LAB 6:

FAMILY: ENTEROBACTERIACEAE

Taxonomy:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

The prefix entero- refers to the Gastrointestinal (GI) tract. Thus, the name Enterobacteriaceae implies that members of this family are bacteria having some association with the GI tract. Some members of the family are indigenous microflora of the colon, and of these, most are opportunistic pathogens. Other members of the family are not indigenous microflora; they are virtually always pathogenic when ingested.

Although there are literally hundreds, perhaps even thousands, of species within the Enterobacteriaceae family, the following are the most clinically significant members:

1- *E. coli*

2- *Klebsiella*

3- *Enterobacter*

4- *Serratia*

General characteristics:

Straight Gram-negative bacilli or coccobacilli, Facultative anaerobes, Non–spore forming, Biochemically active, able of fermenting glucose,

Catalase positive (except *Shigella dysenteriae*), Oxidase negative (except *Plesiomonas shigelloides*), Capable of reducing nitrate to nitrite and Capable of growing on MacConkey agar.

Classification:

1- Serological classification upon antigens:
   *Somatic antigen (O-Ag)
   *Flagellar antigen (H-Ag)
   *Capsular antigen (K-Ag)
2- Biochemical reactions and sugar fermentation.
3- DNA-DNA hybridization and G:C ratio.

Escherichia coli

live in the human gut and are usually harmless but some are pathogenic causing diseases as a result of ingestion of contaminated food or water:

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.

These serotypes include:

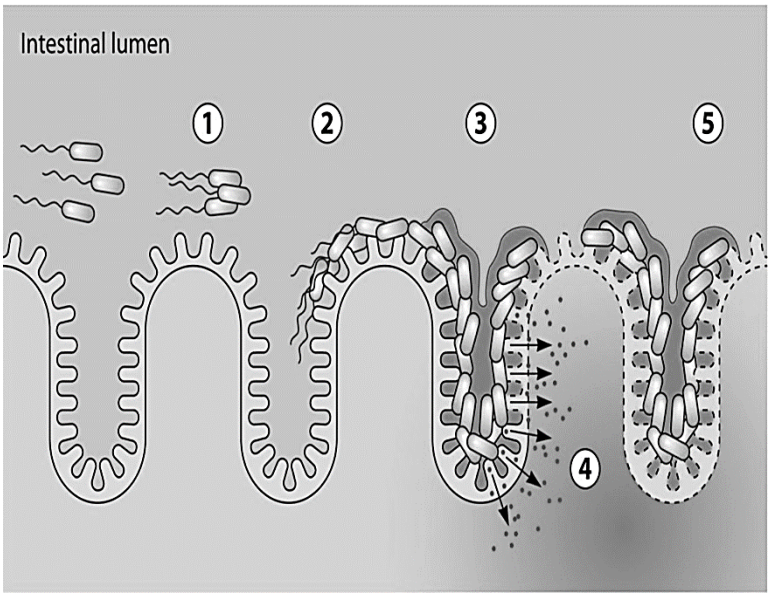
1- Enteropathogenic *E. coli* (EPEC): Certain serotypes are commonly found associated with infant diarrhea.

2- Enterotoxigenic *E. coli* (ETEC): causes diarrhea resembling cholera but much milder in degree. They also cause "travelers' diarrhea".

3- Enteroinvasive *E. coli* (EIEC): causes a syndrome that is identical to shigellosis, with profuse diarrhea and high fever. EIEC are highly invasive, and they use adhesin proteins to bind to and enter intestinal cells.

4- Enterohemorrhagic *E. coli* (EHEC): These are usually serotype O157:H7. These organisms can produce a hemorrhagic colitis (characterized by bloody and copious diarrhea with few leukocytes in afebrile patients). The organisms can disseminate into the bloodstream producing systemic hemolytic-uremic syndrome (hemolytic anemia, thrombocytopenia and kidney failure) which is often fatal.

5- Enteroaggregative *E. coli* (EAggEC): cause acute and chronic diarrhea in both the developed and developing world. EAEC are defined by their "stacked-brick" pattern of adhesion to the intestinal mucosa and form biofilms, thus allowing the bacteria to persistently colonize the intestinal tract.



(1) Agglutination of planktonic EAEC bacteria.

(2) Adherence to the intestinal epithelium and colonization of the gut.

(3) Formation of biofilm.

(4) Release of bacterial toxins, inducing damage to the epithelium and increased secretion.

(5) Establishment of additional biofilm.

E. coli colony on MacConkey agar are dry, pink (lactose positive) and surrounded by pink area.
Ferments glucose, lactose, trehalose, and xylose.
Positive for indole and methyl red tests, negative for Voges-Proskauer and Simmons citrate test (+ + - -).
Does NOT produce H2S or phenylalanine deaminase.

2- *Klebsiella*:

Klebsiella are Lactose fermenter and the cells are capsulated and the polysaccharide is very thick where produce mucoid colonies on agar, capsule conceded as a virulent factor and make them resistant to phagocytosis. *Klebsiella* produce enterotoxin that cause diarrhea, respiratory tract infection and septicemia as well as pneumoniae. *Klebsiella* also ranked as the second agent after *E. coli* that cause urinary tract infections in older patients.

3- *Enterobacter*:

Enterobacter are ubiquitous in nature; their presence in the intestinal tracts of animal's results in their wide distribution in soil, water, and sewage. They are also found in plants. In humans, multiple *Enterobacter* species are known to act as opportunistic pathogens, including *E. cloacae*, *E. aerogenes*, *E. gergoviae*, and *E. agglomerans*. Pathogenic Enterobacter can cause any of a variety of conditions, including eye and skin infections, meningitis, bacteremia (bacterial blood infection), pneumonia, and urinary tract infections. In many instances, illness caused by E. cloacae or by E. aerogenes is associated with exposure to the organisms in nosocomial settings, such

as hospitals or nursing homes. *Enterobacter* very similar to *Klebsiella* in biochemical reaction but they differ in motility (Klebsiella non-motile while *Enterobacter* is motile. Colonies of Enterobacter strains may be slightly mucoid. They are catalase positive and oxidase negative. Nitrates are also reduced.

4- *Serratia*: widely distributed in nature, primarily in soil and water. Most infections caused by *Serratia* spp. are nosocomial infections. The most commonly isolated species is S. marcescens. Although most strains of *S. marcescens* are nonpigmented, some produce a red pigment. marcescens causes a wide variety of infections including: UTIs; respiratory tract, eye, and postoperative wound infections; otitis externa; septicemia; endocarditis; arthritis;
osteomyelitis; and meningitis.

Lab. Diagnostic tests:

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

2- MacConkey agar: as a *selective and **differential media. The Indicator is neutral red which appear Yellow in alkaline pH and Pink in acid pH.

*Selective:

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

**Differential: Differentia between lactose fermenter (pink colony) and non-lactose fermenter (pale colony).

3- Eosin Methylene Blue (EMB)

Eosin Methylene Blue (EMB) agar is both selective and differential. It

contains the dyes eosin Y and methylene blue, which inhibit the growth of gram-positive bacteria and therefore select for gram-negative bacteria. It also contains the lactose, which allows differentiation of gram-negative bacteria based on their ability to ferment lactose. Since the both dyes in the medium are act as a pH indicator and at an acidic pH medium combine to form a green-metallic precipitate (sheen)

4- Triple Sugar Iron agar (TSI):

The medium contains 1.0% each of sucrose and lactose and 0.1% glucose.

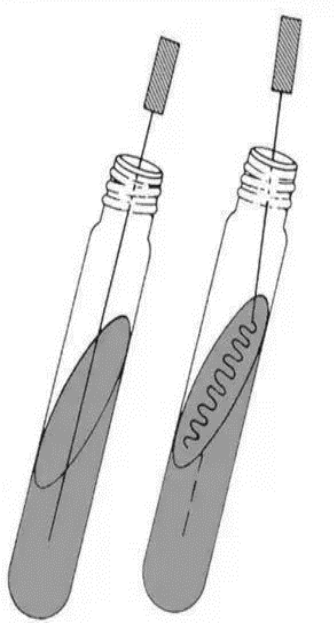
If only glucose is fermented, acid produced in the butt will turn it yellow, but insufficient acid products are formed to affect the phenol red in the slant. However, if either sucrose or lactose are fermented, sufficient fermentation products will be formed to turn both the butt and the slant yellow. If gas is formed during the fermentation, it will show in the butt either as bubbles or as cracking of the agar. If no fermentation occurs (as for an obligate aerobe), the slant and butt will remain red. The medium also contains ferrous sulfate. If the bacterium forms H2S, this chemical will react with the iron to form ferrous sulfide, which is seen as a black precipitate in the butt (a black butt).

Procedure for Triple Sugar Iron Agar (TSI) Test

With a sterilized straight inoculation needle touch the top of a well-isolated colony. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant (see figure below). Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 to 24 hours.

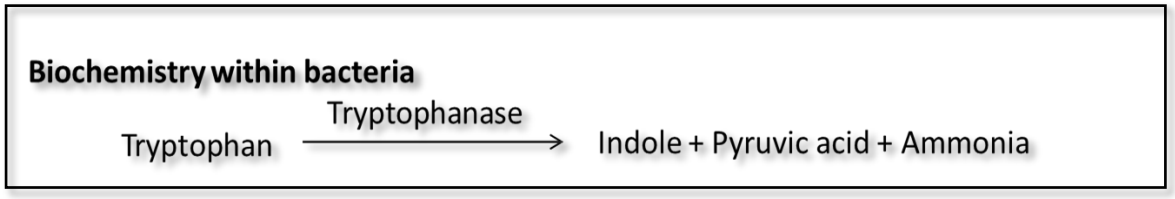
Some examples of Triple Sugar Iron Agar Reactions and inoculation method:

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 (-)

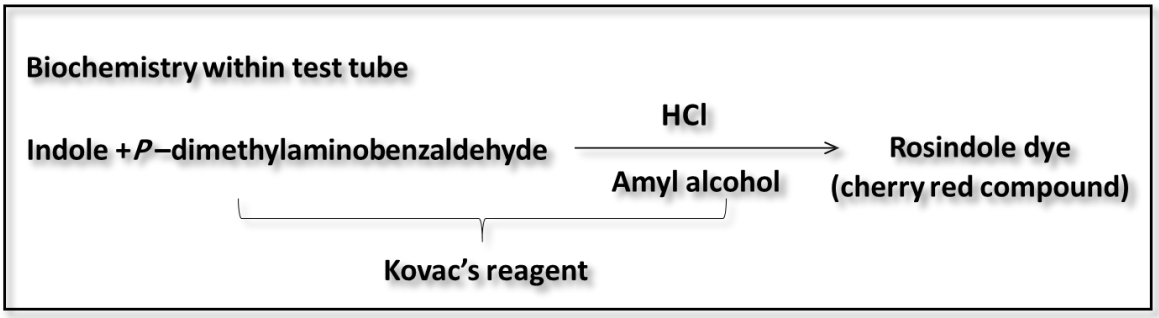


6- IMVIC test (Indole, Methyl red, Vogas Proskauer, Citrate utilization).

1- **Indole test**: it done in peptone water which are rich with tryptophan and it used to determine the ability of an organism to utilize the tryptophan. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products – one of which is indole (see figure below).



Indole production is detected by Kovac's or Ehrlich's reagent (para-dimethylaminobenzaldehyde), this reacts with indole (if present) to produce a red colored compound as a ring in top of the test tube.

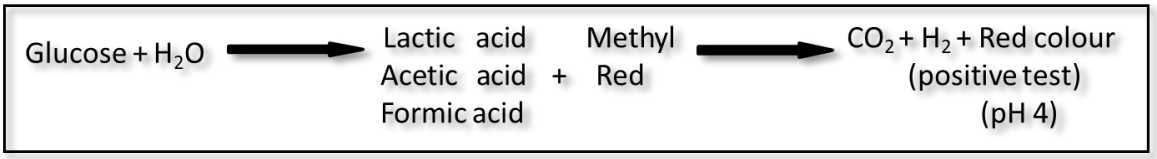


2- MR-VP tests:

Methyl Red (MR) and Voges-Proskauer (VP) tests which are used in the identification of certain fermentative bacteria (e.g. Enterobacteriaceae). These tests are performed together because organisms are generally positive for one of them. These tests are based on the facts that bacteria can ferment glucose into mixed acids or butylene glycol pathway.

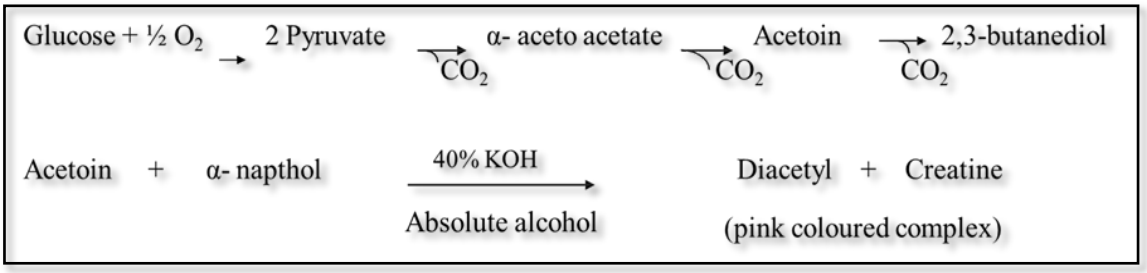
Methyl Red test:

Principle: When grown in a glucose containing medium, some bacteria can produce large amounts of mixed acids such as acetic acid, formic acids or succinic acid from glucose fermentation. The amount of acids produced overcomes the buffering activity of the phosphate buffer included in the glucose phosphate broth thereby rendering the pH of the medium acidic. This acidity is tested by using Methyl Red, a pH indicator. MR reagent is an alcoholic solution of the Methyl Red dye, which remains red at a pH of 4.4 or below.



Voges Proskauer test:

Principle: Some fermenting bacteria undertake the butylene glycol pathway in the fermentation of glucose. These organisms do produce some organic acids but the chief end product of glucose fermentation is 2-3 butylene glycol (2-3 butanediol), a neutral product. However, the VP test detects an intermediate product, the acetyl methyl carbinol (acetoin). Upon the addition of KOH, acetoin is oxidized to diacetyl, which then reacts with the guanidine group of arginine (contained in the peptone) to give rise to a red-colored product.



3- Citrate utilization test: are performed on Simmon citrate agar and used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts (NH4H2PO4) as the sole source of nitrogen.
Bacteria that can grow on this medium produce an enzyme, citrate-permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle.
When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the

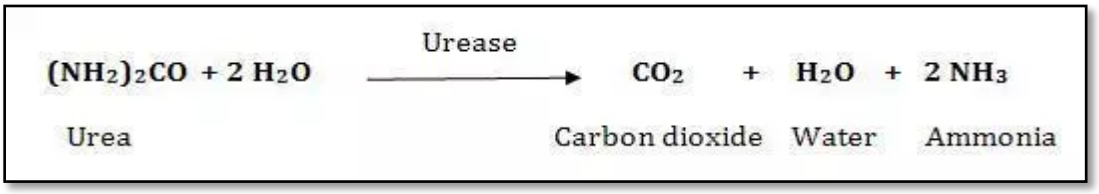
bromothymol blue indicator in the medium from green to blue above pH 7.6.

Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony.

Incubate aerobically at 35 to 37˚C for up 18-24 h, observe a color change from green to blue along the slant.

6- Motility test: Motility by bacterium is demonstrated in semisolid agar by stabbing method.

7- Urease test: Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.



8- Sensitivity test: Muller-Hinton agar

Sensitive to Aminoglycoside (tobramycin), piperacillin, cephalosporines, and chloramphenicol.

Some cultural and biochemical character for some members of *Enterobacteriaceae

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