment and new advances in the diagnosis criteria in field of microbiology. 63 random samples were taken received in Fortis Escorts Hospital Amritsar were evaluated for isolation porposes under manual and automated techniques like VITEK 2. In which 72.0% were found positive under culture method and rest were sterile. All type of organisms including Gram positive and Gram negative were found in the isolation technique but SA was most common etiological agent isolated from samples. Percentage of SA isolation in these samples was around 50.0%. Another important data that was observed during this period was out of 45 positive cases 26 were taken from inside the hospital, this means around 60.0% samples were taken froms patients that were admitted in hospital. This data indicates that there was false positive indication for the presence of nosocomial infection because staphylococcal infections are very common in health care community populations and also has more chance of transmission from person to person.

Pyogenic infections has also relation with the age of the person. As from the above data more than 30 patients were more than the age of 50 that were found positive, results in 70% of patients were found +50 that were infected. This finding indicates that prevalence of this infection increase as age increase because as age increase immunity will surely decrease. Various risk factors also play role in having pyogenic infection that include compromised immune response in case of HIV, person on antibiotic therapy, person with diabetes have more chance for accumulation of pus because of presence of more glucose in blood which increase the growth of bacteria at site of injury. Although along with superficial infections, deep infections are more prone to morbidity if left untreated. In time of antibiotic era every person should get proper awareness and treatment which can save the life of a person.

14. Conclusion

Infections due to *S.aureus* are very common and SA continues to be a serious and formidable challenge to health care providers as their prevalence is reported to be increasing exponentially. In the past, SA infections were reported mostly from hospitalized patients but now they are encountered in community settings as well. Understanding the types of *S. Aureus* infections, their pattern and distribution, as well as the factors associated with their spread are of paramount importance for its management and control.

Staphylococcus aureus infection is also important cause of disseminated infection with multisystem involvement. It can either be health care associated or of community acquired. Early diagnosis and prompt initiation of sensitive antimicrobial agent can avoid mortality & morbidity in these patients. Simple superficial abscesses are not known to be associated with systemic manifestation of infection unless there are other factors such as immune-suppression, diabetes or abuse of injected drugs

Empiric therapy with β-lactam drugs may no longer be adequate for treatment now that MRSA strains are being identified more frequently as the causative agents for SSTIs. It is important for physicians to have a working knowledge of the local antimicrobial susceptibilities to avoid treatment failures and to prevent inappropriate antibiotic usage.

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