Enterobacteriaceae

This is a large group of microorganisms that most of them live as a normal flora in the intestine (colon) of human and other animals and may be other parts of the body, and cause several enteric diseases as well as other diseases that involve the respiratory, genitourinary, the meninges and the skin. The classification and groups belong to this family will be studied during the theory lectures.

General characters;

There are some general characters that are shared by all bacteria belong to this family, these are;

1- Gram negative non spore-forming rods. Some are motile, others are capsule producing.
2- Facultative anaerobes
3- Ferment glucose with or without gas
4- Not fastidious but can grow on ordinary media
5- Oxidase negative

There are certain tests and media used for this group of bacteria for characterization, isolation and differentiation between the different genera and also species in the same genus. Such media are:

1- MacConkey agar:

(The main ingredients; bile salts, lactose and neutral red indicator)

This is one of the important media used as a selective as well as a differential medium. As a selective because it contains bile salts that inhibits the growth of contaminated Gram positive cocci that may be found in the specimen, except for ***Strep.faecalis*** that normally live in the intestine. As a differential, because it contains lactose, therefore; the bacteria that ferment lactose will produce acid and change the color of the indicator to red, and the colony will appear red or pink in color, while the non lactose-fermentor will appear yellowish in color.

(Exercise: try to observe the colors of different genera and species on this medium which indicates whether it is lactose-fermentor or non lactose-fermentor and write it down).

2- Eosin methylene blue (EMB) agar:

(The main ingredients: Bile salts, eosin and methylene blue stains)

This is also a selective and a differential medium. As a selective because it contains bile salts (see above), and as a differential; because there are certain bacteria that have the ability to combine these two stains and produce a violet greenish metallic sheen colonies, others do not posses this ability.
(Excersice: observe this phenomenon and write down the bacteria that can and that can't produce this phenomenon).

3- Triple Sugar Iron (TSI) agar;

(The main ingredients: 3 sugars (glucose, lactose and sucrose), iron salts, and phenol red indicator)
This is one of the important differential media used for enterobacteriaceae. It detects whether the organism has the ability to ferment glucose only or all the 3 sugars, produce gas from fermentation or not, and also if the organism can split sulfur from protein and produce H_2S or not.
Glucose is present in only 0.1%, while the other 2 sugars are 1%. The medium is in the slant form, and the organism will be streaked on the surface and also stabbed by a needle to almost the bottom of the agar. During the first 6-8 hours all groups of bacteria will utilize and ferment glucose (by definition) with production of acid which will change the color of the indicator (phenol red) from pink to yellow in the whole tube.

Since glucose is only 0.1% therefore; after further incubation (18-24 hours) it will be soon exhausted, and if the bacteria capable of utilizing and fermenting the other two sugars, this means will continue to produce acid and the color will remain yellow at the slant and at the butt of the tube (slant: acidic, butt: acidic). On the other hand, if the bacteria are not capable of utilizing the other two sugars will switch to peptones present in the medium and splitting it to amino acids and then to ammonia as end product. Since the bacteria on the

surface (slant) grow faster (aerobic) than those at the bottom of the tube (anaerobic), therefore glucose will be exhausted at the slant first and ammonia produced will re-change the color of the indicator to pink, therefore the tube will be read as (slant: alkaline (pink), butt: acidic (yellow)).
The gas produced **(CO_2)** by fermentation will lead to cracking or even pushing the medium upwards, and this is a qualitative test for gas production.
Some species of bacteria has the ability to split sulfur from proteins and produce **H_2S** as an end product; this will combine with iron ions forming **FeS** as a black precipitate coloring the medium.
N.B**.; Kligler Iron Agar** has the same principle of reaction except it contains two sugars (glucose and lactose) instead of three.
(Exercise: try to observe and study all types of fermentations on this medium, and write down the organisms that produce them, and notice the gas production as well as H_2S production and indicate the organisms that do so).

4- Sugar fermentation tests:

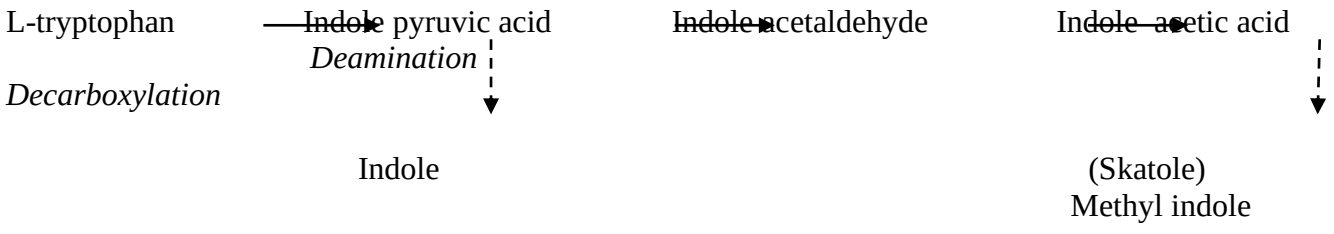
(The main ingredients, broth medium with a single sugar, phenol red indicator, Durham tube)
This test is used to detect the ability of an organism to ferment a specific sugar with or without production of gas. If the organism ferment the sugar the medium will look turbid, and the color of the indicator will be changed from pink to yellow. If there is gas produced from the bacteria at the bottom of the tube, it will be collected by the inverted Durham tube, and amount of the gas collected (which is quantitative) depend on the type of the organism, which could be used as a distinctive character.

5- IMViC test :
I : Indole production
M: Methyl red test
V: Voges Proskeur test
C: Citrate utilization

This test is considered as the most practical test for identification and differentiation between thedifferent species belong to this family.

I. Indole production:

(the medium used is called peptone water which contains the amino acid tryptophan)
Certain bacteria has the ability to split indole from tryptophan molecule. Tryptophan is an amino acid that can be oxidized by certain bacteria to form 3 major indole metabolites: Indole, Methyl indole (skatole) and indole acetic acid (indole acetate). Various enzymes are involved which are collectively called; tryptophanase:



Indole split from the tryptophan molecule , could be detected by a reagent which is either Kovac's reagent (or Erlich reagent) ;

Kovac's reagent:

Pure amyl or isoamyl alcohol (or butyl alcohol) …….. 150 ml.
P-dimethylaminobenzaldehyde…….. 10.0 gm.
Conc. HCl…… 50.0 ml.

Indole combines with the aldehyde present in either Kovac's or Ehrlich's reagent to give a **red colour** (rosindole) floating on the alcohol layer, the negative will give a **yellow colour**.

(Exercise: add the reagent to a 24 hour old culture on peptone water and observe the rings and write down the organisms that give +ve or –ve tests).

II. Methyl red & Voges Proskeur tests:

(The medium used is called MR/VP medium)

This test is used to determine the ability of an organism to continue fermenting the sugars and Producing stable acids despite the pH has reduced to a very low level (less than 4) by ,mostly, mixed acid type of fermentation. Methyl red indicator gives red color under pH of lower than 4, therefore addition of few drops of it to a 48-72 hours old culture will give a dark red color. On the other hand, other bacteria has no such ability, therefore as soon as the acids accumulated and the pH reduced they start to break down the initial fermentation products and also the pyruvic acid by decarboxylation and produce more neutral compounds, such as acetyl methyl carbinol (acetoin) which is a step prior to the 2,3 –butylene glycol product, leading to raise the pH to around 6. This will give methyl red test –ve and Voges Prokeur test +ve. In other words, when methyl red test is +ve, VP will be negative and vice versa.

One molecule of acetoin is formed by the decarboxylation of 2 molecules of pyruvic acid;

2 Pyruvic acid → Acetolactic acid + CO_2 Acetoin → + CO_2 2,3-butylene glycol

NADH

NAD

V.P. reagent;

5% α – nephthol in absolute ethanol alcohol……. 6 drops

40% KOH + 0.3 gm. creatine …… 2 drops

α – nephthol is first added to which combines with acetoin to form diacetyl in the presence of air (O_2) by shaking. Then KOH is added to facilitate the oxidation with the formation of a red-pinkish colour.

(Exercise: try to do these tests, and write down the organisms that give +ve or –ve reactions).

III- Citrate utilization test:

(The medium used is called Simmon citrate medium with bromothymol blue indicator)

The principle of the test depends on the ability of the organism to utilize citrate as the only carbon source in the medium, therefore; the organism will have no other choice either utilize it or it will mostly die.

4 Citrate → acetate + 5 CO_2 + Formate + Succinate

The medium is prepared as a slant in a tube and the organism is streaked on the surface and incubated for 24 hours. The organism that can utilize citrate as a carbon source has also the ability to utilize peptone as a nitrogen source leading to production of ammonia making the medium alkaline and lead to change the color of the indicator (bromothymol blue) from greenish to blue color giving the +ve test. On the other hand, if the organism has no such ability, it will not grow and no color change occurs.

(Exercise; try to do the test and observe the color differences and write down the organisms that give +ve and –ve reactions)

E coli and klebsiella

SALMONELLA & SHIGELLA

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which grade in all directions (i.e.peritrichous).

Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, and foodborne illness.

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause enteritis, systemic infection, and enteric fever.

Salmonella Typhi causes typhoid fever

S choleraesuis cause bacteremia

Salmonella Typhimurium causes enterocolitis

Diagnostic Laboratory Tests

A. SPECIMENS

Blood , Bone marrow , Stool .

B. BACTERIOLOGIC METHODS FOR ISOLATION OF SALMONELLAE

1. **Differential medium cultures**— EMB, MacConkey's, or deoxycholate medium permits rapid detection of lactose nonfermenters. Bismuth sulfite medium permits rapid detection of salmonellae which form black colonies because of H2S production. Many salmonellae produce H2S.

2. **Selective medium cultures**— The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, XLD, or deoxycholate-citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae.

3. **Enrichment cultures**— The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is plated on differential and selective media.

4. **Final identification**— Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

C. BIOCHEMICAL TESTS

1- TSI agar (triple sugar iron) results are read as follow: **Slant/ butt - or + gas , - or + H2S**

Salmonella typhimurium alk /acid +gas, + H2S

Salmonella typhi alk /acid - gas, +H2S

The black color in the tube indicates the production of H2S.

2- IMViC TEST for salmonella is - + - +

D. SEROLOGIC METHODS

Serologic techniques are used to identify unknown cultures with known sera and may also be used to determine antibody titers in patients with unknown illness, although the latter is not very useful in diagnosis of salmonella infections.

1. **Agglutination test**— In this test, known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1, C2, D, and E.

2. **Tube dilution agglutination test (Widal test)**— Serum agglutinins rise sharply during the second and third weeks of salmonella infection. At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer. Serial (twofold) dilutions of unknown serum are tested against antigens from

representative salmonellae. The results are interpreted as follows: (1) High or rising titer of O (= 1:160) suggests that active infection is present. (2) High titer of H (= 1:160) suggests past immunization or past infection. (3) High titer of antibody to the Vi antigen occurs in some carriers.
Results of serologic tests for salmonella infection must be interpreted cautiously. The possible presence of cross-reactive antibodies limits the use of serology in the diagnosis of salmonella infections.

Shigella

Shigella species are classified by four serogroups:

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

The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery. Shigellae are slender gram-negative, non motile rods; facultative anaerobes All shigellae ferment glucose. With the exception of Shigella sonnei , they do not ferment lactose. The inability to ferment lactose distinguishes shigellae on differential media. Shigellae form acid from carbohydrates but rarely produce gas.

Diagnostic Laboratory Tests

A. SPECIMENS

Fresh stool, mucus flecks, and rectal swabs for culture.

B. CULTURE

differential media (eg, MacConkey's or EMB agar) and selective media (Hektoen enteric agar or salmonella-shigella agar),. Colorless (lactose-negative) colonies are seen on MacConkey's . these organisms fail to produce H2S, that produce acid but not gas in the butt and an alkaline slant in triple sugar iron agar medium, and that are nonmotile.

C. BIOCHEMICAL

1- TSI alk / butt –gas –H2S

2- IMViC ± + - -

D. SEROLOGY

Normal persons often have agglutinins against several shigella species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is not used to diagnose shigella infections.

Proteus

Proteus ; Gram-ve , actively motile pleomorphism rods.

Causes UTI, cystitis, wound infections.

Four speices ; *Proteus morgani, Proteus vulgaris, Proteus mirabilis* and *Proteus rettgeri* are distinguished from each other on the bases of fermentation of maltose, mannitol, sucrose and the production of indole.

All Proteus species are indole +ve except *Proteus mirabilis.*

All Proteus do not ferment mannitol except *Proteus rettgeri* (late fermenter).

Labrotory diagnosis;

Specimen; urine, exudates, swabs.

Smear; G-ve bacilli.

Culture;

1- MacConkey…Proteus appear as a non- lactose fermenter.(colorless colonies).
2- EMB…. There is no metallic sheen on EMB.
3- S.S agar….Proteus appear as pale colonies with black centers.
4- Blood agar…highly motile (swarming), therefore they produce swarming spread overgrowth on blood agar plate .swarming is characterized by expanding rings (waves) over the surface of blood agar.

Biochemical tests;

1- I M Vi C test

– – –

\+ + - +

2- **TSI ; K / A Gas +ve H2S +ve**

Or ; A / A Gas +ve H2S +ve

3- Urease test; + ve .. Converting the slant of urea agar from yellow color to pink- purple color due to the utilization of urea by urease enzyme(produced by proteus) and the formation of ammonia converting the medium into an alkaline pH and producing a pink purple color by a change in the phenol red indicator.

Serology; certain serotypes of proteus have cross-reacting Ags with some Rickettsias, It is purely coincidental, but serves as a useful clinical tool to determine if a person has been infected with rickettsia . Mixing the serum of a patient suspected of having a rickettsial disease with Ag from Ab Ag will agglutinate ,indicating +ve results; (weil- felix test).

Typhus group rickettsiae reacts with *P. vulgaris* OX19, and scrub typhus reacts with *P. mirabilis* OXK. The spotted fever group rickettsiae reacts with *P. vulgaris* OX2 and OX19, to varying degrees, depending on the species.

The Weil-Felix Test can be done as either a slide or a tube test. The antigens necessary (OX2, OX19, and OXK) can be obtained commercially.