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Isolation and identification of pathogenic bacteria from burn wound infection

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Summary

Out of 50 burn wound swabs samples taken from hospitalized patients in city hospital. The result showed that *Pseudomonas aeruginosa* was found to be the most common isolate followed by *Staphylococcus aureus,Enterobacter spp., Proteus vulgaris, Micrococcus sp., Escherichia coli,* and*Klebsiella spp.*

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Chapter one

Introduction

Introduction

A burn is damage to your body's tissues caused by heat, chemicals, electricity, sunlight, or radiation. Scalds from hot liquids and steam, building fires and flammable liquids and gases are the most common causes of burns. Another kind is an inhalationinjury, caused by breathing smoke.

There are three types of burns:

- First-degree burns damage only the outer layer of skin
- Second-degree burns damage the outer layer and the layer underneath
- Third-degree burns damage or destroy the deepest layer of skin and tissues underneath

Burns can cause swelling, blistering, scarring and, in serious cases, shock, and even death. They also can lead to infections because they damage your skin's protective barrier. Treatment for burns depends on the cause of the burn, how deep it is, and how much of the body it covers. Antibiotic creams can prevent or treat infections. For more serious burns, treatment may be needed to clean the wound, replace the skin, and make sure the patient has enough fluids and nutrition.

Globally, burns are considered devastating forms of trauma in patients with serious thermal injury.[1,2] They can be caused by scalds, thermal, electrical, gas or chemical agents.[1,4] Patients with serious burn injury require immediate specialized care in order to minimize bacterial infection, which is a major cause of morbidity and mortality in burn patients.[5-8]

Much progress has been made with respect to infection control and burn wound management, however, burn wound infection still poses a major clinical challenge in most developing countries, where wound site infections are a major source of post-operative illness and mortality among burn patients.[8] The consequential effect of burn wounds contaminated with pathogenic bacteria can delay wound healing, cause wound breakdown and herniation of the wound or complete wound dehiscence.[9-10] Although in most cases the source of contamination is the patient’s normal flora or exogenous contamination from contaminated wound dressing devices in or from the hospital environment, various

groups of microorganisms have been reported to be associated with wound infections. [10-11] A study carried out by Patil et al.[11] in India revealed that Pseudomonas aeruginosa, Methylene Resistant Staphylococcus aureus (MRSA), Acinetobacterbuamanni, Klebsiella pneumonia, Proteus mirabilis, Citrobacter sp., Coagulase negative Staphylococci, Enterobacter sp. and Escherichia coli were commonly associated with the wounds of burn patients. Whilst some studies have reported Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella sp. and Escherichia coli as predominant bacteria associated with burn wounds,[12-14] the exact number of burn injuries is very difficult to estimate. Although some studies have shown that adult females and children (1-9 years) are at a greater risk of burn-related injuries than adult males,[15-18] burn wound is an important cause of disability and mortality in all ages and in both developed and developing countries.[19,20]

Burn wound infection

*Infection is an important cause of morbidity and mortality in hospitalized burn patients,in patients with burn over more than 40% of the total body surface area,75% of all deaths following thermal injuries are related to infections.the rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient,age of the patient,extent of injury,and depth of burn in combination with microbial factors such as type and number of organisms,enzyme and toxin production, colonization of the burn wound site,systemic dissemination of the colonizing organisms.Moreover the larger area of tissue is exposed for a longer time that renders patients prone to invasive bacterial sepsis. In extensive burns when the organisms proliferate in the eschar,and when the density exceeds 100,000 organisms per gram of tissues, they spread to the blood and cause a lethal bacteremia.Therapy of burn wound infections is therefore aimed at keeping the organisms burden below 100,000 per gram of tissues which increases the chances of successful skin grafting.*The denatured protein of the burn eschar provides nutrition for the organisms.Avascularity of the burned tissue places the organisms beyond the reach of host defense mechanisms and systemically administered antibiotics.In addition, cross-infection results between different burn patients due to overcrowding in burn wards.Also thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune

responses are pivotal factors contributing to infectious complication in patients with severe burn.Burn wound infections are largely hospital acquired and the infecting pathogens differ from one hospital to another.The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous origin, thermal injury destroys the skin barrier that normally prevents invasion by microorgsnisms.This makes the burn wound the most frequent origin of sepsis in these patients.Burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms, gram-positive bacteria that survive the thermal insult,such as S. aureus located deep within sweat glands and hair follicles, heavily colonize the burn wound surface within first 48 h.

Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi.Gastrointestinal and upper respiratory tract and the hospital environment.Followingcolonization,these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient's immunosuppression.If sub-eschar tissue is invaded, disseminated infection is likely to occur, and the causative infective microorganisms in any burn facility change with time.Individual organisms are brought into the burns ward on the wounds of new patients.These organisms then persist in the resident flora of the burn treatment facility for a variable period of time,only to be replaced by newly arriving microorganisms.Introduction of new topical agents and systemic antibiotics influence the flora of the wound.The aim of the present study was to obtain information about the type of isolates,identification and antimicrobial sensitivity of bacterial wound infections in burn patients.

Chapter Two

Materials & Methods

CultureMedia:media used for bacterial isolation and identification are ordinary media such as Blood agar,Nutrient agar, triptic soya agar,and special media pseudomonas agar, salmonella-shigella agar. MacConkeyagar,Mannitol salt agar and Eosin methylene blue agar.

Samplecollection:

Sample Collection and Inoculate 50 burn wound swabs were taken from burned patients, who presented invasive burn wound infection, from both sex,and average age 10-55 year, admitted to burn unit of teaching medical center of city hospital.The most preferred areas were the upper and lower extremities.

How do you swab a wound?

In primary care, a swab is the most common method used for sampling a wound.Although biopsy or aspirates of pus are the "gold standard" techniques, wound swabs can provide acceptable samples for bacterial culture provided that the correct technique is used.If the wound is not purulent it should be cleaned prior to swabbing.If the wound is not clean it often leads to the isolation of multiple organisms which may not be relevant and can generate laboratory results reporting "mixed bacterial flora" rather than individual species.Cleaning removes the organisms present in the surface material,which are often different from those responsiple for the pathology, and allows for more accurate culture results.Wounds should be washed with sterile saline and then superficially debrided with a cotton, alginate or rayon-tipped swab.ideally, the patient should not have received recent antibiotic treatment before swabbing a wound as this can affect the microbiological results.

The recommended swabbing procedure is as follows:

1. Applied sterile saline to moisten the head of the swab to increase the adherence of bacteria.

2.Passed the swab over the wound area in a zigzag motion while twisting the swab so that the entire head of the swab comes into contact with the wound surface.

3.Swabbed from the centre of the wound outward to the edge of the wound.

4.The swab should be pressed firmly enough that fluid is expressed from the wound tissue(this may be painful for the patient).

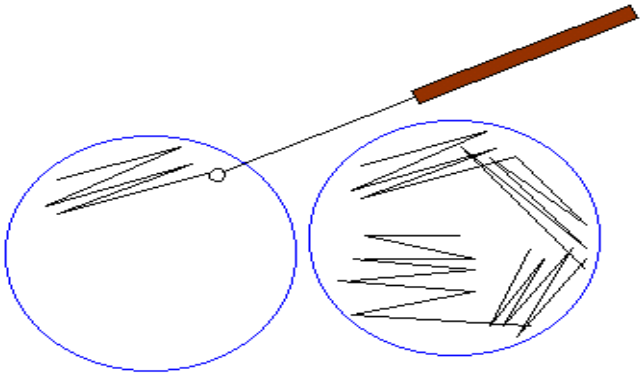
5.Repeated the process with a separate swab if a pocket or sinus is present in the wound.

Once the sample has been collected it should be labelled with the patient identification details,date and time of the sample and wound site.The specimens were transported in sterile, leak-proof container.All specimens were inoculated on 5% blood agar, MacConkey agar and chocolate agar plates and incubated overnight at 37°C aerobically. The sample was also put into liquid media and was subcultured after overnight incubation onto blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method.

Method of inoculating the solid culture media:

Method used for inoculating the solid media depends upon the purpose of inoculation- whether to have isolated colonies or to know the bacterial load of the sample (quantitative analysis).

For obtaining the isolated colonies streaking method is used, the most common method of inoculating an agar plate is streaking.



Streaking method

Streaking method steps:

1. A small amount of sample is placed on the side of the agar plate (either with a swab, or as a drop from an inoculating loop).

2.A sterile loop is then used to spread the bacteria out in one direction from the initial site of inoculation.This is done by moving the loop from side to side,passing through the initial site.

3.The loop is then sterilised (by flaming) again and the first streaks are then spread out themselves.

4.This is repeated 2-3 times, moving around the agar plate as shown in the figure.

In this method single bacterial cells get isolated by the streaking, and when the plate is incubated, forming discrete colonies that will have started from just one bacterium each.

Identification methods:

The most important task of a bacteriology is to identify the pathogens from the clinical sample so that appropriate treatment can be instituted.

There are several methods to identify the different type of bacteria:

1.Staining reaction
2.Morphology of bacterial colony
3.Cultural characteristics

4.Metabolism
5.Biochemical properties

1.Staining reaction: Simple stains bring out the best morphology.Differential and special stains are necessary to bring out characteristics like: gram negative and gram positive bacteria, Acid fast and non acid fast, spirochetes, capsule and flaggella, etc.

a.Gram stain: gram stain divides the bacteria into gram positive and gram negative.

Procedure:

1.Took a heat fixed bacterial smear.

2.Flooded the smear with crystal violet for 1min, then wash with water.

3.Flooded the smear with iodine for 1min, then wash with water.

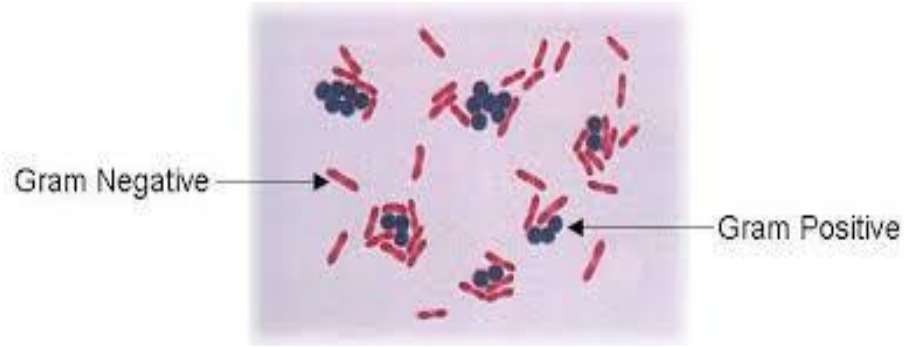
4.Flooded the smear with ethanol-acetone, quickly, then wash with water.

5.Flooded the smear with safranin for 1minute, then wash with water.

6.Blotted the smear, air dry and observe.

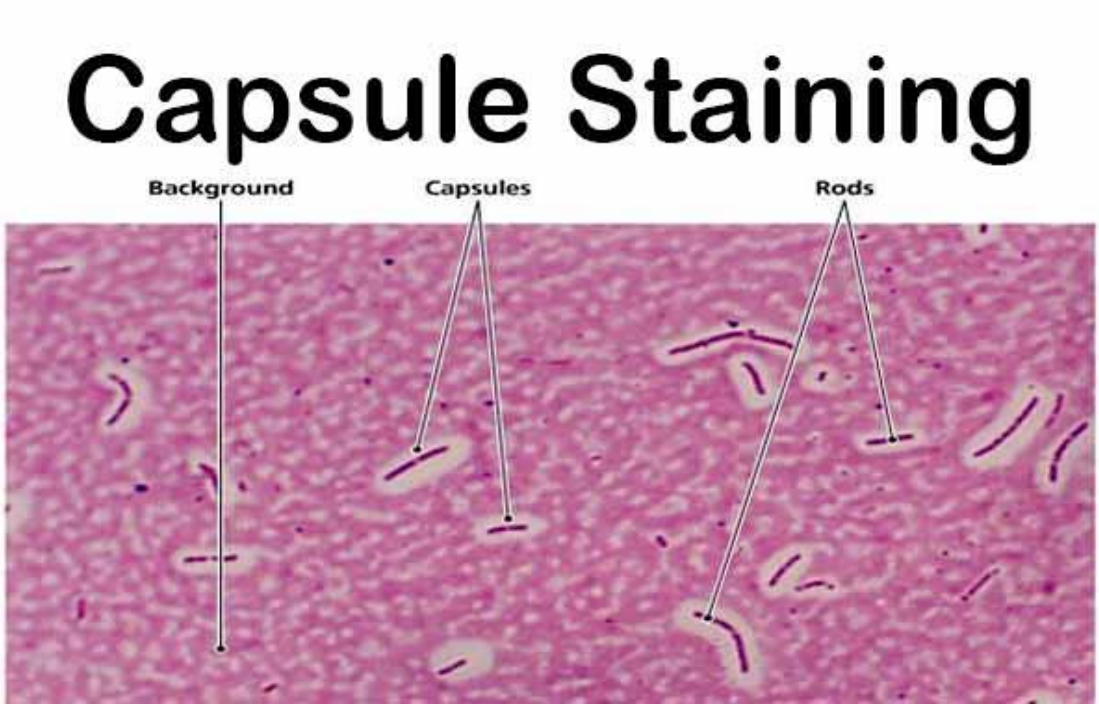
7.Examined under microscope:

Gram positive bacteria: violet
Gram negative bacteria: pink



B.capsule stain:

The capsule stain employs an acidic stain and a basic stain to detect capsule production.
Capsule are formed by organisms such as Klebsiellapneumoniae.Most capsules are composed of polysaccharides, but some are composed of polypeptides.



2.Morphology of the bacterial colony:

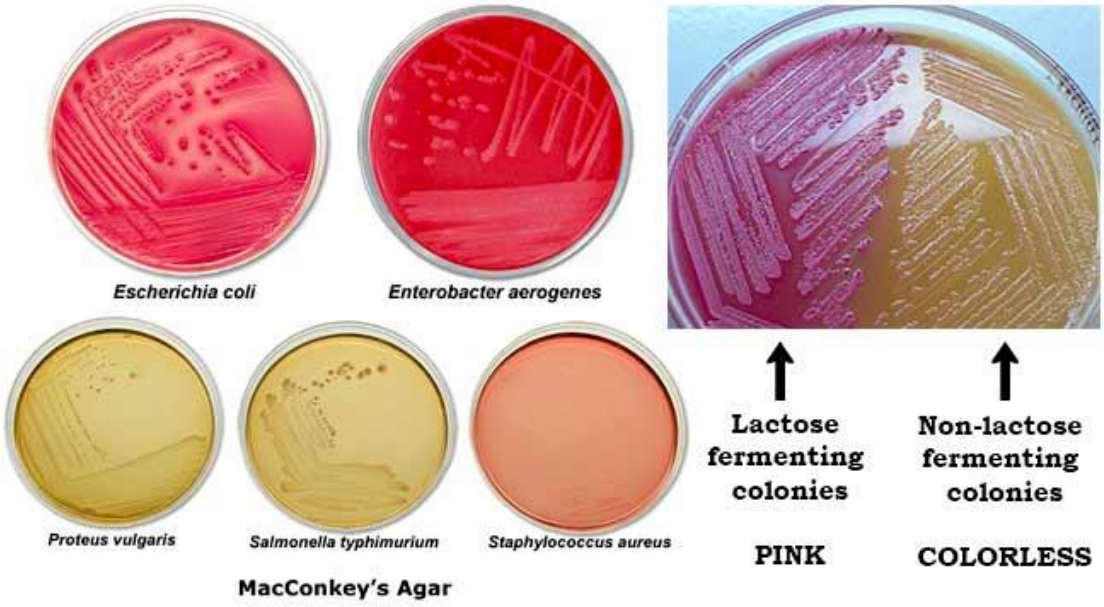
- Shape: circular, irregular, radiate or rhizoid.
- Size: diameter in mm.
- Elevation: flat, raised, low convex, dome shaped.
- Margin: entire, wavy, lobate, filiform.
- Surface: smooth, wavy, rough, granular, papillate, glistening, etc.

3. Cultural characteristics:

These provide additional information for the identification of a bacterium.

A. On solid medium the following characters are observed:

- Shape: circular, irregular, radiate or rhizoid.
- Size: the size of the colony can be a useful characteristic for identification.The diameter of a representative colony may be measured.
- Elevation:
- Margin: entire, wavy, lobate, filiform.
- Surface: smooth, wavy, rough, granular, papillate, glistening, etc.
- Size in mm.
- Texture: dry, moist, mucoid, brittle, viscous, butyrous (buttery).
- Color: colorless, pink, black, red, bluish-green.



B. In a fluid medium following characters are observed:

- Degree of growth: Absence, scanty, moderate, abundant, etc.
- Present of turbidity and its nature.
- Presence of deposit and its character.
- Nature of surface growth.
- Ease and disintegration and odor.

4.Biochemical Tests:

- Indole test
- Methyl red/Vogesproskauer
- Citrate

- Motility
- Lactose fermantation
- Sucrose fermentation
- Glucose fermentation&gas production
- Triple Sugar Iron Agar (TSI) test
- Mannitol Salt Agar (MSA)
- Test for enzymes:
    - Catalase test
    - Oxidase test
    - Urease test
    - Coagulase test
    - Nitrate reduction

Indole Test:

This test is performed to help differentiate species of the family Enterobacteriaceae.

Procedure of Indole Test:

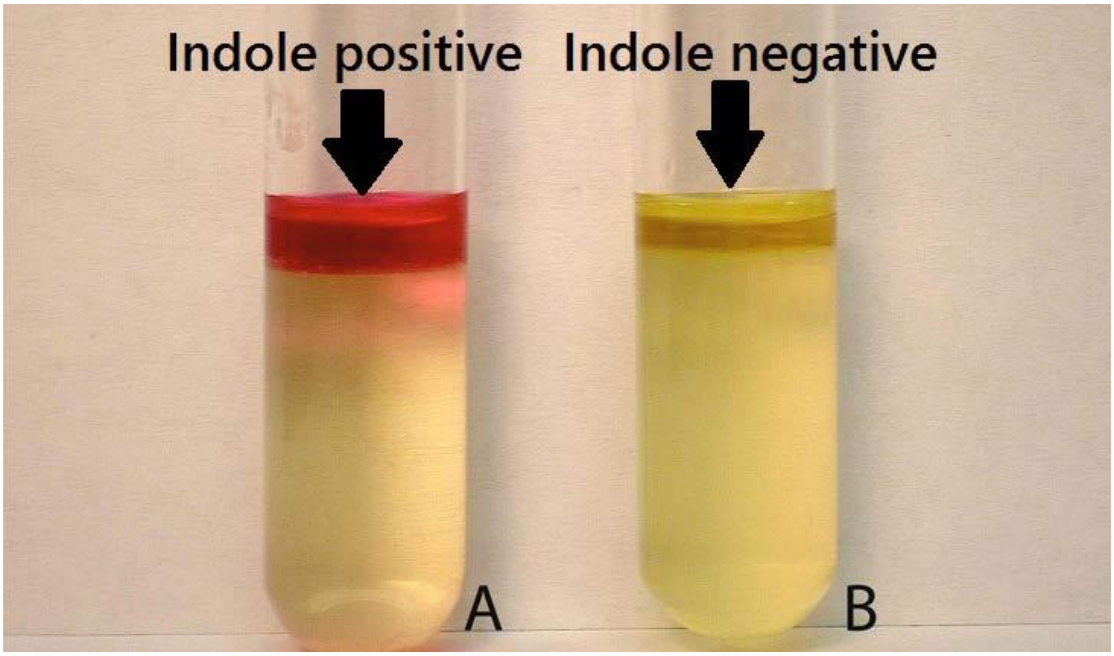
1.Took a sterilized test tubes containing 4 ml of tryptophan broth.

2.Inoculated the tube aseptically by taking the growth from 18 to 24 hrs culture.

3.Incubated the tube at 37°C for 24-28 hours.

4.Added 0.5 ml of Kovac's reagent to the broth culture.

5.Observed for the presence or absence of ring.



Indole positive:E.coli, Proteus vulgaris
Indole negative: Salmonella spp., Klebsiella spp., Enterobacteraerogens

Citrate Utilization test:

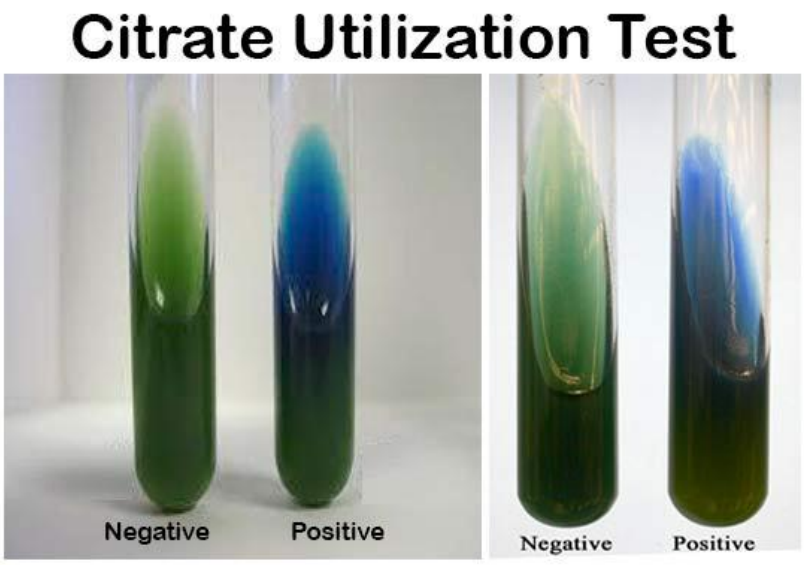
This test is used to assist in the identification of enterobacteria.The test is based on the ability of an organism to use citrate as its only sole source of carbon and ammonia as its only source of nitrogen.

Procedure:

1.Inoculated simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old.

2.Incubated at 35^{o}C to 37^{o}C for 18 to 24 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.

3.Observed the development of blue color; denoting alkalinization.

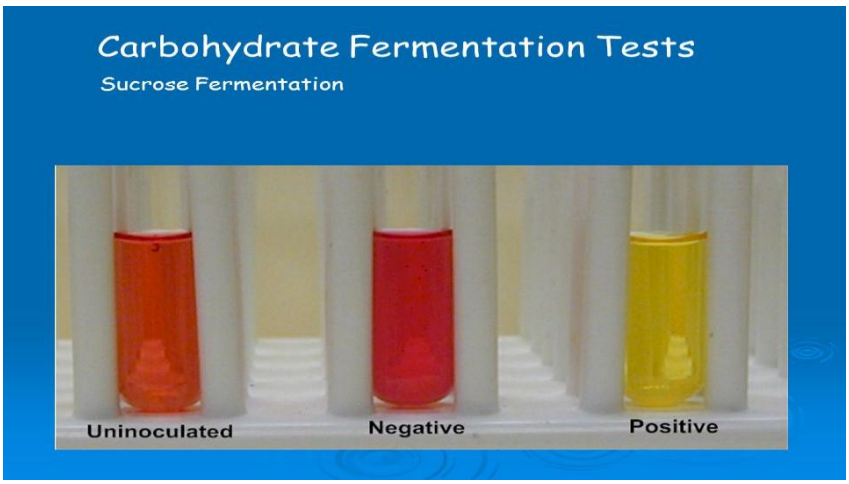


Lactose Fermentation:

This test is used to determine the ability of bacteria to ferment lactose.

How is the test performed?

An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube is incubated at 35-37 C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a p*H* change to acidic.



Triple Sugar Iron Agar (TSI) Test:

TSI agar is used to determine whether a gram negative rod utilizes glucose and lactose or sucrose fermentatively and forms hydrogen sulphide (H2S). The production of H2S requires an acidic environment and is indicated by blackening of the butt of the medium in the tube.

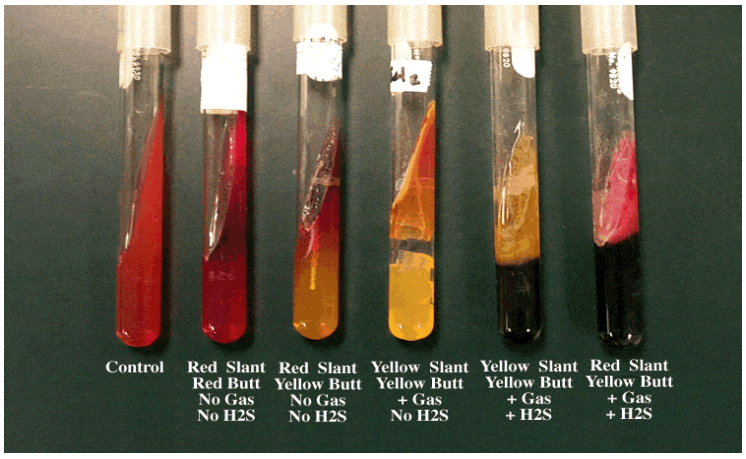
Procedure:

1.Touched a well isolated colony with a sterile straight wire.

2.Inoculated TSI by first stabbing through the centre of the medium to the bottom of the tube and then streak the surface of the slant.

3.Left the cap loose and incubate the tube at 35 in ambient air for 18 to 24 hours.

4.Observed the reaction.



Some example of Triple Sugar Iron (TSI) Agar Reactions:

Name of the organisms	Slant	Butt	Gas	H2S
Escherichia, Klebsiella, Enterobacter	Acid (A)	Acid (A)	Pos (+)	Neg (-)
Shigella, Serratia	Alkaline (K)	Acid (A)	Neg (-)	Neg (-)
Salmonella, Proteus	Alkaline (K)	Acid (A)	Pos (+)	Pos (+)
Pseudomonas	Alkaline (K)	Alkaline (K)	Neg (-)	Neg (-)

Mannitol Salt Agar (MSA):

This test is used to determine the ability of bacteria to tolerate 7% salt concentration and ferment mannitol.The media is selective because it selects for salt tolerant bacteria.

Media and Reagents: MSA media contains nutrient agar, mannitol, 7% sodium chloride and phenol red indicator.

How to perform test: inoculate an MSA plate using streak plate method and incubate 24-48 hours.

MSA Results:

- If the organism is tolerant to salt, it will grow.
- If the organism is not tolerant to salt, it will not grow.
- If the salt tolerant organism can ferment mannitol, then there will be yellow zones around the colonies.
- If the salt tolerant organism cannot ferment mannitol, then the media will remain pink.

MANNITOL SALT AGAR

NEGATIVE MSA TEST

POSITIVE MSA TEST

POSITIVE TEST IS GROWTH AND FERMENTATION OF MANNITOL (YELLOW)

Test for enzymes:

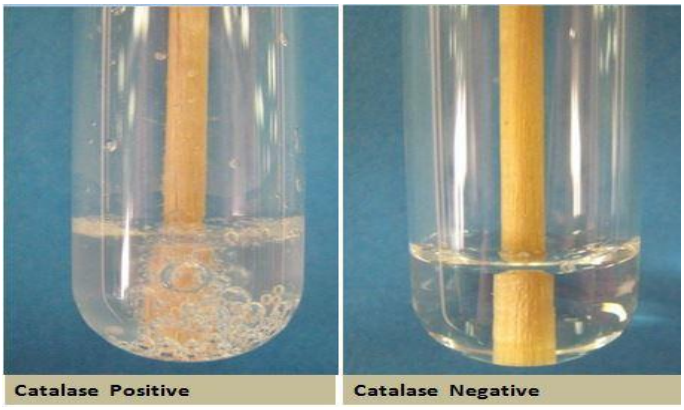
Catalase test:

This test is used to demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produces an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture while 15% H_2O_2 is used for detection of catalase in anaerobes.

Methods:

1. Slide method

2. Tube method



Positive: Copious bubbles produced, active bubbling
Negative: No or very few bubbles produced.

Oxidase test:

This test is used to determine the presence of bacterial cytochrome oxidase enzyme using the oxidization of thesubstrate" tetramethyl-p-phenylenediaminedihydrochloride" to indophenol a dark purple colored end product.

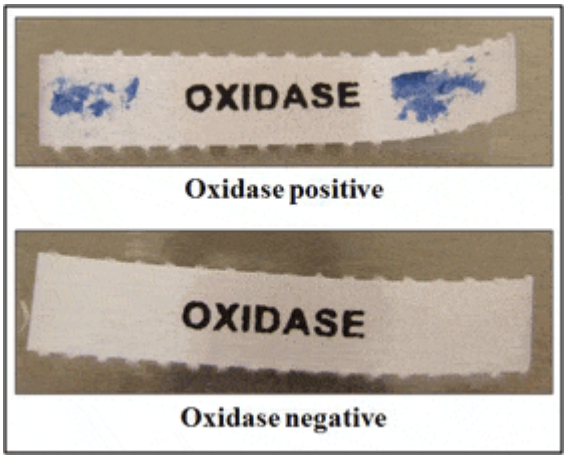
Procedure:

1.Took a filter paper soaked with the substrate tetramethyl-p-phenylenediaminedihydrochloride.

2.Moistened the paper with a sterile distilled water.

3.Picked the colony to be tested with wooden or platinum loop and smear in the filter paper.

4.Observed inoculated area of paper for a color change to deep blue or purple within 10-30 seconds.



Positive: Pseudomonas spp., Aeromonas spp., Vibrio spp., Alcaligenes spp., Neisseria spp.

Negative: Enterobacteriaceae, Acenitobacter spp.

Urease test:

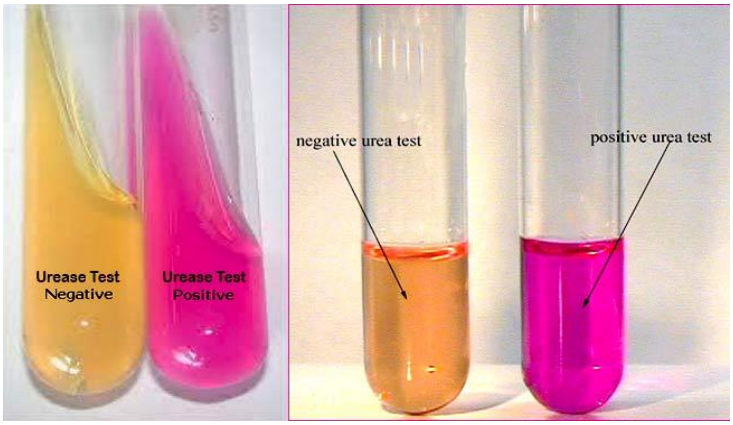
This test is done to determine the ability of bacteria to hydrolyze urea to make ammonia using enzyme urease.

Procedure:

1.Streaked the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture.

2.Left the cap on loosely and incubate the tube at 35°-37°C in ambient air for 48 hours to 7 days.

3.Examined for the development of a pink color for as long as 7 days.



Positive Reaction: Development of an intense magenta to bright pink color in 15 min to 24 h.
Negative Reaction: No color change.

Coagulase test:

This test is used to differentiate Staphylococcus aureus (positive) from coagulase negative Staphylococci.

Method:

A. Slide test: (for bound coagulase)
1.Placed a drop of coagulase plasma on a clean,dry glass slide.
2.Placed a drop of distilled water or saline next to the drop of plasma as a control.
3.With a loop or wooden stick,emulsified a portion of the isolated colony being tested in each drop.
4.Mixed well and rock the slide gently for 5 to 10 seconds.

B. Tube test: (for free coagulase)

1.Emulsified several colonies in 0.5 ml of rabbit plasma (with EDTA) to give a milky suspension.

2.Incubated tubes at 35 in ambient air for 4hrs. Check for clot formation.

3.If negative at 4hrs, incubated at room temperature overnight and checked again for clot formation.

A. Slide test:

-positive: Macroscopic clumping in 10 seconds or less in coagulated plasma drop and no clumping in saline or water drop.
-negative: No clumping in either drop.

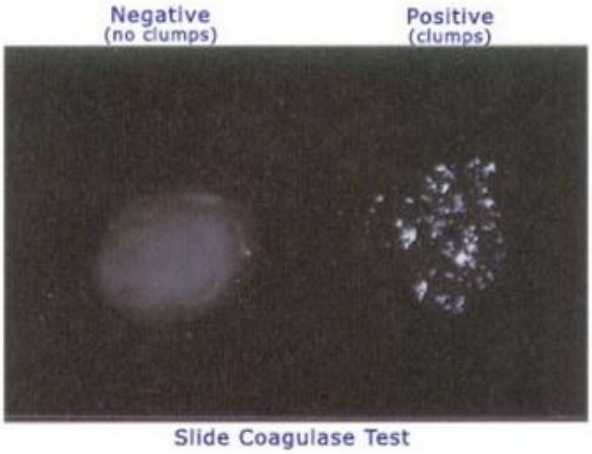
B. Tube test:

-positive: Clot of any size

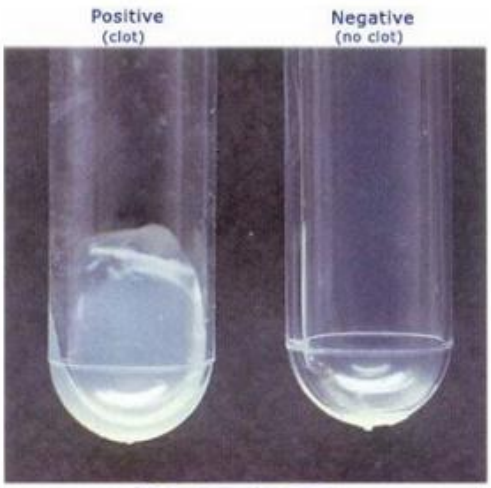
-negative: No clot

Coagulase positive: Staphylococcus aureus

Coagulase negative: Staphylococcus epidermidis



Slide Coagulase Test



Tube Coagulase Test

Nitrate reduction test:

This test is used to determine the ability of the bacteria to reduce nitrate to nitrites or free nitrogen gas.

Procedure:

1.Inoculated nitrate broth with a heavy growth of test organism using aseptic technique.

2.Incubated at an appropriate temperature for 24 to 48 hours.

3.Added one dropperfull of sulfanilic acid and one dropperfull of a α-naphthylamine to each broth.

-At this point, a color change to RED indicates a POSITIVE nitrate reduction test. If you get a red color, then you can stop at this point.

-No color change indicates the absence of nitrite. This can happen either because nitrate was not reduced or because nitrate was reduced to nitrite, then nitrite was further reduced to some other molecule. If you DO NOT get a red color, then you must proceed to the next step.

4.Added a small amount of zinc (a toothpick full) to each broth. Zinc catalyzes the reduction of nitrate to nitrite.

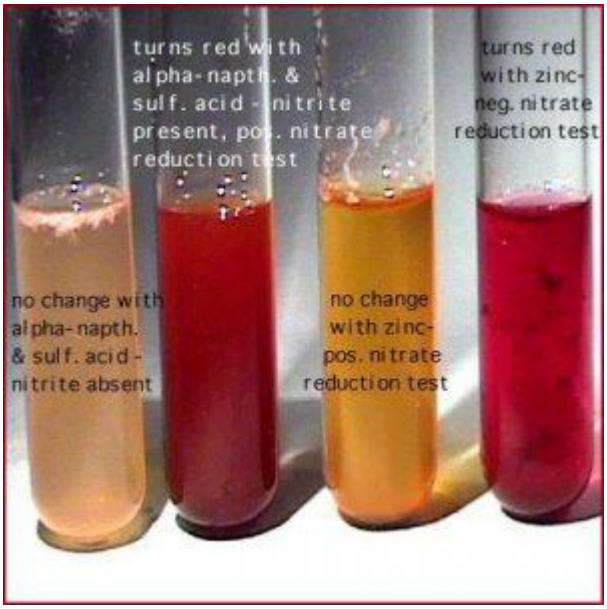
-At this point, a color change to RED indicates a NEGATIVE nitrate reduction test because this means that nitrate must have been present and must have been reduced to form nitrite.

-No color change means that no nitrate was present. Thus no color change at this point is a POSITIVE result.

Results:

1.Nitrate Reduction Positive: (**Red** after sulfanilic acid + alpha-naphthylamine; no color after zinc).

2.Nitrate Reduction Negative: (**No color** after sulfanilic acid + alpha-naphthylamine followed by Red after zinc).



Chapter three

Results&Discussion

Results:

The various types of bacteria isolated from burn wound culture of total 50 wound swabs were shown in the below table(1) Bacterial isolates were found in 35 (70%) wound swabs, and only 15 samples (30%) were negative in bacterial growth.The results showed that *P. aeruginosa* was the commonest isolate (16 isolates, 45.71%) followed by *S. aureus* (8 isolates, 22.85%),*Citrobacterbraakii*(4 isolates,11.42%).Enterobacter spp. , coagulase-negative Staphylococci,and Proteus vulgaris were 3 isolates for all (8.57%).*Corynebacterium* spp., *Micrococcus* spp.,Streptococci spp., and *Proteus mirabilis* were 2 isolates for all(5.71%). *E.coli, Enterococcus faecalis, Serratiamarcescens, Serratiarubidia*, and *Klebsiella* spp. were 1 isolate for all(2.85%).Most of the isolates showed mixed infection as showed in the table.

Table(1) : Genus of bacteria that isolation from burn patients

Name of isolated bacteria	Number & frequency	Total No.	Percentage
Pseudomonas aeruginosa	**6 single isolates & 10 mixed with other bacteria**	**16**	**45.71**
Staphylococcus aureus	**3 single isolates & 5 mixed with other bacteria**	**8**	**22.85**
Citrobacterbraakii	**1 single isolates & 3 mixed with other bacteria**	**4**	**11.42**
Enterobacterspp	**3 single isolates**	**3**	**8.57**
Coagulase-negative Staphylococci	**2 single isolates & 1 mixed with other bacteria**	**3**	**8.57**
Proteus vulgaris	**1 single isolates & 2 mixed with other bacteria**	**3**	**8.57**
Corynebacterium spp.	**1 single isolates & 1 mixed with other bacteria**	**2**	**5.71**

Micrococcus spp.	**1 single isolates & 1 mixed with other bacteria**	**2**	**5.71**
Streptococci spp.	**2 isolates mixed with other bacteria**	**2**	**5.71**
Proteus mirabilis	**2 isolates mixed with other bacteria**	**2**	**5.71**
E. coli	**1 single isolates**	**1**	**2.85**
Enterococcus faecalis	**1 single isolates**	**1**	**2.85**
Serratiamarcescens	**1 single isolates**	**1**	**2.85**
Serratiarubidia	**1 isolate mixed with other bacteria**	**1**	**2.85**
Klebsiella spp.	**1 isolate mixed with other bacteria**	**1**	**2.85**

Discussion:

Bacteria isolated from only 35 burn wound swabs from the total 50 swab indicated that 70% of examined burn patients had invasive burn wound infections, this idea supported the investigation of reference (27), who explained that the burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury, also reference (28), demonstrated that the infectious

complications are considered a major causes of morbidity and mortality and the type and amount of microorganisms on and in the injured tissues influence wound healing.Most of the isolates in our research had mixed with other bacterial species and some of these have shown to be resistant to many antimicrobials, and this indicates the high contamination of burn wounds in our hospitals. In the present study, the most commonly isolated organisms from burned patients were *P. aeruginosa* followed by *S. aureus, C. braakii* and *Enterobacter* spp. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn trauma. This evidence was consistent with previous observation mentioned by some workers. Initially, the immunologic response to severe burn injury is proinflammatory but later becomes predominately anti-inflammatory responses in an effort to maintain homeostasis and restore normal physiology, cytokines and cellular response mediate both of these phases (29). Systemic responses to burn occur by proinflammatory cytokines (30), but the anti-inflammatory responses and the subsequent immunosuppression following burn injury are characterized by a set opposing cells and cytokines, the production and release of monocytes/ macrophages are decreased following burn injury and sepsis (21). the nosocomial transmission of microorganisms to the burn wound occurred by transfer from the hands of health care personnel and through immersion hydrotherapy treatment. Our results of bacterial isolation from burn wound were in accordance with other previous studies. Reference(22) and (31) reported that Pseudomonas species was the commonest pathogen isolated from burn wound followed by *S. aureus*, *Klebsiella* spp., and *Proteus* species. Reference (23),(31) reported that Enterobacter spp. is the main isolate from burn wound sample, *Micrococcus* spp. and *E. coli*. Microbial infection is one of the major serious complications in wound patients, our results showed that the rate of isolation of gram-negative organism was more than gram-positive, these results are consistent with those reported by reference (24) and (26), reported that the rate of gram-negative bacterial isolation from burn wound was more than gram-positive.

Chapter four

References

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