Enterobacteriaceae

The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (escherichia, shigella, salmonella, enterobacter, klebsiella, serratia, proteus, and others). Some enteric organisms, eg, *Escherichia coli,* are part of the normal flora and incidentally cause disease, while others, the salmonellae and shigellae, are regularly pathogenic for humans. The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. these bacteria may also be called coliforms.

Typical Organisms

The Enterobacteriaceae are short gram-negative rods. Typical morphology is seen in growth on solid media *in vitro*, but morphology is highly variable in clinical specimens. Capsules are large and regular in klebsiella, less so in enterobacter, and uncommon in the other genera(Table-1).

Escherichia

Is a Gram negative rod-shaped, motile bacterium that is commonly found in the lower intestine, *E coli* typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *E coli* by its hemolysis on blood agar, typical colonial morphology with an iridescent "sheen" on differential media such as EMB agar. Over 90% of *E coli* isolates are positive for β-glucuronidase using the substrate 4-methylumbelliferyl-β-glucuronide (MUG). Optimal growth of E. coli occurs at 37°C.

***E. coli* is responsible primarily for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases. The common strains of *E. coli* are:**

1-Enteropathogenic Escherichia coli (EPEC).

2-Enterotoxigenic Escherichia coli (ETEC).

3-Enterohaemorrhagic Escherichia coli (EHEC)

Klebsiella-Enterobacter-Serratia Group

Most infections are due to K. pneumonia ssp. include UTI, pneumonia, septicemia, meningitis (in neonates). Klebsiella species exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate.

Most enterobacter species give positive tests for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Enterobacter aerogenes* has small capsules. Serratia produces DNase, lipase, and gelatinase.

Klebsiella, Enterobacter, and Serratia usually give positive Voges-Proskauer reactions.

Citrobacter

These bacteria typically are citrate-positive and differ from the salmonellae in that they do not decarboxylate lysine. They ferment lactose very slowly.

Table–1: Rapid, Presumptive Identification of Gram-Negative Enteric Bacteria.
Lactose Fermented Rapidly ***Escherichia coli:*** **metallic sheen on differential media; motile; flat, nonviscous colonies (figure.1).** ***Enterobacter aerogenes:*** **raised colonies, no metallic sheen; often motile; more viscous growth** ***Klebsiella pneumoniae:*** **very viscous, mucoid growth; nonmotile (figure.2).**

Diagnostic Laboratory Tests

Specimens

Specimens include fresh stool, urine , and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically. Serum specimens, if desired, must be taken 10 days apart to demonstrate a rise in titer of agglutinating antibodies.

Culture

The specimens are streaked on blood agar and differential media (eg, MacConkey's or EMB agar). Culture on "differential" media that contain special dyes and carbohydrates (eg, eosin-methylene blue [EMB], MacConkey's, or deoxycholate medium) distinguishes lactose-fermenting (colored) from non-lactose-fermenting colonies (nonpigmented) isolates.

E coli **and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. Enterobacter colonies are similar but somewhat more mucoid. Klebsiella colonies are large and very mucoid and tend to coalesce with prolonged incubation. Some strains of** ***E coli*** **produce hemolysis on blood agar.**

IMVC Tests

The identification of enteric (intestinal) bacteria is of prime importance in determining certain food-borne and waterborne diseases. Many of the bacteria that are found in the intestines of humans and other mammals belong to the family Enterobacteriaceae.
The differentiation and identification of these enteric bacteria can be accomplished by using the IMViC tests (Indole, Methyl red, Voges-Proskauer, and Citrate).

Indole Production

The amino acid tryptophan is found in nearly all proteins. Bacteria that contain the enzyme tryptophanase can hydrolyze tryptophan to its metabolic products, mainly, indole, pyruvic acid, and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs; indole is not used and accumulates in the medium. The presence of indole can be detected by the addition of Kovacs' reagent. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium (figures.3).

Methyl Red Test

All enteric bacteria catabolize glucose for their energy needs; however, the end products vary depending on the enzyme pathways present in the bacteria. The pH indicator methyl red detects a pH change to the acid range as a result of acidic end products such as lactic, acetic, and formic acids. This test is of value in distinguishing between *E. coli* (a mixed acid fermenter) and *E. aerogenes* (a butanediol fermenter).
Mixed acid fermenters such as *E. coli* produce a mixture of fermentation acids and thus acidify the medium. Butanediol fermenters such as *E. aerogenes* form butanediol (acetoin), and fewer organic acids. The pH of the medium does not fall as low as during mixed acid fermentation. As illustrated in figure.4, at a pH of 4, the methyl red indicator turns Red (a positive methyl red test). At a pH of 6, the indicator turns yellow (a negative methyl red test).

Voges-Proskauer Test

The Voges-Proskauer test identifies bacteria that ferment glucose, leading to 2,3-butanediol accumulation in the medium. The addition of 40% KOH and a 5% solution of alpha-naphthol in absolute ethanol (Barritt's reagent) will detect the presence of acetoin a precursor in the synthesis of 2,3-butanediol.
In the presence of the reagents and acetoin, a cherry-red color develops. Development of a red color in the culture medium with 15 minutes following the addition of Barritt's reagent represents a positive VP test; absence of a red color is a negative VP test (figure.5).

Citrate Utilization Test

The citrate utilization test determines the ability of bacteria to use citrate as a sole carbon source for their energy needs. This ability depends on the presence of a citrate permease that facilitates transport of citrate into the bacterium. Inside the bacterium, citrate is converted to pyruvic acid and CO_2. Simmons citrate agar slants contain sodium citrate as the carbon source, NH_4^+ as a nitrogen source, and the pH indicator bromothymol blue. This test is done on slants since O_2 is necessary for citrate utilization. When bacteria oxidize citrate, they remove it from the medium and liberate CO_2. CO_2 combines with sodium (supplied by sodium citrate) and water to form sodium Carbonate (an alkaline product). This raises the pH, turns the pH indicator to a blue color, and represents a positive citrate test; absence of a color change is a negative citrate test (figure.6). Citrate-negative cultures will also show no growth in the medium.

Procedure

Indole Production Test

1. Inoculate some tubes of peptone water medium with the bacterium
2. Incubate the tubes for about 24 hours at 37°C.
3. Remove the tubes from the incubator and add 0.5 ml (about 10 drops) of Kovacs' reagent to each tube, and shake the tube gently. A deep red develops in the presence of indole. Negative reactions remain colorless or light yellow.

Methyl Red -Voges Proskauer Tests

1. Using aseptic technique, inoculate a tubes of the MR-VP broth media with the appropriate bacterium by means of a loop inoculation.
2. Incubate all tubes at 37°C for 24 to 48 hours. For slow fermenters, it may take four to five days.
3. Transfer 1/3 of each culture into an empty test tube and set these aside for the Voges-Proskauer test.
4. To the 2/3 of the culture remaining in each tube, add 0.2 ml (about 4 to 5 drops) of methyl red indicator. Carefully note any color change (a red color is positive).
5. Use the 1/3 aliquot from the methyl red test. add 0.6 ml of Barritt's reagent to each culture, and shake vigorously to aerate. Alternatively, about 15 drops of 40% KOH followed by 5 drops of 5%alpha-naphthol.
6. Positive reactions occur at once or within 20 minutes and are indicated by the presence of a red color.

Citrate Utilization Test

1. Using aseptic technique, inoculate the Simmons citrate agar slants with bacteria by means of a stab-and-streak inoculation.
2. Incubate these cultures for 24 to 48 hours at 37°C.
3. Examine the slant cultures for the presence or absence of growth and for any change in color from green to blue. The development of a deep blue color is a positive test.

Motility test (Tube Method):

Prokaryotes move by means of flagella unique to bacteria. Almost all spiral bacteria and must bacilli are motile, whereas essentially none of the cocci bacteria are motile.
Semi sold media used for motility test, this media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. The non-motile bacteria will only grow in the soft agar tube and only the area where they are inoculated. But in the case of motile bacteria, they will grow along the area of stab and will also swim out away from the stabbed area. Thus, a negative result is detected by growth in a distinct zone directly along the stab. A positive result is indicated by diffuse or cloudy growth mostly at the top and bottom of the stab. (figure 7)

The API 20E System

The API 20E System is a standardized, miniaturized version of conventional biochemical procedures used in the identification of Enterobacteriaceae and other gram-negative bacteria. A total of 127 taxa can be identified with this system. The microtubes system that performs 22 standard biochemical tests on pure bacterial cultures from appropriate, primary isolation media.
This system consists of a strip containing 20 chambers (figure 8), each consisting of a microtube and a depression called a cupule. The tubes contain dehydrated substrates. The substrates are rehydrated by adding a bacterial saline suspension. To create anaerobic conditions, sterile mineral oil is added to several of the microtubes. The strip is then incubated for 18 to 24 hours at 35° to 37°C so that the bacterium can act on the substrates.
The strip is read by noting color changes after the various indicator systems have been affected by the metabolites or added reagents. The identification of the unknown bacterium is achieved by determining a seven-digit profile index number and consulting the API 20E Profile Recognition System or the API 20E Profile Index Booklet.

Klebsiella sp

E. coli

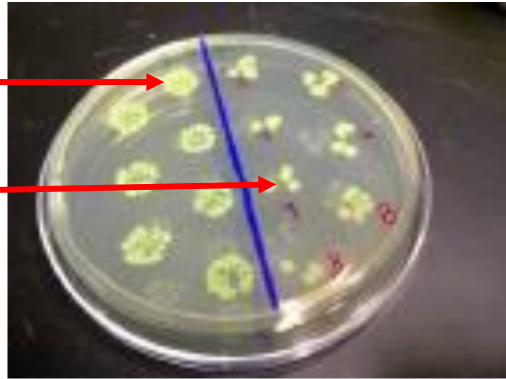


Figure.1A: ***E. coli and Klebsiella sp.***
On nutrient agar medium

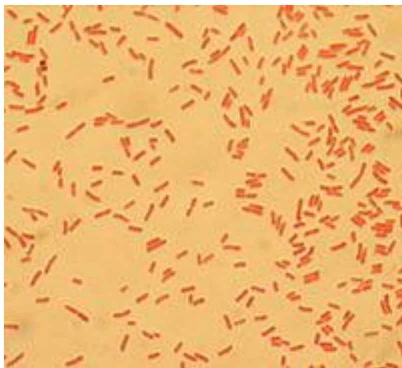
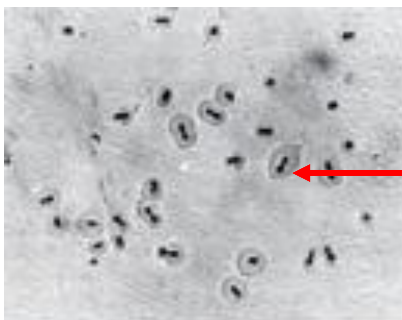


Figure.1B: ***E. coli***

Viscosity due to the Capsule of Klebsiella sp.

Figure.2A: Klebsiella sp.



Swelling due to the Capsule of Klebsiella sp.

Figure.2B: Klebsiella sp.

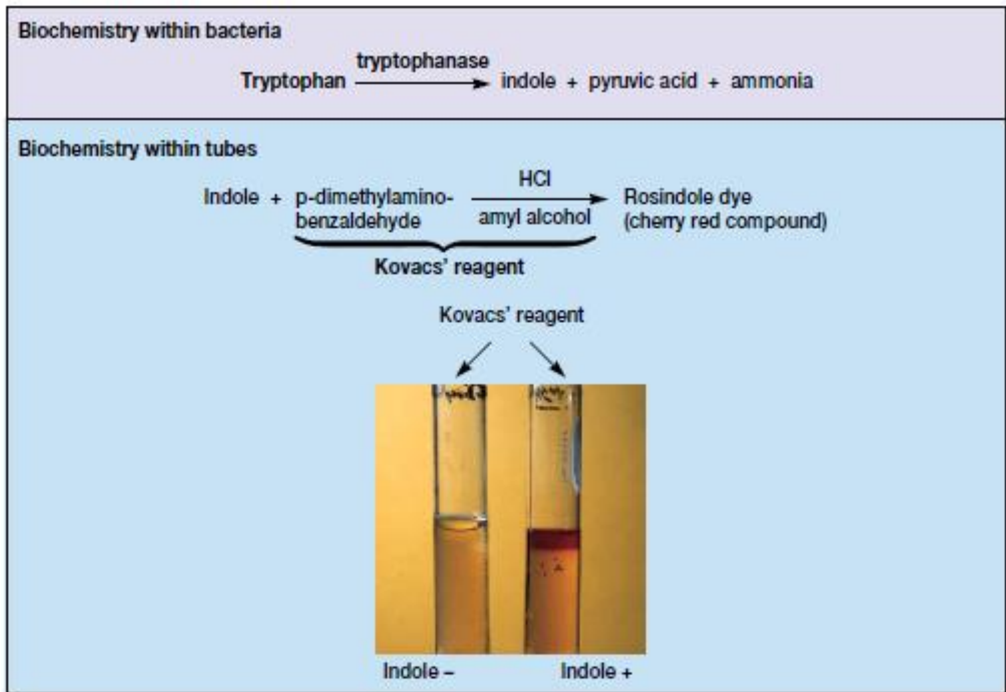


Figure.3: Indole Test.

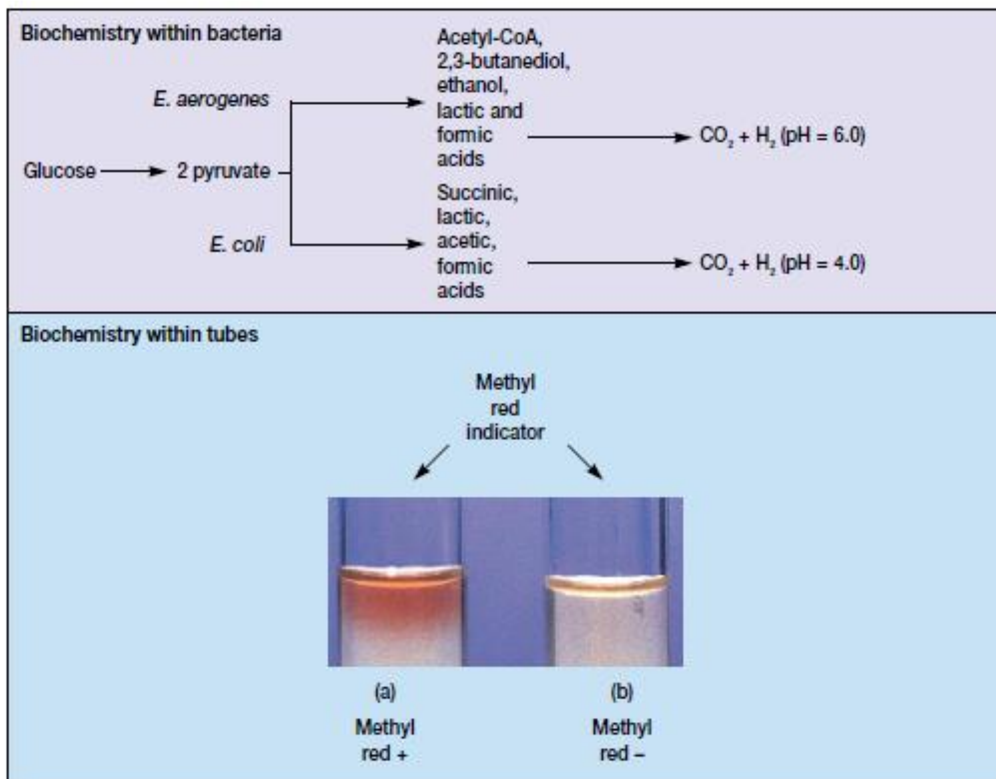


Figure.4: Methyl Red Test.

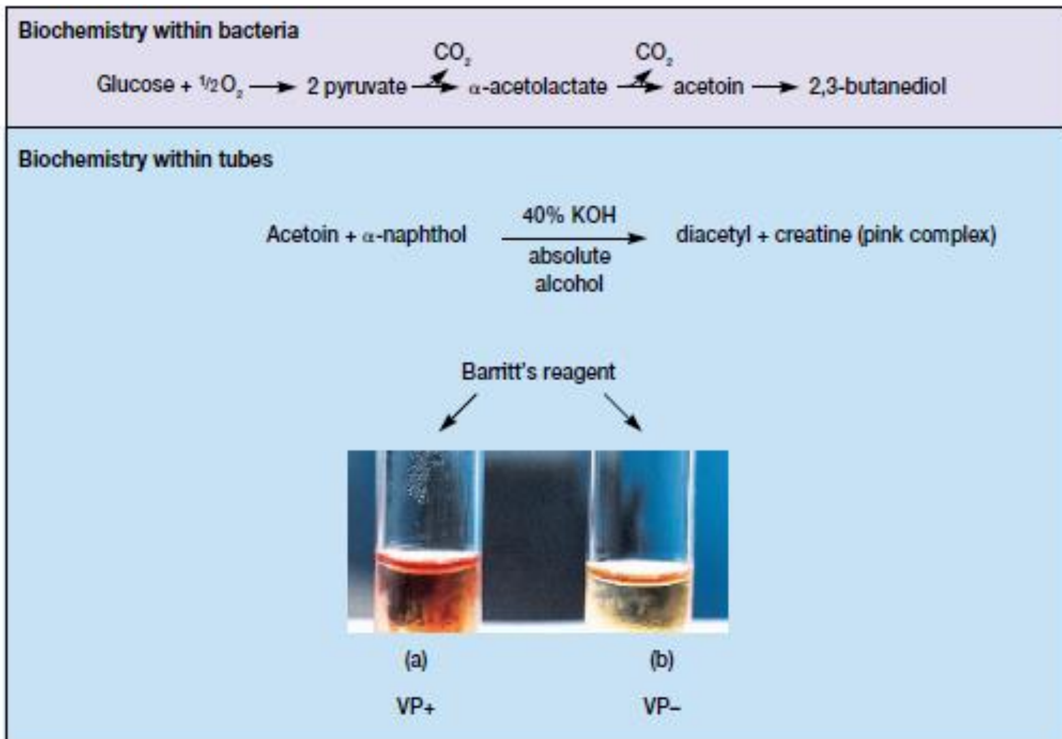


Figure.5:Voges-Proskauer Test.

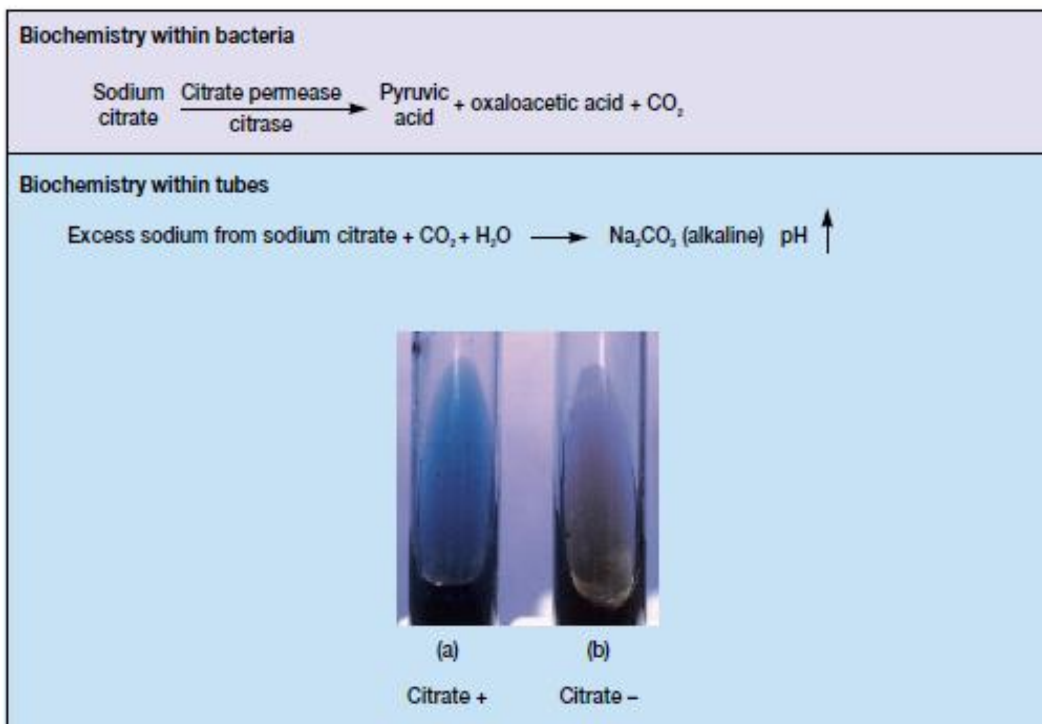


Figure.6: Citrate Test.

Fig. 7: motility test

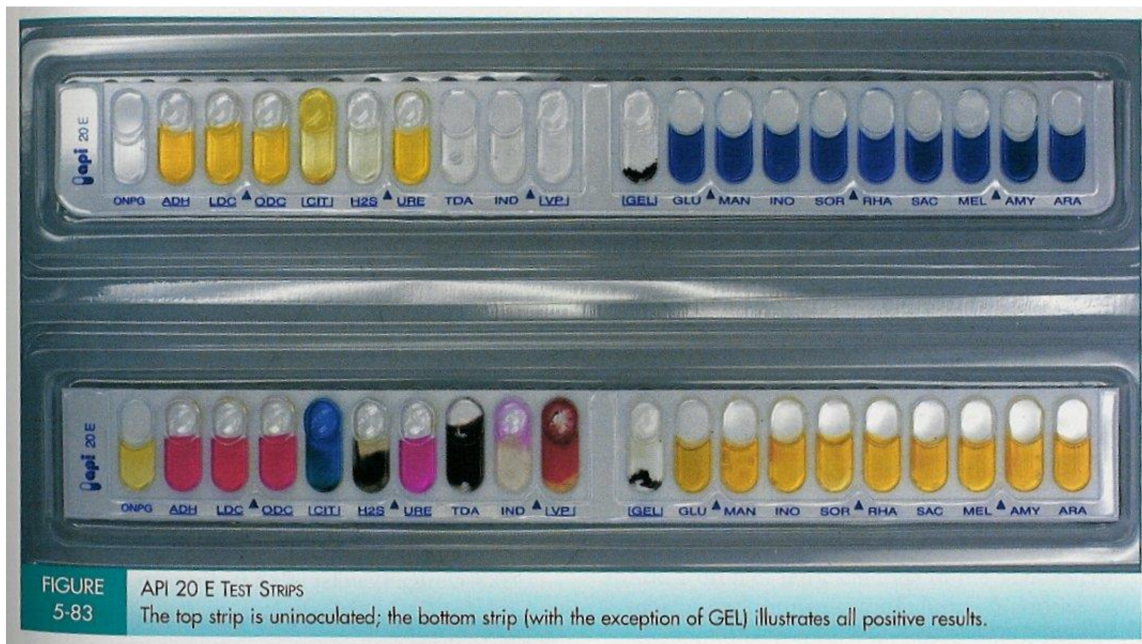


Figure.8: The API 20E strip