

## LAB: 6

## Practical Pathogenic bacteria

### Family: Enterobacteriaceae

Enterobacteriaceae is a large family of Gram-negative bacteria. It was first proposed by Rahn in 1936, and now includes over 30 genera and more than 120 species.

Enterobacteriaceae includes, along with many harmless symbionts, many of the more familiar pathogens, such as Salmonella, Escherichia coli, Klebsiella, and Shigella. Other disease-causing bacteria in this family include Enterobacter and Citrobacter. Members of the Enterobacteriaceae can be referred to as enterobacteria or "enteric bacteria",

Members of the Enterobacteriaceae are bacilli (rod-shaped), and are typically 1–5 µm in length. They typically appear as medium to large-sized grey colonies on blood agar, although some can express pigments.

Most have many flagella used to move about, but a few genera are nonmotile. Most members of Enterobacteriaceae have peritrichous, type I fimbriae involved in the adhesion of the bacterial cells to their hosts. They are not spore-forming.

### Some Genera

Citrobacter, Enterobacter, Escherichia, Hafnia, Morganella, Providencia, Klebsiella, Proteus, Salmonella, Shigella and Serratia.

## General characteristics

- 1- Gram negative
- 2- Coccobacilli
- 3- Facultative anaerobes
- 4- None spore forming
- 5- Capsule \ + or –
- 6- Motility \ + or –
- 7- Catalase +
- 8- Oxidase –

## Identification

To identify different genera of Enterobacteriaceae, a microbiologist may run a series of tests in the lab. These include:

- Phenol red
- Tryptone broth
- Phenylalanine agar for detection of production of deaminase, which converts phenylalanine to phenyl pyruvic acid
- Methyl red or Voges-Proskauer tests depend on the digestion of glucose. The methyl red tests for acid end products. The Voges Proskauer tests for the production of acetylmethylcarbinol.
- Catalase test on nutrient agar tests for the production of enzyme catalase, which splits hydrogen peroxide and releases oxygen gas.

- **Oxidase test** on **nutrient agar** tests for the production of the enzyme **oxidase**, which reacts with an aromatic amine to produce a purple color.
- Nutrient gelatin tests to detect activity of the enzyme **gelatinase**.

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- Nutrient gelatin tests to detect activity of the enzyme **gelatinase**.

## Lactose fermenting

Genus: Escherichia

Genus: Klebsiella

Genus: Enterobacter

### *Escherichia coli*

*E. coli* is a genus of Gram-negative, oxidase-negative, catalase positive. Facultative anaerobe, nonsporulating coliform bacterium. Cells are typically rod-shaped, and are about 2.0 µm long and 0.25–1.0 µm in diameter, with a cell volume of 0.6–0.7  $\mu\text{m}^3$ .

The flagella which allow the bacteria to swim have a peritrichous arrangement.

### Culture growth

Optimum growth of *E. coli* occurs at 37 °C, but some laboratory strains can multiply at temperatures up to 49 °C . *E. coli* grows in a variety of defined laboratory media, such as lysogeny broth, or any medium that contains glucose, ammonium phosphate monobasic, sodium chloride, magnesium sulfate, potassium phosphate dibasic, and water. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide, and trimethylamine N-oxide. *E. coli* is classified as a facultative anaerobe. It uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. Respiration type is managed in part by the arc system. The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates.

**Escherichia coli** bacteria normally live in the intestines of healthy people and animals. Most types of *E. coli* are harmless or cause relatively brief diarrhea. But a few strains, such as ***E. coli* O157:H7**, can cause severe stomach cramps, bloody diarrhea and vomiting.

You may be exposed to *E. coli* from contaminated water or food — especially raw vegetables and undercooked ground beef. Healthy adults usually recover from infection with ***E. coli* O157:H7** within a week. Young children and older adults have a greater risk of developing a life-threatening form of kidney failure.



**Figure : *Escherichia coli* on MacConkey agar (Dry pink colonies )**

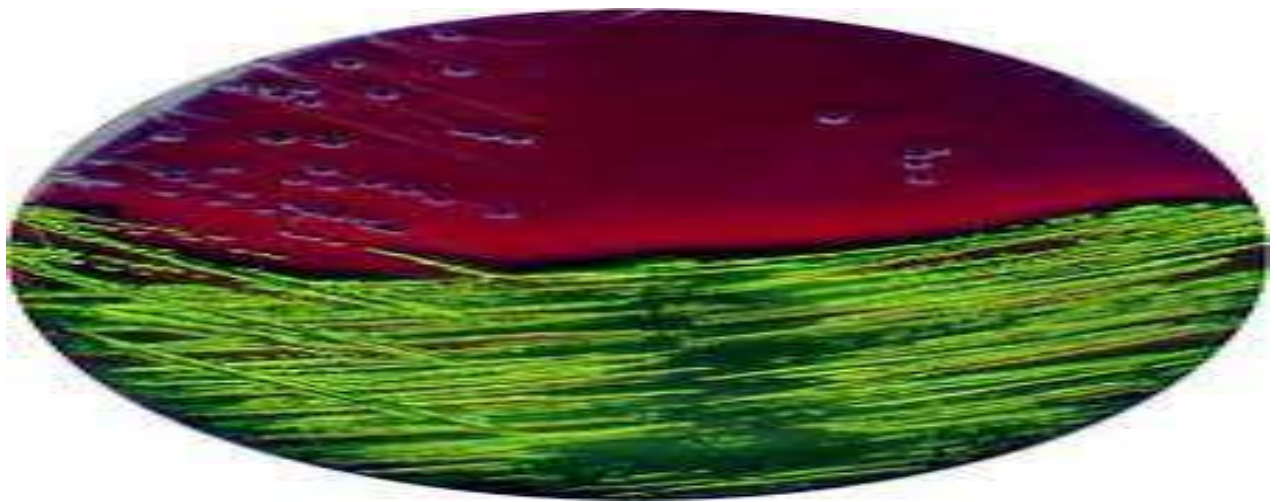


Figure : *Escherichia coli* on EMB agar (Dark colony and Green metallic sheen ).

### characteristics

- 1- Gram negative
- 2- Coccobacilli
- 3- Facultative anaerobes
- 4- None spore forming
- 5- Capsule: have microcapsule
- 6- Motility : 80% motile , 20% none motile
- 7- Catalase : positive
- 8- Oxidase : negative
- 9- Urease : negative
- 10- IMViC test : positive , positive . negative , negative
- 11- TSI agar test : Acid \ Acid , CO<sub>2</sub> + , H<sub>2</sub>S –
- 12- Lactose fermenting (**Dry pink colonies**)

## Klebsiella spp

*Klebsiella* is a genus of Gram-negative, oxidase-negative, catalase positive rod-shaped bacteria with a prominent polysaccharide-based capsule.

*Klebsiella* species are found everywhere in nature. They can be found in water, soil, plants, insects and other animals including humans.

The members of the genus *Klebsiella* are a part of the human and animal's normal flora in the nose, mouth and intestines. The species of *Klebsiella* are all gram-negative and usually non-motile. They tend to be shorter and thicker when compared to others in the family Enterobacteriaceae. The cells are rods in shape and generally measures 0.3 to 1.5 µm wide by 0.5 to 5.0 µm long. They can be found singly, in pairs, in chains or linked end to end. *Klebsiella* can grow on ordinary lab medium and do not have special growth requirements, like the other members of Enterobacteriaceae. The species are aerobic but facultative anaerobic. Their ideal growth temperature is 35° to 37 °C, while their ideal pH level is about 7.2.

They have no specific growth requirements and grow well on standard laboratory media, but grow best between 35 and 37 °C and at pH 7.2. The species are facultative anaerobes, and most strains can survive with citrate and glucose as their sole carbon sources and ammonia as their sole nitrogen source.

Members of the genus produce a prominent capsule, or slime layer, which can be used for serologic identification, but molecular serotyping may replace this method.

Members of the genus *Klebsiella* typically express two types of antigens on their cell surfaces. The first, O antigen, is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. Both

contribute to pathogenicity and form the basis for [serogrouping](#). Based on those two major antigenic determinants several vaccines have been designed.

- *K. pneumoniae* (type-species)
- *K. granulomatis*
- *K. oxytoca*
- *K. michiganensis*
- *K. quasipneumoniae*
- *K. grimontii*
- *K. variicola*



Figure : *Klebsiella* on MacConkey agar (mucoid pink colonies )





Figure : *Klebsiella* on MacConkey agar (mucoid pink colonies )



Figure : *Klebsiella* on EMB agar (mucoid purple colonies )

## General characteristics

- 1- Gram negative
- 2- Coccobacilli
- 3- Facultative anaerobes
- 4- None spore forming
- 5- Capsule: positive
- 6- Motility : none motile
- 7- Catalase : positive
- 8- Oxidase : negative
- 9- Urease : positive
- 10- IMViC test : negative , negative, positive ,positive
- 11- TSI agar test : Acid \ Acid , CO<sub>2</sub> + , H<sub>2</sub>S –
- 12- Lactose fermenting (**muroid pink colonies**)

## *Enterobacter spp*

*Enterobacter* is a genus of common Gram-negative, facultative anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. It is the type genus of the order Enterobacteriales. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermo tolerant coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts.

The genus *Enterobacter* ferments lactose with gas production during a 48-hour incubation at 35-37 °C in the presence of bile salts and detergents. It is oxidase-negative , catalase- positive , indole-negative, and urease-variable .



Rough and smooth colony growth of *Enterobacter cloacae* bacteria on Tryptic Soy agar

## General characteristics

- 1- Gram negative
- 2- Coccobacilli
- 3- Facultative anaerobes
- 4- None spore forming
- 5- Capsule : V
- 6- Motility : motile
- 7- Catalase : positive
- 8- Oxidase : negative
- 9- Urease : V
- 10- IMViC test : negative , negative, positive ,positive
- 11- TSI agar test : Acid \ Acid , CO<sub>2</sub> + , H<sub>2</sub>S –
- 12- Lactose fermenting (**muroid pink colonies**)



The encapsulated strain of *Enterobacter aerogenes* on MacConkey medium



*Enterobacter aerogenes* on MacConkey medium

*Enterobacter cloacae* is a facultative anaerobic Gram-negative bacterium of size 0.3-0.6 x 0.8-2.0 µm. It is lacking capsule and spore and it is motile due to flagella which is a member of the [Enterobacteriaceae family](#). *E. cloacae* is the normal gut flora of many humans. It is one of the common nosocomial pathogens capable of causing a wide variety of infections, like pneumonia, urinary tract infections (UTIs), and septicemia. Most of *Enterobacter* species are common carbapenem-resistant (meropenem, imipenem, and ertapenem).

## Biochemical Reactions of *Enterobacter cloacae*

Basic Features	Properties
1. Gram Staining	Gram-Negative Rods (GNRs)
2. Spore	Non-Sporing
3. Capsule	Negative
4. Motility	Motile
5. Pigment	Negative
7. Catalase test	Positive
8. Oxidase test	Negative
9. Nitrate reduction test	Positive

10. MR (Methyl Red) test	Negative
11. VP (Voges- Proskauer) assay	Positive
12. OF (Oxidative-Fermentative) test	Fermentative
13. Gas	Positive
14. H <sub>2</sub> S production	Negative
15. Indole formation	Negative
16. Urease/ urea hydrolysis test	Negative
17. Citrate/ citrate utilization	Positive
18. DNase test	Negative
19. Glucose fermentation	Positive
20. Maltose fermentation	Positive
21. Lactose fermentation	Negative
22. Sucrose fermentation	Positive
23. Xylose fermentation	Positive
24. Mannitol fermentation	Positive

## Biochemical Reactions of Enterobacter aerogenes

Basic Features	Properties
1. Gram Staining	Gram-Negative Rods (GNRs)
2. Spore	Non-Sporing
3. Capsule	Small capsule
4. Motility	Motile
5. Pigment	Negative
7. Catalase test	Positive
8. Oxidase test	Negative
9. Nitrate reduction test	Positive
10. MR (Methyl Red) test	Negative
11. VP (Voges- Proskauer) assay	Positive
12. OF (Oxidative-Fermentative) test	Fermentative
13. Gas	Positive
14. H <sub>2</sub> S production	Negative
15. Indole formation	Negative



<b>16. Urease/ urea hydrolysis test</b>	<b>V</b>
<b>17. Citrate/ citrate utilization</b>	<b>Positive</b>
<b>18. DNase test</b>	<b>Negative</b>
<b>19. Glucose fermentation</b>	<b>Positive</b>
<b>20. Maltose fermentation</b>	<b>V</b>
<b>21. Lactose fermentation</b>	<b>Positive</b>
<b>22. Sucrose fermentation</b>	<b>Positive</b>
<b>23. Xylose fermentation</b>	<b>Positive</b>
<b>24. Mannitol fermentation</b>	<b>Positive</b>

<b>Biochemical characteristics</b>	<b><i>E. coli</i></b>	<b><i>E. aerogenes</i></b>
Indole production	+	-
Methyl Red	+	-
Voges Proskauer	-	+
Citrate utilization	-	+
Catalase production	+	+
Galactose	AG	AG
Glucose	AG	AG
Lactose	AG	AG
Maltose	AG	AG
Mannitol	AG	AG
Sucrose	A	AG
Starch	Nil	AG

<b>Biochemical test</b>	<b><i>E. coli</i></b>	<b><i>K. pneumoniae</i></b>
Catalase	+	+
Triple sugar agar TSI	+	+
Indole production	+	-
Methyl red	+	-
Urease	-	+
Voges proskauer	-	+
Simmon's citrate	-	+
H <sub>2</sub> S	-	-

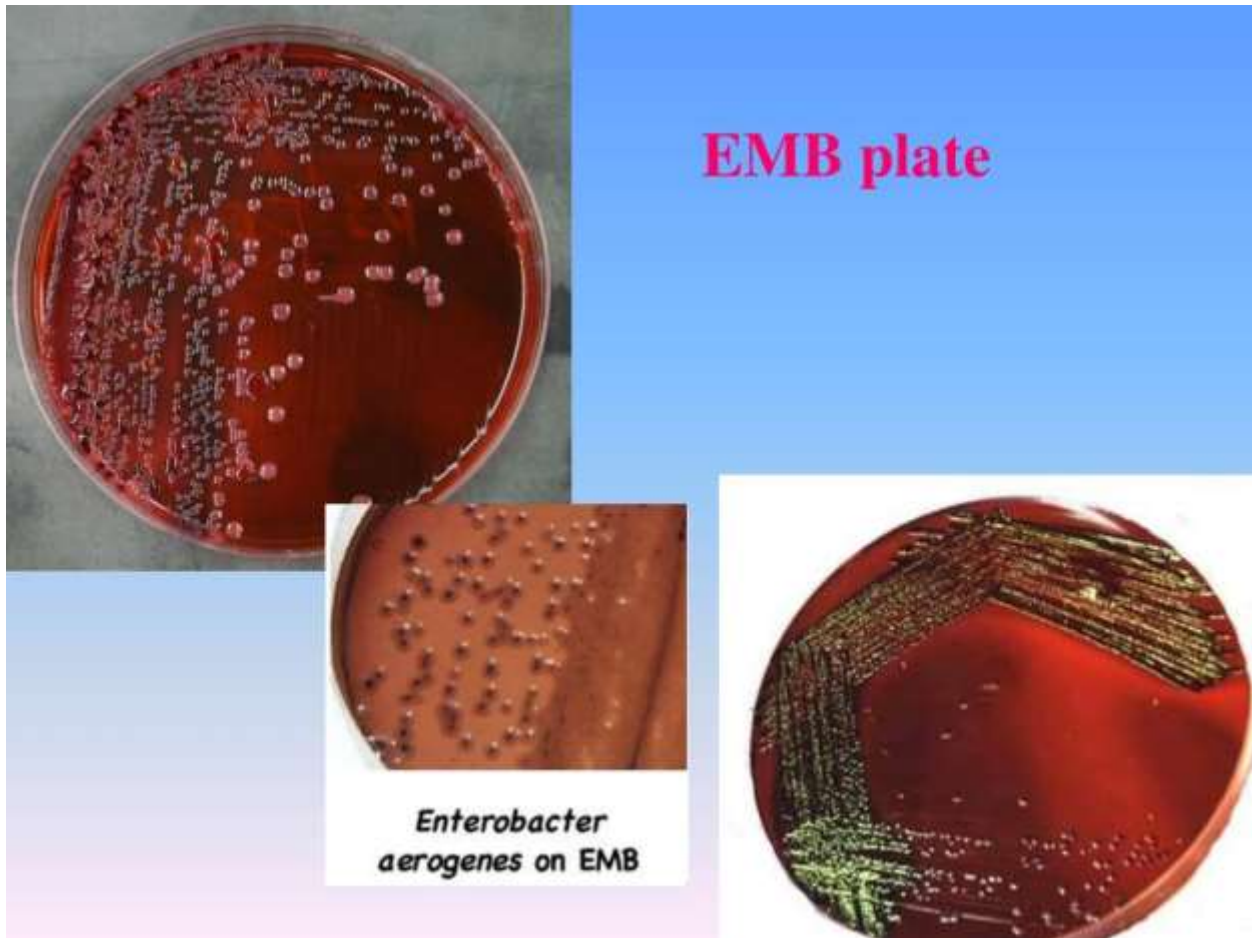
\*Results based on the types of substrate utilization: Positive (+) and negative (-).



***Enterobacter cloacae***



***Enterobacter aerogenes***



Note :

### Eosin Methylene Blue Agar (EMB)

**\*Selective/Differential\***

**Selective for: Gram (-)**

**\*Components:\***

**Eosin dye/MB dye; Lactose**

**Results:**

**\*Differentiates\***

**between lactose and non-lactose fermenters:**

- ( Some acid = pink )
- ( Large acid = metallic green )
- ( No acid = colorless )

**(E. aerogenes = Pink, E. coli = Green (Lactose fermentation),  
Pseudomonas ssp = Clear.)**

Tests	E.coli	Klebsiella	Enterobacter
Catalase	+	+	+
Oxidase	-	-	-
Lactose	F	F	V
Urease	-	+	V
IMViC	+,+,-,-	-,-,+,+	-,-,+,+
TSI	A\A CO2+ ,H2S -	A\A CO2+ ,H2S -	A\A CO2+ ,H2S -
Motility	+	-	+
Spore	-	-	-
Capsule	microcapsule	have	V
H2S	-	-	-
EMB agar	Green metallic sheen	Mucoid pink to Purple	Pink to Purple
MacConkey agar	Pink	Mucoid pink	Pink
Glucose	F	F	F

## Lab Diagnostic tests

1-gram stain

2-IMViC test

3-TSI agar test

4-Catalase test

5-Oxidase test

6-Urease test

7-MacConkey agar

8-EMB agar

9-Blood agar .....10-Motility test