الجامعة المستنصرية

كلية العلوم

قسم علوم الحياة

المرحلة الرابعة

بكتريا مرضية. عملي

يطلب من:

مكتبة حسنين

السعر 1250.

Level :4 **Pathogenic bacteria**

Lab :1

Bacteria: microscopic organisms whose single cells 0.5-2.0 micron in diameter ,can be seen under 100x power (oil immersion) of light microscope.

Pathogenic bacteria: Bacterial that can enter tissues of human & animals multiply their and cause a disease .

Classification of Bacteria

1-Shape : The bacteria are classified according to the

*Cocci (Spherical) shape

Diplococci ex : *Streptococcus pneumoniae*

Chain (Cocci) ex : *Streptococcus pyogenes*

Cluster or Grape like shape ex : *Staphylococcus aureus*

*Bacilli

Short Bacilli ex : *Bacillus subtilis* .

Long Bacilli ex : Lactobacillus .spp

Cocco Bacilli ex : Enterobacteriaceae ex : *Escherichia coli* , Shigella ,Salmonella .

*Kidney shape ex : *Neisseria gonorrhea* .

*Comma shape ex : *Vibrio cholera.*

** Spiral shape ex : *Helicobacter pylori*

2- Ability to form spores :The Bacteria are divided in two groups according to the ability to form spores .

1- spore - former Bacteria : ex – Bacillus

Clostridium .

2-Non spore - former Bacteria : ex – Staphylococcus . spp, *Escherichia coli* , Streptococcus .spp

3- Oxygen requirements :

1-Obligates (strict)Aerobes .ex- Bacillus ,Pseudomonas .

2- Obligates (strict)Anaerobes. ex- Clostridium.

3-Facultative Anaerobes .ex- Enterobacteriaceae ex : *Escherichia coli* , Shigella ,Salmonella . Staphylococcus spp.

4-Microaerophiles .ex- *Helicobacter pylori* .

5- Aero tolerant .ex- Streptococcus

4-Reaction to the Gram stain : The Bacteria are divided in two groups according to the reaction with Gram stain.

1- Gram Positive Bacteria. ex- Streptococcus , Staphylococcus , Bacillus , Clostridium .

2- Gram Negative Bacteria. ex- All the members of Enterobacteriaceae (*Escherichia coli* , Shigella ,Salmonella , ...).

5-Nutritional requirements.

6-Method of energy production (glycolysis ,cellular respiration).

Laboratory diagnosis of bacterial disease :

It depended on clinical specimens reaching to the lab

1-Blood :septicemia .

2-Urine :urinary tract infections .

3-Stool : gastrointestinal infections .

4-Sputum : respiratory infection

5-Vaginal swabs : Vaginal infections .

6-Nose &ear swabs : Nose &ear infection

7-Cerebral spinal fluid (CFS) .CNS infections .

8-Food &vomit :food poisoning .

9-Pus : Acne, wounds ,burns .

10-Seminal fluid, urethral discharge .

Bacterial diagnosis :

1-Phenotypic characters &morphological tests .

2-Biochemical Tests.

3-Serological tests .

4- Growth requirements .

Lab .Directions :

1-Wear lab .Coat .

2-Wash hands.

3-Use alcohol.

Culture Media

By knowing environmental and nutritional factors that affect the growth of specific prokaryotes, it is often possible to provide the appropriate conditions for their cultivation.

***Culture media:** it is the environment where the microorganism lives and gets its own nutrients from nutritional materials forming that environment in order to grow and reproduce in laboratory

***Components of the Typical Culture Medium:**

1. Carbon source
2. Nitrogen source
3. Phosphate source
4. Water source
5. Source of different minerals e.g. iron, magnesium, sodium, potassium and traces of zinc and manganese.

Note: Some m.o. may need a source of vitamins and amino acids which are important in building cellular components of m.o.

***Culture Media Importance:**

1) Isolation and preservation of m.o
2) Reproducing a m.o and studying its characteristics.
3) Encouragement and induction of the m.o to produce materials of industrial importance like production of antibiotics and some organic acids.

***Division of Culture Media :**

A// Depending on its consistency:

1) Liquid media or broth: these are media that do not contain any percentage of agar. They are usually used in the extraction of active compounds produced by m.o. e.g toxins.
2) Solid media: these are media that contain (1.5 - 2) %. agar. They are used for the isolation of m.o in the form of pure colonies and for isolation of two kinds (or more) of bacteria.
3) Semisolid media: these are media that contain less than 1% of agar, about (0.7 – 0.8) %. This amount of agar is added to the liquid medium so it becomes

gelatinous. These media are used for studying of the bacterial motility and their demands for O_2 in order to know if these m.o are aerobic , anaerobic , micro aerobic , or facultative anaerobic .

Note: In order to obtain solid culture media , we use the following materials

1)) Gelatin:

1- It is added to the medium in a percentage of (5-10) %.

2- Its usage is limited because it liquefies at incubation temperature (37)°C and solidifies at (25)°C. Further, some kinds of bacteria can utilize it and dissociate it because they have "gelatinase" enzyme .

2)) Agar:

1- A complex carbohydrate material extracted from the red algae.

2- It is considered a typical solidifying material because it doesn't have nutritional value for the bacteria so it isn't attached or utilized by the bacteria. In addition it liquefies at (100) °C and solidifies at (40- 45) °.

B// Depending on its nature or components or contents:

1) Natural media: these are media that contain natural materials e.g. plant or animal tissues , milk , diluted blood , fruit and vegetables juice (like tomato and potato) and meat extract . The components of these media are accurately unknown e.g. tissue culture which is prepared from chicken embryo and used for cultivation of viruses.

2) Artificial media: these are divided into:

a) Synthetic or simple or defined media: these are media which contain chemical substances that we know their composition and concentration accurately. These media are used for studying:

1- The nutritional requirements of m.o .

2- The effect of each of the substances forming these media on m.o .

b) Semi synthetic or complex media: these are synthetic media supplemented with natural components of unknown chemical composition like the addition of meat extract, yeast extract, peptone or serum.

These media are used when the studied bacteria are sensitive to synthetic media so they are cultured or grown on media supported with natural sources.

3) Living media: these are media in which living cells are used as culture media like the use of chicken embryo &Hela cell for cultivation of viruses.

C//Depending on the purpose of uses:

1) General purpose media: these are media in which many m.o are grown . They are used for many purposes e.g. nutrient broth.
2) Selective media: these are media that are used for the cultivation and isolation of certain species of m.o from a mixture of different species . These media are divided into two kinds :

A) Depressive selective media: these are media which are used for the selection of the certain species of m.o by depressing the undesirable (un wanted) species . There are several ways of depression like:

1- Addition of some depressive materials to the medium like :

a- The addition of certain dyes or stains e.g. crystal violet, methylene blue, basic fuchsine which inhibit the growth of G+ve bacteria without affecting the G-ve growth .

b- The addition of certain antibiotics e.g. cycloheximide which inhibits the growth of saprophytic fungi and allows the growth of medical fungi when it is added to sabouraud agar .

2- Using certain growth conditions and changing them according to the growth conditions of the desirable species e.g. temperature , ventilation, and pH.

B) Enrichment selective media: these are media which are used for the selection of the desirable species of m.o. by induction the growth of these species in a better way than the other species which are grown in the same medium and that is done by adding stimulatory materials which enrich the media like the addition of blood to the nutrient agar medium in order to form blood agar medium. These media are used for the cultivation of fastidious bacteria.

3) Differential or identification media: these are media which differentiate between two different groups of m.o. and allow the diagnosis of m.o. depending on its biological characters (it means that these media contain certain material allows the detection of certain m.o. depending on a metabolic action done by that m.o.) e.g. MacConkey agar which differentiate between lactose fermented bacteria and non-lactose fermented. The colonies of lactose fermented bacteria appear pink while the colonies of non-lactose fermented bacteria appear colorless (it means that these colonies have a color which is similar to the culture media color).

*Lactose fermented bacteria --------- >pink colonies: E. coli

*Non-lactose fermented b. --------> pale or colorless colonies: Neisseria , Proteus

Pseudomonas

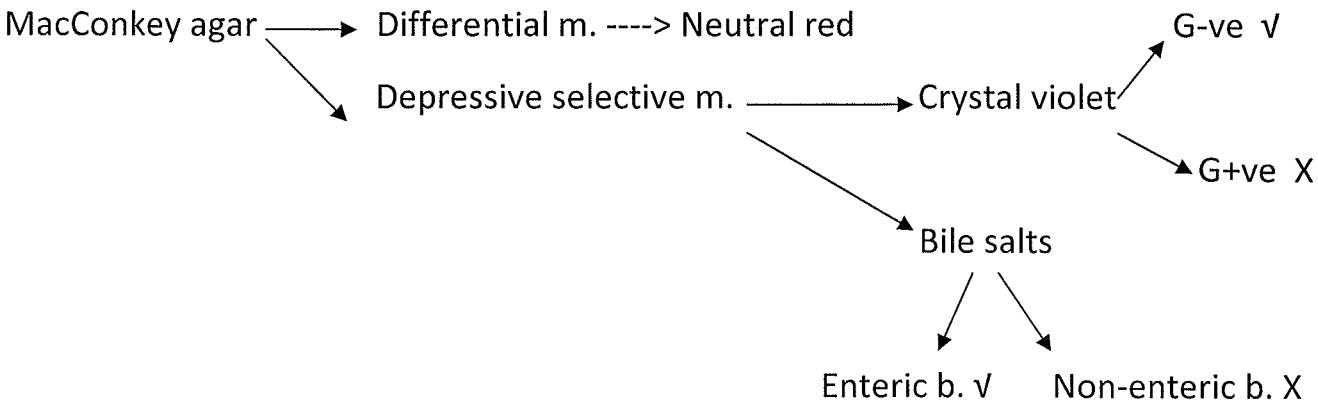
*pH - indicator----------> neutral red

*Sugar --------> lactose

Note: - MacConkey agar is considered a depressive selective medium
it permits the growth of G-ve enteric bacteria and inhibits the growth of G+ve non-enteric bacteria.

Why? This medium contains (crystal violet) which is a dye that inhibits G+ve bacteria, and the medium is also contains (bile salts) which inhibit non-enteric bacteria and both of them (crystal violet and bile salts) do not affect the growth of enteric G-ve bacteria because this bacteria is adaptable to live with the presence of bile salts in the intestine .

Note:- Reagents or indicators are added to the differential media to differentiate between different types of m.o. which are grown on these media. Usually these reagents are dyes which investigate the change of the medium acidity as a result of a metabolic action done by the m.o. and this ehange in acidity is manifested by noticing the color change of the reagent (the dye) which is added to the medium and these reagents are called (PH-indicators).



4) Maintenance media: these media are used for maintenance and storage of m.o. for along period by adding materials in a certain ratio. These materials maintain the persistence and viability of m.o. for a longer time e.g. glycerol or tween-80 which leads to the slow growth of m.o. because fast growth is followed by fast death and that is not desirable. There is a special medium for the maintenance of each m.o.and that medium is maintained in the freezer e.g. nutrient broth, brain-heart infusion broth.

5) Transport medium: these media that are cultivated with the sample temporarily in order to transport it from its isolation source (human, soil, water, ...etc)

to the laboratory for maintaining and keeping its viability and other characteristics e.g. stuart medium which is used for transporting Gonnorhoea bacteria (Gonococci) and glycerol saline medium which is used for transporting stool samples.

6) Assay media: these media are used for performing a particular test (assay) like the medium that is used for performing antibiotic sensitivity test which is called Muller-Hinton age.

7) Stimulatory media: these are media that stimulate the production of certain materials or structures inside the m.o. cell like: toxins, pigments and endospores.

***Preparing the Culture Medium:**

Note: You must read the instructions found on the container of the culture medium before preparing it .

1- Weigh the required amount of the medium powder by using the balance and put it in a flask or other containers.
2- Add the required amount of distilled water to the flask .
3- Dissolve the medium powder in the distilled water by using hot plate with magnetic stirrer or by using the flame of benzene burner or without using heat .
4- Adjust the pH of the medium to the required value (+ 0.1 or + 0.2).
5- Distribute the medium in the tubes or flasks or any other containers according to necessity .
6- Sterilize the culture medium by autoclave
7- Cool the medium after sterilization and keep it in refrigerator at (4) °C until using it.

Note:

1- It is preferred not using containers that made of cupper when you prepare the medium, instead of these you can use containers made of glass which are heat resistant.

2- It is preferred using the distilled water for instead of the tap water for preparation of medium.

3- It is preferred to adjust or fix the pH of the medium before sterilizing it in autoclave.

Q) Why you adjust the pH of the medium before sterilization to the required value with the addition of 0.1 or 0.2 ?

The pH of the medium is adjusted before sterilization, so that it will be not contaminated when using pH-paper or when adding solutions used for fixing pH after sterilization.

On the other hand, the reasons of increasing the value to the mentioned degree is related to the fact that pH or hydrogen ion concentration is affected by heat, it means that the concentration decreases when the temperature increases by heating medium in the autoclave (sterilization), therefore you must elevate (increase) the value before sterilization to reach the required value after sterilization.

Q) How do you adjust (fix) the medium pH ?

1. By using the pH - indicator paper.
2. By using the pH- meter.

*Adjustment of the medium pH is done as follows:-

1. By adding a couple of drops of NaOH in the concentration of 1N .
2. By adding a couple of drops of HCl in the same concentration.

Note: You must cool the medium to (60)° C before the adjustment of the PH because the medium temperature affect the efficiency of the procedure especially when using the pH-meter.

Pathogenic Bacteria **Lab3Level 4**

Preparation and Staining of the Microbial Smear

Microbial Smear: it is a very little amount of microbial growth (which is taken from a liquid or solid medium) and spreaded on a clean glass slide. After the smear is completely dried by air, it is passed for several times over the flame of benzen burner to fix the microbe on the slide and prepare it for staining.

NOTE: The reason of passing the smear for several times over the flame of benzene burner is to fix the microbial cells and prevent removal of these cells during washing between two sequent steps of staining.

Steps of Microbial Smear Preparation

1- Handle a clean slide by its edge, label the target place at the bottom side of the slide by drawing a circle with a diameter about 2 cm using a marker.

2-Sterilize the loop until reaching to the red heat.

3- If the bacteria are grown in liquid medium, shake the culture and transfer 2 loopfuls of the liquid culture to the center of the slide and spread over the target circle.

While if the bacteria are grown on solid medium, place 1 or 2 loopfuls of water on the slide (at the center of target circle) and then use an inoculating loop to spread the organisms in the water in order to get a homogenized smear.

4- Sterilize the loop.

5-Leave the smear to dry at room temp. (By air).

6- After drying, pass the slide over the flame for several times to fix the organisms on the slide (avoid prolonged heating of the slide). This step is called (heat fixation).

NOTE: It is preferred that the microbial culture used in the staining process is a recent one, since the old one gives false results.

Types of Stains (Dyes)

Staining of m.o is done by using stains or chemical compounds in order to study them under the microscope.

Stain: is a chemical compound which is composed of positive and negative ions, one of them carries the color of the stain; this is called (chromophore).

***Stains are divided into 2 groups according to the ions charge which carry the color of the stain.**

These 2 groups are:

1-Basic dyes or cationic dyes: these dyes act well on bacteria because they have color bearing ions that are positively charged. These ions will attract to the bacterial cell wall which is negatively charged, combine with it and stain the cell e.g. methylene blue, safranin, crystal violet, malachite green.

2-Acidic dyes or anionic dyes: these dyes that are negatively charged (have anionic chromophore) do not attract to the bacterial cell wall (which is negatively charged), therefore they do not stain the cell, this process is called "negative staining'"

Negative stains like: nigrosin, india ink, eosin, rose bengal do not penetrate the bacterial cell but they make the background area around a cell opaque or dark.

So, these stains show or clarify the shape and size of the cell and extracellular structure such as : capsules.

***Stains are divided into 3 groups according to the purpose of use.**

These groups are:-

1- Simple stains or simple staining.

2-Differential stains or differential staining.

3- Special stains or special staining.

1-Simple stains or simple staining

In this process, only one stain is used for staining. The most commonly used stains for simple staining are crystal violet, methyene blue, basic fuchsin.

This method of staining is useful in determining basic morphology and the presence or absence of certain kinds of granules.

Steps of simple staining

1- Prepare a fixed smear.

2-Stain the smear with crystal violet by putting a couple of the stain drops and let for 1 min.

3- Wash off by tap water gently; leave it to dry at room temp (by air).

4- Put one drop of cedar oil on the fixed smear examine directly under oil immersion lens.

2-Differential stains or differential staining

In this process, more than one stain and some chemical solutions are used, it is the most important stains in bacteriology named according to the name of its discoverer scientist "Hans Christian Gram" in 1884 .

This technique separates bacteria into 2 groups:- Gram-positive and Gram-negative bacteria.

Steps of Gram staining

1-Prepare a fixed smear.

2-cover the smear with crystal violet and let it stand for 1 min.

3-Wash off the stain by tap water gently.

4-Cover the smear with Gram's iodine solution and let it stand for 1 min.

5-Wash off by tap water gently.

6-Cover the smear with 95% alcohol by putting a couple of drops of this solution on the smear and let it stand for 30 sec.

7-Wash off gently.

8-Cover the smear with safranine for 30 sec.

9-Wash off gently.

10- Let it dry by air.

11- Examine the slide under oil immersion lens to see: Gram – positive bacterial cells which appear as purple cells, and Gram – negative bacterial cells which appear as red cells.

Stains and solutions used in Gram staining

1-Crystal violet: this stain colors all vegetative cells with purple color, it is called "primary stain".

2-Iodine solution: this solution combines with crystal violet and forms an insoluble complex (I-C.V. complex).

This complex is not removed from G+ ve bacterial cells but is removed from G- ve cells by alcohol solution which is used for fixation of the primary stain, therefore, it is called '"mordant solution".

3-Ethanol: it is an organic solvent which is used in concentration about 95% and removes or decolorizes the purple color of the primary stain from G-ve cells but it cannot remove this stain from G+ve cells, this process is called "decolorization" and ethanol is called decolorizer agent. Aceton can be used as alternative decolorizer.

4-Safranine: this stain is used to restain the cells which lost the primary stain after treating with alcohol. These cells are colored with red color, this stain is called "secondary stain".

Factors affecting the efficiency of Gram staining process

1-Thickness of the bacterial smear: a smear with an appropriate amount of m.o must be prepared because thick smear does not allow us to determine the cells shape, arrangement and other details. Furthermore, the thick smear cannot be removed by washing. In contrast, thin smear may lose stains easily, therefore it may lead to false results.

2-Concentration and purity of the solutions and reagents used in the staining: these solutions should be filtered before using.

3-The nature and age of the bacterial culture: it is important to use cultures that are recently cultivated because old cultures may contain dead cells and give false results (G+ve convert into G- ve or reverse).

4-The amount of washing water: the slide must be washed off during subsequent steps of staining with suitable amount of water because excessive water on a smear may dilute the solutions of reagents used in the process of staining.

NOTE: False results that may resulted from staining are:-

1- False positive staining G-ve ---- appear ------> G+ve

Because ethanol is used less than 30 sec and the complex (I.C.V.) has not been removed.

2-False negative staining G+ ve -----appear----> G-ve

Because iodine solution is used less than 1 min and the complex has not been formed perfectly, not contacted to the wall therefore crystal violet has been easily removed by alcohol then the cells stain with safranine and appear G- ve.

Theories explaining the Gram staining

First theory: the chemical complex (iodine- crystal violet) is formed in the bacterial cytoplasm. Because of the high percentage of lipids in the bacterial cell wall of G-ve bacteria which are dissolved after treating by alcohol and permit the complex to exit with the dissolved lipids .

While the percentage of lipids in G+ve cell wall is low, so, the effect of alcohol is low too and this will not allow the complex to exit outside the bacterial cell wall.

Second theory:G+ ve bacteria have a thick layer of peptidoglycan which act as a barrier that prevents the I.C.V . complex from exit during decolorization process .

This layer is also called (murein layer). In contrast, the G-ve bacteria have a thin murein layer, so the bacteria keep the stain for a short time then loose it quickly.

NOTE: Removal of murein layer from G+ve bacteria convert them into G-ve bacteria because they cannot keep the complex.

3-Special stains or special staining

In this process, special stains like: malachite green, carbol fuchsin, india ink are used for diagnosis of some structures and parts of bacterial cells e,g. spores and capsules.

3-Negative staining:

The acidic dye nigrosine will be used to visualize the capsular or sheath that surrounds some bacteria in a process called negative staining. In general ,the size &shape of microorganisms is often less distorted with indirect staining procedures ,especially when sampled from a broth culture. Therefore negative staining is useful whenever the morphology of individual bacteria is in question .

1-After preparing a clean ,greaseless slide ,a small drop of nigrosine is mixed with a small drop from a broth culture or with a quantity of dry material.

2- The drop is spread across the slide using the edge of another slide as a spreader .This same procedures is used for blood smears .

3-After air drying ,the smear is observed using the high dry lens ,or oil immersion if desired .The back ground should be blue –gray . bacteria bacteria will be evident by the absence of any color .

4-Spore staining :

The Schaeffer-Fulton Stain : Spores are extremely resistant structures ,difficult to destroy with spore coats heat or physical and chemical agents only a few genera of bacteria like Clostridium and Bacillus have a spore.

The basic procedure goes like this:

1-Primary stain :malachite green (steam over boiling water for 5mintes)heating allow malachite green to enter the spore coat (,like acid –fast cell walls are resistant to must staining reagents).Vegetative cells take up malachite green as well.

2- Water wash .

3- Spore coats of endospores retain stain (endospores remain green).

4- Water washes malachite green from vegetative cells (Vegetative cells become clear).

5-Counter stain : safranin(20 seconds).

6- endospores retain the malachite green & remain green .

7- Vegetative cells take up the safranin & become clear .

5-Acid fast staining –Ziehl Neelson Method :

Acid fast organisms contain waxes like lipoid all material affecting staining quality and they resist decolorization with acid alcohol .

The basic procedure goes like this:

1-Cover smear with carbolfuchsin steam over boiling water for 8mintes .Add additional stain if stain boils off .

2- After slide has cooled decolorize with acid alcohol for 15 to 20 seconds .

3- Stop decolorization action of acid-rinsing briefly with water .

4- Counter stain with methylene blue for 30 seconds .

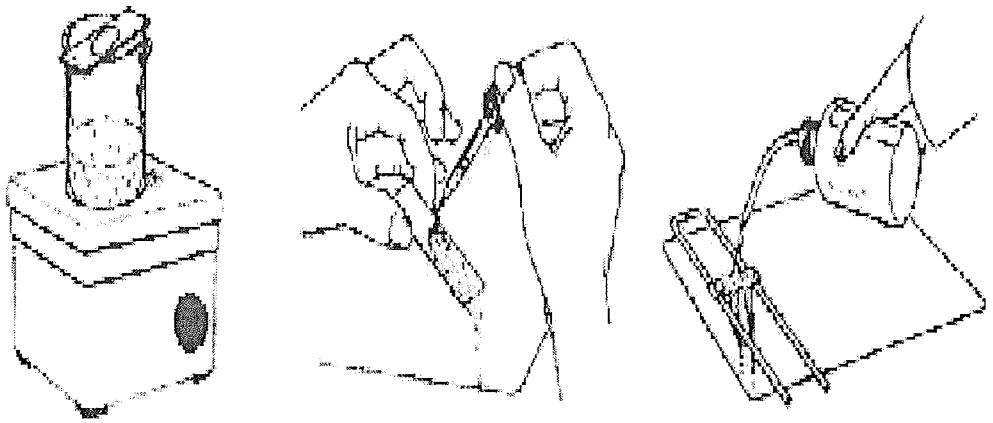
5- rinse briefly with water to remove excess methylene blue .

6- Blot dry with bibulous paper. Examine directly under oil immersion .

6-Flagella stain.

7- Staining of nucleoides .

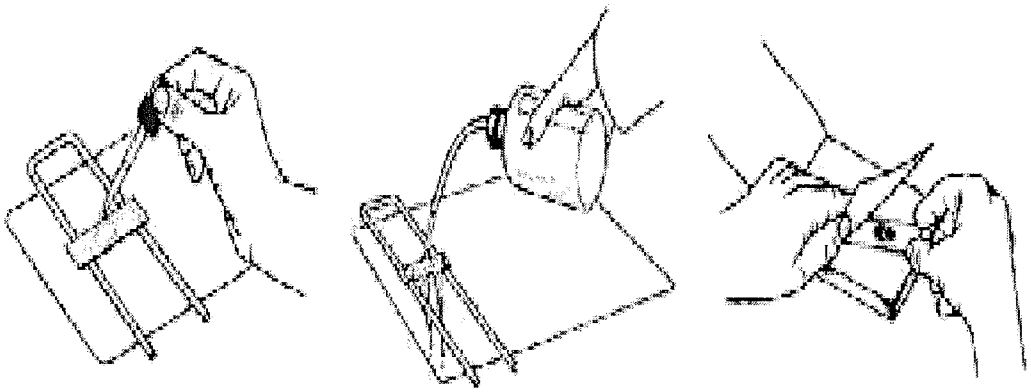
****



1 Cover smear with carbolfuchsin. Steam over boiling water for 8 minutes. Add additional stain if stain boils off.

2 After slide has cooled decolorize with acid-alcohol for 15 to 20 seconds.

3 Stop decolorization action of acid-rinsing briefly with water.



4 Counterstain with methylene blue for 30 seconds.

5 Rinse briefly with water to remove excess methylene blue.

6 Blot dry with bibulous paper. Examine directly under oil immersion.

Ziehl-Neelsen acid-fast staining procedure

Lab. 4

Genus:*Staphylococcus*

Species; *Staph. aureus*
Staph. epidermidis
Staph. saprophyticus

General characteristics

Gram positive, cocci, arranged in cluster, aerobic to facultative anaerobic ,non spore former,uncapsulated,0.8-1 μ in diameter, some are member of the normal flora of the skin & mucous membrane.

Staphylococcus aureus

It is human major pathogen causes infection ranging from minor skin infections to sever infections (septicemia).

Infections caused by *Staph. aureus* :

1. Skin infection including :acne,impetigo,infection of surgical wounds
2. Bacteremia: endocarditis,meningitis,osteomylitis
3. pneumonia
4. Disease associated with toxin production:

*food poisoning→ enterotoxin
*Scalded skin syndrome→ exfolative toxin
* Toxic shock syndrome → TSST-1

- **Specimens → blood, pus, wound swab, tracheal aspirate**

Lab. Diagnostic tests:

1- Gram stain
2- Blood agar →for hemolysis
3- Milk agar → for pigments
4- Nutrient agar → sensitivity & catalase

Catalase Test

$$H_2O_2 \longrightarrow H_2O + O^-_2$$

$$2O^-_2 + 2H^+ \longrightarrow H_2O + O_2$$

Bac. Growth + H_2O_2 (30%) ⟶ bubble (+ve)
(on slide)

5- Staph. 110 media (tolerance to 7.5% Nacl) selective media for Staph.

6- Mannitol salt agar (selective & differential) selective for *Staph.* : Because it contain 7.5% Nacl, differential for *Staph. aureus* because it ferment mannitol (indicator is phenol red).

7- Gelatin test (liquefaction of gelatin)

8- Coagulase test

A. Bounded Coagulase

Fibrinogen ⟶ fibrin

Slide method

One drop of plasma + drop of D.W +1 colony ⟶ clot (+ve)

B.Free coagulase (extracellular enzyme)

Coagulase ⟶ CRF coagulase reactive factor (thrombin) ⟶ coagulase CRF complex + fibrinogen ⟶ fibrin (clot) +ve

Tube method

Add 0.1 ml of broth or few colonies to 0.5 ml citrated or oxalate, human or rabbit plasma after dilution to 1:5 ,incubate in water bath,37C° for 4 hrs (or incubate)to 24 hr. examine for clot formation.

9- DNase test: inoculate DNase plate by 1 line streaking incubate for 24 hrs at 37 C° ,add toludin blue or 0.1 % hydrochloric acid (pink color surround the colonies) +ve

***Drug of choice for *St. aureus* ⟶ cephalosporine, amoxicillin

Catalase test not done on blood agar?

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
pigment	Golden	white	Light yellow
M.S.A. growth	+	+	+
M.S.A. fermentation	+	-	-
coagulase	+	-	-
DNase	+	-	-
Aerobic growth in thioglycolate	+ (after 24 hr)	+(moderate down tube)	
Hemolysis	ß -hemolysis	-	-
catalase	+	+	+
Gelatin liquification	+	+	+
Staph. 110	+	+	+

Lab.5

Genus:*Sreptococcus*

Morphology

Gram positive, cocci, arranged in chain or in pairs, non spore former, non motile, anaerobic to facultative anaerobe,fastidious,the presence of CO_2 enhance the growth as well as humidity, colonies appear very small in blood agar imbedded in the agar like pin point, some time appear Gram negative, rod this variation on shape depend on age of culture.

** Due to autolysis of the cells.

Diseases caused by *Streptococcus*

Group	Hemolysis	Name	Diseases
A	ß	***Strept. pyogenes***	Tonsilitis,bronchopneumonia,scarlet Fever,cellulites,complication: Glomerlonephritis,rheumatic fever
B	ß	***Strept. agalatiae*** (CAMP+ve)	neonatal endocarditis & meningitis
C	ß	***Strept. equisimilis***	throat infection,puerperal fever
D	α	***Strept. faecalis***	UTI
	α	***Strept. viridans***	opportunistic.sub acute endocarditis to heart failure
	α	***Strept pneumoniae*** (optochin +ve)	pneumonia

***Specimens**: throat swab,sputum,pus blood for culture, serum for antibodies determination.

Lab. Diagnostic tests:

1- Gram stain: G+ ve cocci
2- Blood agar →for hemolysis
3-Bile solubility test: presence of bile salt (Na-deoxy cholate or Na-tauro cholate),the surface tension of the salts causes release of autolysis enzymes causes lysis of the cells.
(+) of the test is cell lysis (no growth)
(-) of the test is (growth of the cells)

4-Streptokinase test: indicator for this test is the lysis of the plasma clot (inverse coagulase), we make clot in plasma by adding $Cacl_2$ & we add growth to see streptokinase production.

##All species of ***Strept.*** Produce streptokinase except ***Strept pneumoniae*** (-)

This differentiate between ***Strept*** & ***Staph.*** beside coagulase.
Clot test = 0.2 ml plasma +0.25 ($Cacl_2$) + 0.8 saline +0.5 ml culture
Control = 0.2 ml human plasma +0.25 ($Cacl_2$) + 0.8 saline +0.5 ml saline.

5- Carbohydrate fermentation: to differentiate among ***Strept. ssp.*** Because ***Strept.*** are fastidious Muller-Hinton agar with CHO is used for fermentation, the indicator is bromothymol blue. Better than phenol red, the sugar are glucose, insulin ,mannitol & lactose.

6- Bacitracin & Optochin test: 2 kinds of antibiotics are used in media.

Strept. **pyogenes** (bacitracin +ve),***Strept. pneumoniae*** (optochin +ve).

7- CAMP test

8- Growth in 6.5 % Nacl

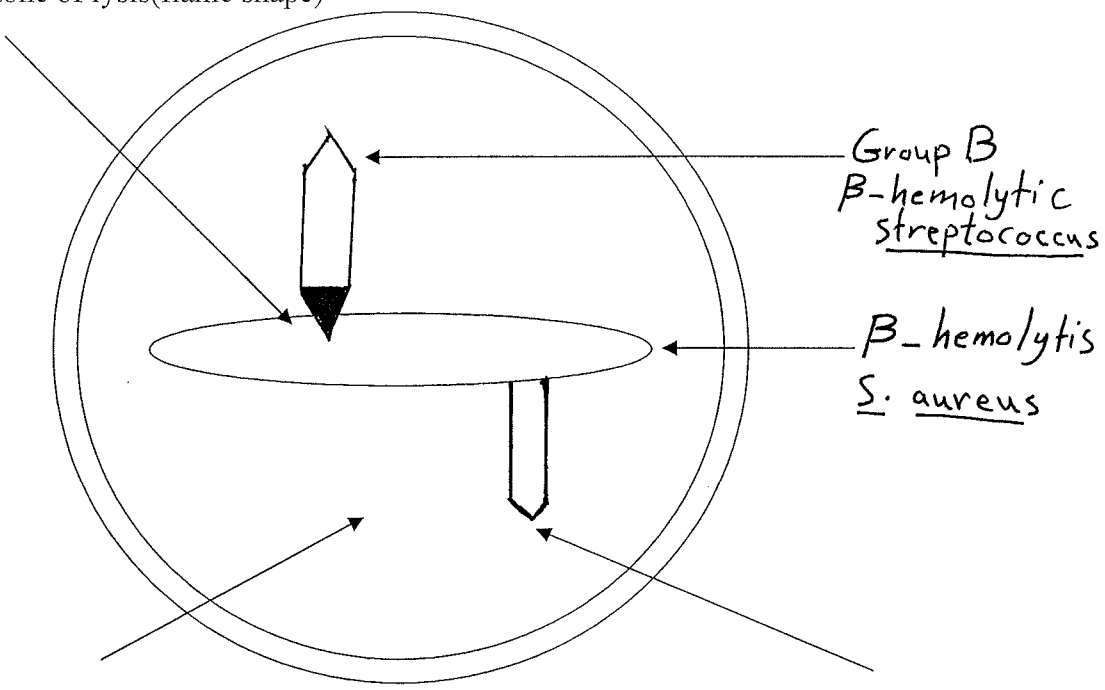
(+ve)⟶turbidity ⟶ ***Strept. faecalis***
(- ve) no growth.

Test	*Strept pneumoniae*	*Strept. viridans*	*Strept. faecalis*	*Strept. pyogenes*
Inulin	+	+\-	-	-
Lactose	-	+	+	+ no gas
Mannitol	-	-	+	+ no gas
Glucose	-	+	+	+ no gas
Bile solubility	(+) no growth	(-) growth	(-) growth	(-) growth
Optochin	+(S)	- (R)	-	-
Bacitracin	-	-	-	+
CAMP	-	-	-	-
Hemolysis	α	α	α	β
Growth at 6.5% Nacl	(-) no growth	(-) no growth	(+) growth	+\-
Streptokinase	-	+	+	+

9- Quelling reaction: this is performed for capsulated ***Strept. pneumoniae*** ,by mixing bacterial growth with standard specific anti-capsular Ag.,swelling of the capsule indicates (+) result.

10- CAMP reaction: we can identify group B ß-hemolytic ***Strept.***,by streaking of ***S. aureus*** that cause ß-hemolysis in the middle of sheep blood agar plate, and by streaking perpendicularly a line of standard strain of group B ß-hemolytic ***Strept.***and on the other side of ***S. aureus*** line, we streak unknown group of ***Strept.*** strain ,hemolysis of ***Staph. aureus*** interact with hemolysis of group B ß-hemolytic ***Strept.*** to form a flame shape or arrow head zone if increased hemolysis between the 2 genera, other group B ß-hemolysis dose not show this shape.

Arrow head zone of increased hemolysis,
Enlarged zone of lysis(flame shape)



Sheep blood agar

The unknown group of ***Strept.***

CAMP reaction

Lab. 6

Family :*Enterobacteriaceae*

Genus :1- *Escherichia coli*

2- Klebsiella pneumoniae

Sub sp. A) K. pneumoniae aerogenes

B) K. pneumoniae adwardi

C) K. pneumoniae atlantae

D) K. pneumoniae oxytoca

E) K. pneumoniae chinoscheromatis

3- *Enterobacter*

4- *Serratia*

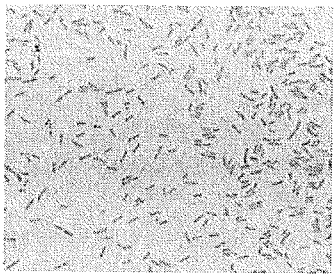
General characteristics

G-ve,bacilli or coccobacilli,catalase +ve, oxidase –ve,capsulated or uncapsulated, motile or non motile, non spore former, aerobic or facultative anaerobe ,pathogenic for human ,animal and plants. Intestinal parasite, normal flora, reduce nitrate to nitrite.

Classification:

1-serological classification upon antigens (O-Ag,H-Ag,K-Ag).
2-biochemical classification & sugar fermentation.
3- DNA-DNA hybridization\ G: C ratio.

Escherichia coli



G-ve,coccobacilli or bacilli,non spore former,motile,facultative anaerobes,can cause:

1- Urinary tract infection (UTI).
2-Neonatal Meningitis.
3-Gastrointestinal infection: Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties.Virotypes includes:

1- Enterotoxigenic ***Escherichia coli***(ETEC):watery diarrhea in infants and travelers,no fever.
2- Enteropathogenic ***Escherichia coli*** (EPEC): infant diarrhea (child under 3 years old).
3- Enterohaemorrhagic ***Escherichia coli*** (EHEC): bloody diarrhea in adult.
4- Enteroinvasive ***Escherichia coli*** (EIEC): invasive ***Escherichia coli*** (toxin producer)dysentray-like diarrhea(mucous,blood),fever.
5- Enteroaggregative ***Escherichia coli*** (EAggEC)): non invasive, persistent diarrhea in young children with out inflammation, no fever.

2- Klebsiella:

G-ve,bacilli,capsulated,the polysaccharide is very thick(mucoid colonies),virulent, resistant to phagocytosis,produce enterotoxin cause diarrhea ,also cause respiratory tract infection and septicemia as well as pneumoniae.

3-Enterobacter aerogenes:

Mostly are non pathogenic, very similar to ***Klebsiella*** in biochemical reaction but they differ from ***Klebsiella*** in motility (***Klebsiella*** non motile while ***Enterobacter*** is motile),it may cause UTI.

Lab. Diagnostic tests:

1- Gram stain:G-ve bacilli or coccobacilli.

2- MacConkey agar:selective & differential

**Selective:

- crystal violet(inhibit G+ve)
- Bile salt(inhibit G-ve other than enteric bacteria)

**Differential: Differential between lactose fermenter (pink colony) & non lactose fermenter(pale colony).

Indicator: neutral red
#Yellow in alkaline pH
#Pink in acid pH.

3- Eosin Methylen Blue(EMB)

**Selective for Enterobacteriaceae(contain methylene blue inhibit G+ve).

Differential for ***E. coli* (green metallic sheen).

Lactose fermentation $\longrightarrow$ Alcohol + Eosin $\longrightarrow$ green metallic sheen.

4- Triple Sugar Iron agar(TSI)

Contain glucose 1%,sucrose & lactose 10%, used for sugar fermentation,H_2S production

##Indicator: phenol red
#Yellow in acid pH
#Pink in alkaline pH.

Gas $\longrightarrow$ bubbles if fermentation occurs (CO_2).

Na-thiosulfate $+H_2 \longrightarrow H_2S$

$H_2S + FeSO_4 \longrightarrow FeS^{+}$ (black precipitate).

6- IMVIC test (Indol,Methyl red,Vogas proskawer,Citrate utilization).

***Indol** test:deamination of tryptophane to pyrovic acid

Medium:peptone water
Substrate:tryptophane
Indicator:Kovacs or Erlich (para dimethyl amine benzaldehyde)
(+) red ring
(-) no change

** **Methyl red-Vogas proskawer**

- Methyl red (MR) glucose full fermentation.
- Vogas proskawer(VP) glucose partial fermentation

Substrate(MR,VP):glucose.

Glucose full fermentation (pyruvic acid+lactic acid+formic+succinic+acetic)

Indicator:MR 2 drops +0.5 ml culture

Red pH< 5 (full fermentation)
Yellow pH> 5.8

Glucose+ H_2O ⟶ Lactic acid / Acetic acid / Formic acid ⟶ $CO_2 + H_2$ methyl red indicator (red color)

Glucose partial fermentation(acetyl methyl carbinol or 2,3 butyleneglycol + acetone) (pH 4.4'0
Indicator: 40% KOH(3 drops),α-naphthol(6 drops) to 1 ml culture(10-20 min).

Glucose+½ O_2 ⟶ Acetic acid ⟶ 2,3 butane diol+acetyl methyl carbinol+ CO_2+ H_2

indicator Pink-red (turbid)

***Citrate utilization**: ability to utilize Na- citrate as the only carbon source in the medium,indicator is(6-7.6)bromothymolblue if utilized pH increase(basic alkaline)the color change to blue.
Bacteria contain a transport system which is begin with utilizing the citrate molecule by citrase enzyme
\Citrate lyase\ enzyme and then make it possible to enter the bacteria cell wall by permease enzyme.

Citric acid —citrase→ oxaloacetic acid+acetic acid ⟶ pyrovic acid +excess of CO_2 +

excess in Na^+ ions

** What is the pH indicator in the citrate test? What color changes occur? Why?

6- Motility test: stab method in semisolid media
7- Urease test: production of ammonia from utilized urea by urease enzyme which increases the pH and covert the color from yellow to pink (rose).

**medium: urea agar.
**Indicater: phenol red.(pH:6.8-8).
+ve(pink in alkaline)
-ve(yellow in acid)

8- Sensitivity test:Muller-Hinton agar
**Sensitive to Aminoglycoside (tobramycin), piperacillin, cephalosporines, chloramphenicol.

Test	*E.coli*	*K. pneumoniae*	*Enterobacter*
Indol	+	-	-
MR	+	-	-
VP	-	+	+
citrate	-	+	+
Urease	-	+	-
Motility	+	-	+
TSI	A/A+ -	A/A+ -	A/A+ -
MacConkey agar	Pink smooth colony	Large than E. coli mucoid colony	Pink colony
EMB	Green metallic sheen	No green metallic sheen large colony	Pink colony

Lab. 7

Family: Enterobacteriaceae

Genus: 1- Proteus

Spp. A) Proteus vulgaris(UTI,wound infection)
B) Proteus mirabilis(UTI,wound infection,nonocomial infction)
C) Proteus myxofacieus (non pathogenic)
D) Proteus inconstans (non pathogenic)
E) Proteus.rettgeri (gastroenteritis)
F) (Morganella) Proteus morganii (summer diarrhea)

General characteristics

G-ve,bacilli,coccobacilli,pleomophic,actively motile with peritrichous flagella,lactose non fermenter,facultative anaerobes,noncapsulated,non spore former, swarming on agar,growth at 25-37 C°.

Natural habitat: some are free living in water,sewage,soil and vegetable,some are normal intestinal flora,pathogenic strain cause :

1- Mainly UTI
2- otitis media
3-wound infection
4- Summer diarrhea in infant

##One of the most causative agents of nosocomial infection?

Urease activity is an important factor determining it is pathogenicity in UTI,forming of NH_3 from urea make urine alkaline which affect the kidney cause kidney infection,NH_3 inactivate complement ($C4^+$),alkalinity cause deposition of phosphate stone in bladder and ureters.

Serological classification is not dependable because of the cross reactivity with Rikettsia (typhus fever).

Differentiation among 4 biotype of ***Proteus***(***mirabilis***, ***vulgaris***, ***myxofacieus***, ***inconstans***)by carbohydrate fermentation.

Enzyme produced by *Proteus*

Proteolytic enzymes which are protease include gelatinase (liquification of gelatin),phenylalanine,deaminase,urease & hemolysin.

Type of hemolysis

1-α- hemolysis : partial appears as agreen zone around the colony.
2-ß – hemolysis : complete hemolysis appear as a clear zone around the colony.
3-γ – hemolysis : non hemolysis .
4-γ – hemolysis : between α and ß ,green and clear zone.

**Highly sensitive to piperacillin,cefotaxime & garamycin.
***Drug of choice → piperacillin

Some factors inhibits the swarming phenomena :

1- 4% agar concentration.
2- Presence of bile salts (MacConkey agar).
3- Anaerobic conditions.

Lab. Diagnostic tests:

1- Gram stain:G-ve bacilli or coccobacilli pleomorphic.
2- Inoculation MacConkey agar.
3- Blood agar (swarming & hemolysis).
4- TSI
5- Urease test
6- IMVIC
7- Gelatin liquification.
8- Phenylalanine.

Phenylalanine $FeCl_3$

Phenylalanine $\xrightarrow{\text{deaminase}}$ phenyl pyruvic $\longrightarrow$ green complex.....1

Phenylalanine $\longrightarrow$ Phenylalanine + NH_3 (indicator converted from green to blue)....2

**indicator /bromothymol blue

9- Maltose (differentiation & fermentation)
10- Glucose (differentiation & fermentation)
11- Sensitivity test.

Test	*Proteus vulgaris*	*Proteus mirabilis*
Urease	+	+
TSI	K/A - +	K/A - +
MacConkey agar	L.N.F.	L.N.F.
Indol	+	-
MR	+	+
VP	-	-
Citrate	+	+
Gelatin	+	+
Phenylalanine	+	+
Maltose	+	-
Glucose	+	+
Motility	+	+
Blood agar	+swarming /α hemolysis	+swarming /α hemolysis

Lab. 8

Family: *Enterobacteriaceae*

Genus: Shigella, Salmonella

A: ***Shigella*** *:Shigella* species are classified to four serogroup:

- Serogroup *A*: ***Sh. dysenteriae*** (12 serotypes)
- Serogroup *B*: ***Sh. flexneri*** (6 serotypes)
- Serogroup *C*: ***Sh.boydii*** (23 serotypes)
- Serogroup *D*: ***Sh. sonnei***(1 serotype)

General characteristics

Gram negative,rod,cylindrical,non motile, non spore former,uncapsulated ,non lactose fermenter,the colonies appear pale on MacConkey agar,they are facultative anaerobic considered as intestinal normal flora of human if present in small number, about 200 cells can pass to the intestine causing infection(highly virulent) the infection caused by contaminated food with fecal materials, growth temp. ranged between(10-42C°),opt. 37C°, *Shigella* causes dysentery that results in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. all *Shigella* ferment glucose without gas except *Sh. flexneri* ,all *Shigella* are L.N. F. exhibit *Sh. sonnei* which are L.F.

Specimens:

Stool during 4-5 days of infection or mucous and blood from intestine or rectal swab for the detection of cells.

Drug of choice Severe dysentery can be treated with ampicillin,TMP-SMX,fluoroqinolones such as ciprofloxacin .

Lab. Diagnostic tests:

1- Gram stain.
2- MacConkey agar (pale colonies)
3- S-S agar (selective & differential):selective for *Salmonella & Shigella* ,differential for *Shigella*,colonies appear pale while *Salmonella* give black colour,the media contain brilliant green inhibitor for other group of enterobacteriaceae and bile salt inhibitor for G+ve,G-ve,the indicator is thiosulfate & ferric citrate for the production of H_2S.
4- IMVIC test
5- Motility
6- Glucose fermentation.
7- TSI test.
8- Mannitol
9- Gelatin.
10- Phenylalanine.
11- XLD agar (xylose,lysine deoxycholate) is a selective media used in the isolation of *Salmonella* and *Shigella* species from clinical samples and from food. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow. Most gut

bacteria, including *Salmonella*, can ferment the sugar xylose to produce acid; *Shigella* colonies cannot do this and therefore remain red. It also contains the sugars lactose and sucrose,sodium thiosulfate for H_2S production.

12- Urease.

13- CDA (citrate deoxycholate agar) selective for *Salmonella & Shigella.*

14- Brilliant green agar (selective & differential):lactose,sucrose,phenol red,brilliant green. All *Salmonella* ssp grow except *Sal. typhi.*

15- Bismuth sulfate agar is used to isolate *Salmonella* species. It uses glucose as a primary source of carbon and bismuth stop gram positive growth.Bismuth sulfite agar tests the ability to utilize ferrous sulfate and convert it to hydrogen sulfide (*S. typhi* which appear as black colonies while other doesn't grow).

Test	*Sh,dysenteriae*	*Sh. flexneri*	*Sh. sonnei*	*Sh. boydii*
Indol	+/-	+/-	-	+/-
MR	+	+	+	+
VP	-	-	-	-
citrate	-	-	-	-
motility	-	-	-	-
glucose	+ no gas	-	+ no gas	+ no gas
manniltol	-	+	+	+
TSI	K/A - -	K/A - -	K/A - -	K/A - -
Phenylalanine	-	-	-	-
Gelatin	-	-	-	-
Urease	-	-	-	-
MacConkey	L.N.F.	L.N.F.	L.F.	L.N.F.
S-S agar	Pale colony	Pale colony	Pale colony	Pale colony

B: Genus : Salmonella

Ssp.:Sal. typhi,Sal.paratyphi A,Sal. paratyphi B,Sal. typhimurium, Sal. enteritidis,Sal. arizona,Sal. choleraesuis,Sal. gallinarium,Sal. schottmuelleri para A

General characteristics

G-ve bacilli, nonspore former,motile except *S. gallinarium*(cause acute enteritis),they are L.N.F.,urease –ve,citrate utilizer, H_2S producer, growth temp.(4-40C°), biochemical test are independable in diagnosis but serotyping is used for identification, all *Salmonella* causes enteritis, *Salmonella* characterized by resistant to some chemical like brilliant green,Na-tetracholate & Na deoxycholate,therefore it is useful to add these chemical to the medium for *Salmonella* isolation & can be used without sterilization source of contamination by human feces , animals, birds & reptiles transferred by direct contact as well as contaminated food & water causing gastroenteritis & food poisoning.

Pathogenicity

A- Acute gastroenteritis 10^5- 10^8 cells caused by *S. typhi* & *S. typhimurium.*

B- Septicemia & complex local infection by all *Salmonella* ssp.

C- Enteric fever (10^4-10^6cells) of *S. typhi.* or *S. prartyphi A* & *B* cause infection.

Specimens:

For isolation: stool, urine, blood & serum for serological identification.

Drug of choice:

Chlormaphenicol & sulfadrug(methabrim).

Biochemical test used to differentiate from *Shigella.*

###Serological diagnosis by widal test for O-Ag & Flagellar Ag (H-Ag) or by phagtyping.

Test	*S. typhi*	*S. typhimurium*	*S. para typhi A*	*S. para typhi B*
TSI	K/A++ gas weak	K/A ++	K/A + -	K/A ++
Indol	-	-	-	-
MR	+	+	+	+
VP	-	-	-	-
Sc	-	+	+	+
Mannitol	+gas	+gas	+gas	+gas
Motility	+	+	+	+
urease	-	-	-	-
S-S agar	Transparent + H_2S	Transparent ++ H_2S	Transparent	Transparent +++ H_2S
MacConkey agar	L.N.F.	L.N.F.	L.N.F.	L.N.F.
Brilliant green	- Pinkish white colony opaque	+	+	+
Bismuth sulfate agar	Black colonies	-	-	-

Lab. 9

Family: Pseudomonadaceae

Genus: 1- ***Pseudomonas***

Spp. A) Pseudomonas aeruginosa
B) Pseudomonas fluorescence
C) Pseudomonas putida
(Fluorescent group)
D) Pseudomonas pseudoalcaligene(opportunistic)
E) Pseudomonas mallei
F) Pseudomonas pseudomallei
G) Pseudomonas cepatia
(Pseudomallei group)

2- ***Acinetobacter***

General characteristics

G-ve,bacilli,motile with polar flagella(monotrichous or polytrichous)& some of them non motile,catalase +ve,oxidase +ve,non spore former, uncapsulated, strick aerobic but can utilize nitrate as a source for respiration.

They are found in soil, any moist areas like water(river and marine),they present in small no. of normal intestinal flora & skin, they are characterized by extracellular pigments, the colors of these pigments differ according to the **S*sp:***

**P. aeruginosa* produce blue green pigment ⟶ pyocyanin

* ***P. fluorescence*** produces yellow to green pigment ⟶ fluorescen (pyoveridin)
Pyoveridin (composed from 2 pigments fluorescen A & fluorescen B).
Other produces red pigment → pyorubin
Some produce black pigment→ pyomelanin.

Pathogenicity

P. aeruginosa is the most important species, it is invasive & toxigenic produce infection in patients with abnormal host defense & is an important nosocomial pathogen, they cause UTI, otitis media & septic shock, and the main infection of ***Pseudomonas*** is burn infection & wound infection.

They may found in antiseptic solution, eye drops, grows well in dettol,heating 55 C° kill ***Pseudomonas*** ,so it could survive in detergents, it shows also resistant to different & multiple antibiotics.

Enzyme & toxin

They are extrecellular include hemolysin,lipase,collagenase,protease,the most important toxin is exotoxin A which cause blockage of protein synthesis which leads to tissue necrosis.

Classification

1- Biochemical.
2- Serological (H-Ag,O-Ag,110 serotype).
3- Pyocin typing ***Pseudomonas*** produce pyocin which is an antimicrobial agent.
4- Phage typing.
5- Sensitivity pattern antibiotics.

Drug of choice: pyopen
Specimens: skin lesion,pus,spinal fluid,sputum & urine.

Lab. Diagnostic tests:

1- Gram stain:G-ve bacilli .
2- Milk agar (for pigmentation).
3- Blood agar (for hemolysis).
4- Kling A,King B (selective & differential)
5- MacConkey agar.
6- TSI.
7- IMVIC.
8- Motility.
9-OF (oxidation-fermentation) contain 1% glucose,bromothymol blue,K_2HPO_4 buffering,add paraffin on the slant to produce anaerobic condition,inoculation by stabing,the colour change to yellow.
10- Nitrate broth.
11- oxidase & catalase.

Test	*P. aeruginosa*	*P. fluorescence*
Indol	-	-
MR	-	-
VP	-	-
SC	+	+
TSI	K/K - -	K/K - -
Nitrate	+	+
Motility	+	+
Growth at 42 C°	+	-
Growth at 4 C°	-	+
King A	+ pyocianin	(- +) - pigment
King B	+ fluorescen	+ fluorescen
MacConkey	L.N.F. transparence, irregular	L.N.F. transparence, irregular
oxidase	+	+
catalase	+	+
OF medium	O (+)/F (-)	O (+)/F (-)

Family: *Vibrioaceae*

Genus: Vibrio

General characteristics

They are curved G-ve(comma shape) ,aerobic rods, motile with single polar flagellum, found in single or in cluster forming S shape, non spore former, on prolong cultivation *Vibrio* may become straight rods.

Vibrio found in nature mostly in water, fishes & food.

Culture:

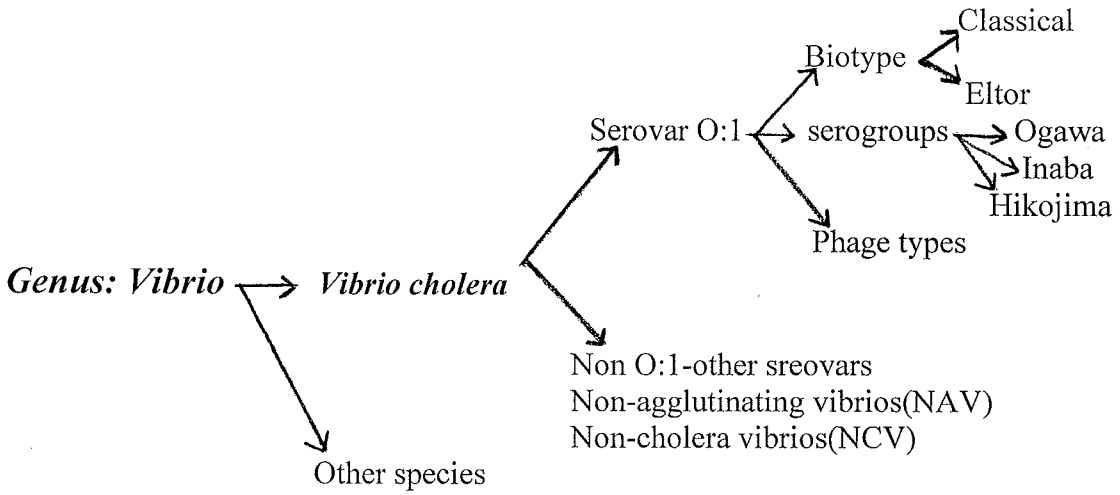
Vibrio produce convex ,smooth round colonies, opaque & granular in transmitted light, they are oxidase +ve,most *Vibrio* grow well at 37° C on media containing mineral salt & amino acids (aspargin,arginin,lysine) as a source of carbon & nitrogen.

These organisms grow at alkaline pH(8.5-9.5) but they are rapidly killed by acid & heating at 55C° for 15 min.,culture containing carbohydrates become sterile after few days.

Vibrio cholra grows well on TCBS (thiosulfate citrate bile sucrose) media.

Serological classification

Vibrio has O & H Ag ***Vibrio cholra*** can be differentiated from other vibrios by O:1 Ag



Vibrio cholera (V.C.): O1,O139
Non vibrio cholera (N.V.C.):O2-O138

V. parahaemolyticus (food-asscciated diarrhea disease)
V. vulnificis(wound infection)
V. alginolyticus (otitis externa,wound infection)

Eltor	Classical
hemolytic	Non hemolytic
Polymixin B (resistant)	Polymixin B (sensitive)
Cause heme agglutination of sheep RBC	dose not
VP. +ve	VP. -ve

Lab. Diagnostic tests:

1-Gram stain. (Weak)
* Motility under dark field (shooting star)

* Cholera immobilization test: bac. (Stool) + Ab ⟶ no movement (+ve)

2- TCBS (Na-citrate,Na-thiosulfate,bile salt,sucrose,bromothymolblue,pH:8)
3- Peptone water NaCl 7%, 0%
4- IMVIC
5- Motility
6-Nitrate reduction test.
7- Kligeler iron agar (TSI without sucrose). +ve green → pink
8- OF media.
9- Mannitol fermentation.
10- Catalase & oxidase test.
11- String test : in the present of bile salt in conc. 5% the growth become elastic if we add on drops to the (taro choate) the colony thread light withdrew by the loop & disappear for 30-60 sec.).
12- Cholera red test.

Tryptophane + conc. H_2SO_4 → nitrosoindole (red color +ve)

Test	*Vibrio cholera*	*Vibrio parahaemolyticus*
Catalase & oxidase	+	+
NO_2 reduction	+	+
Indol	+	+
MR	+ weak	-
VP	-	-
SC	+\-	+\-
Peptone water 7% NaCl	-	+
Peptone water 0% NaCl	+	-
TSI	A\A - -	K\A - -
Motility	+	+
Cholera red	+	-
Mannitol	+ weak	+ weak
String test	+	+
OF media	Oxidation & fermentation	oxidation

13- oxidase test (4,5 tetramethyl paraphenyl diamine dihydro chloride),The oxidase test is based on the bacterial production of an oxidase enzyme.
Transfer 1 colony (not from blood agar) to filter paper by loop then add 1 drop of (4,5 tetramethyl paraphenyl diamine dihydro chloride)
+ve dark violet
-ve no change in color

Lab. 10

Family: *Neisseriaceae*

G: 1-*Moraxella* **(associated with UTI)**
2-*Neisseria*
3-*Branhamella*
4-*Acinetobacter* (mainly associated with abdominal wound infection)

Ssp:• *N. gonorrhea* (also called the gonococcus), which causes gonorrhea.
• *N. meningitidis* (also called the meningococcal), one of the most common causes of bacterial meningitis and the causative agent of meningococcal septicaemia.
• *N. lactamica*
• *Branhaemella catarrhalis (N. catarrhalis* -normal flora of U.R.T.)

General characteristics of *Neisseria*

The *Neisseria* cells appear in pairs, bean like opposite to each other, they are non motile, very fastidious organism, need either serum or heated blood with some supplement (antibiotics specially for the first isolation), it dose not grow well on blood agar because it lake enzymes which destroy (lysis) RBC, optimium condition for growth is present of CO_2 5-10% with moisture & 37C° for the pathogenic strain, they are oxidase, catalase positive & can reduce nitrate. Found associated with or inside polymorphnuclear leukocytes while other *Neisseria* are normally inhibiter of human respiratory tract & occur extracellulary.Gonocci & meningococci are closely related with 70% DNA homology & differentiated by few lab. test & specific characteristic.

***N. meningitides* capsulated, this ssp. Contain 4 serotype Ag, type A is responsible for about 95% of the cases isolated from CSF & from blood.

***N.gonorrhea* include 16 serotype, the Ag of pili is the base of seroclassification,gonococci characterized by ability to produce β–lactamase which inhibit penicillin antibiotic, isolated from urethral discharge & blood.

***Branhaemella catarrhalis*: non capsulated, penicillin sensitive & vancomycin resistant.

Lab. Diagnostic tests:

1- Gram stain.
2- Oxidase test.(+ve)
3- Carbohydrates fermentation (Muller-Hinton + bromothymol blue indicator + pH(6-7)+10% sugar: lactose ,sucrose, glucose ,maltose, fructose)
4- Streaking on chocolate agar (for colonial morphology & pigmentation): The colonies appear very small, concave & greenish on choclate agar.
5- Blood agar
6- Nitrate & nitrite reduction test: inoculate 1-2 loop full of culture to nitrate broth(NO_3) → incubation 24 hr.at 37 C° → 2 ml of nitrate media add 5 drops of Sol.B(α-nephthyl amine)5 drops + sol.A(sulphonilic acid) 5 drops→ after 30 sec.:

Red color ($NO_3 \longrightarrow NO_2$) +ve

No changes in color add trace of Zinc powder:

** No change in color ($NO_3 \longrightarrow N_2$) +ve

** Red color (NO_3 doesn't reduced) –ve

Test	*N. gonorrhea*	*N. meningitidis*	*B. catarrhalis*
Glucose	+	+	-
Maltose	-	+	-
Fructose	-	-	-
Sucrose	-	-	-
Lactose	-	-	-
Pigment	Grayish-white	Grayish-white	Opaque gray
CO_2 requirement	necessary	Not necessary	Not necessary
Growth at 35 C°	+	+	+
NO_2 reduction	-	+/-	+
Morphology	Smooth,non-capsulated	Transparent,flattened,mucoid if capsulated	Opaque,smooth,capsulated

Lab. 11

Genus: Mycobacterium

**Mycobacterium tuberculosis*	*(T.B.)*
**Mycobacterium leprae*	*(leprosy)*
**Mycobacterium bovis*	*(infection similar to T.B.)*
**Mycobacterium avium*	*(potentially pathogen)*
**Mycobacterium marium*	*(potentially pathogen)*
**Mycobacterium phlei*	*(non pathogenic)*
**Mycobacterium smegmatous*	*(non pathogenic)*

General characteristics

Appears rod shape or filamentary bacilli, non spore former, aerobic exhibit *M. bovis* which is microaerophilic, they resist de colorization (acid or alcohol),therefore called acid fast stain bacilli (AFB) .

Morphology & identification

T.B. (tubercles bacilli) in animal tissue are thin straight rods and in artificial medium coccoid and filiaments,the cells characterized by acid fast 95% ethanol alcohol containing 3% HCL while other bacteria are decolorized quicley.this characters depend on the structure of waxy envelopes.

Specimens: sputum or gastric washing, urine, plural fluid, spinal fluid, tissue.

Lab. Diagnosis tests

1-Stained smears: the Zielneelson stain technique of staining is used for identification of acid fast bacteria or by staining with fluorochrome. Mycobacterium appears yellow orange florescent.
2- culture: (Lowenstein –Jensen medium)
* If grow in 1 week (rapid grower).
* If grow in less than 6-8 weeks (potentially pathogenic bacteria)
* If grow more than 8 weeks (pathogenic bacteria)
##If culture is pigmented rather than creamy color (not T. B.)
If culture proliferate in 22-33 C° (not T.B.)
3- Animal inoculation
Note: No serological test used for diagnosis

Lab.12

Genus:Helicobacter pylori

General characteristics

G-ve,spiral,microaerophilic,when cultured in solid media it will become rod or coccoid,highly motile with multiple flagella,most of the species have strong urease activity,catalase +,oxidase +.Species isolated from human *H. pylori,H.cinaedi.H.fennelli.H.heilmannii.*

Ways of transmission:

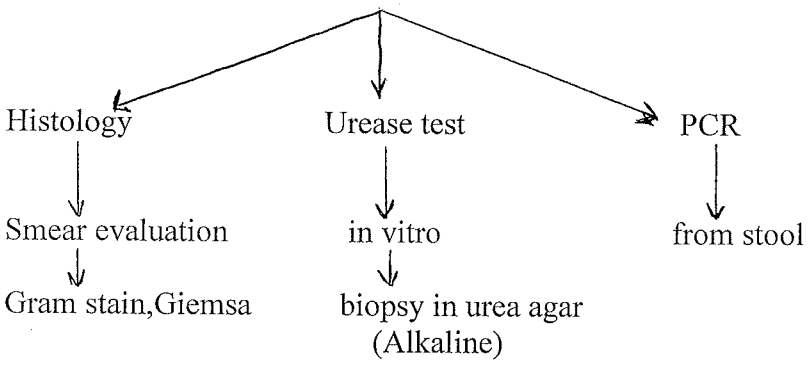
1- fecal-oral route (contaminated food or water)
2-oral-oral route
3-Endoscopist
4-19% in oral cavity
5- *H.cinaedi.H.fennelli* is a part of normal flora in GIT.

Specimens:

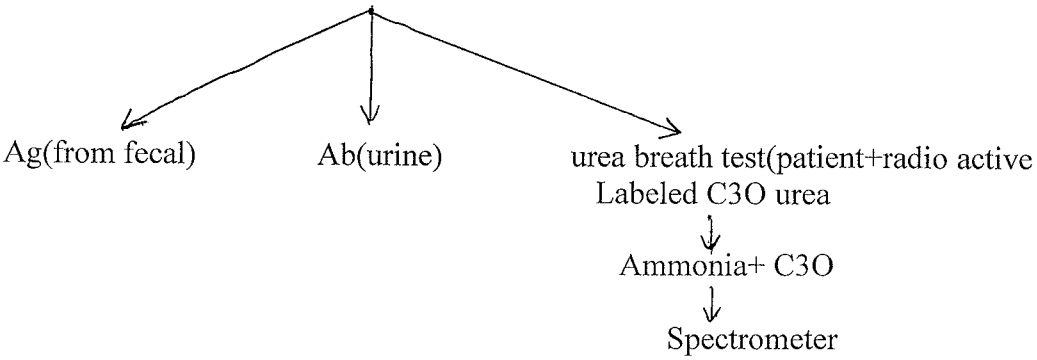
*blood culture (rare).
*Tissue biopsy.
*fecal for *H. pylori* Ag.
*urine (ELISA).
*serum for serology.
*dental plaque.

Lab. Diagnosis tests

1- Invasive LAB methods require biopsy material.



2- Non invasive LAB method →doesn't require biopsy material.



Treatment:Triple drug therapy

Metronidazole + bismuth salt + amoxylline or tetracyciline

Or Metronidazole + opeprazole + clarithromycin for 7-14 days