

Gram-negative rods

Enterobacteriaceae

Lab. 6

General Characters of Enterobacteriaceae:

- Members of the Enterobacteriaceae are gram-negative rods, non-spore forming that grow both aerobically and anaerobically on ordinary media including MacConkey agar (i.e. non-fastidious).
- They are Oxidase negative and Catalase positive. They ferment glucose and other carbohydrate with production of acid or acid and gas, and they reduce nitrates to nitrites.
- They are motile with peritrichous flagella except *Klebsiella*, *Shigella* and *Yersinia*; they are non-capsulated except *Klebsiella* sp.

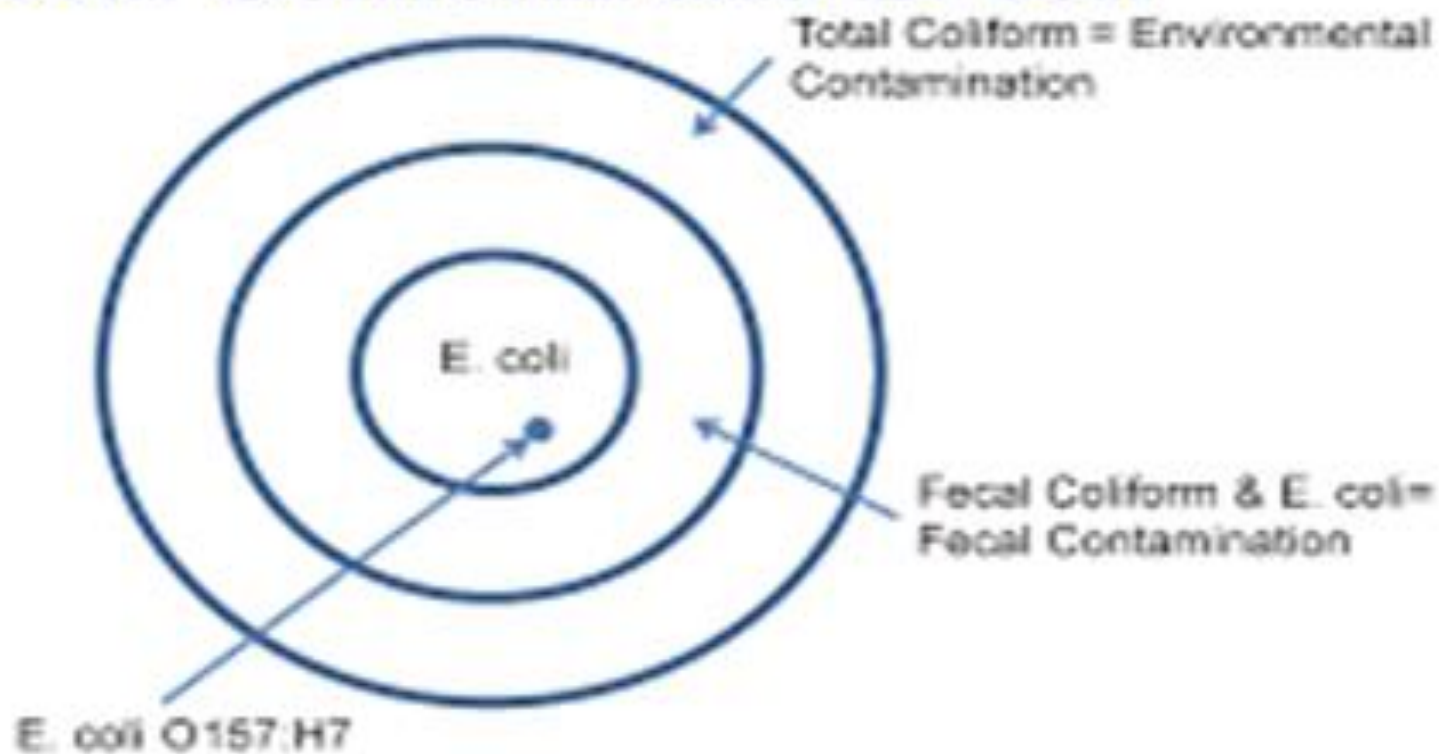
- **Natural Habitat:**

1. Enterobacteriaceae are ubiquitous in nature, Except for few, most are present in the intestinal tract of animals and humans as commensal flora; therefore, they are sometimes call “Coliform group”

Coliform group: Rapid Lactose Fermentating enteric bacteria that are normal and opportunistic include, *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Serratia*, (some strains of *E.coli* are true pathogens).

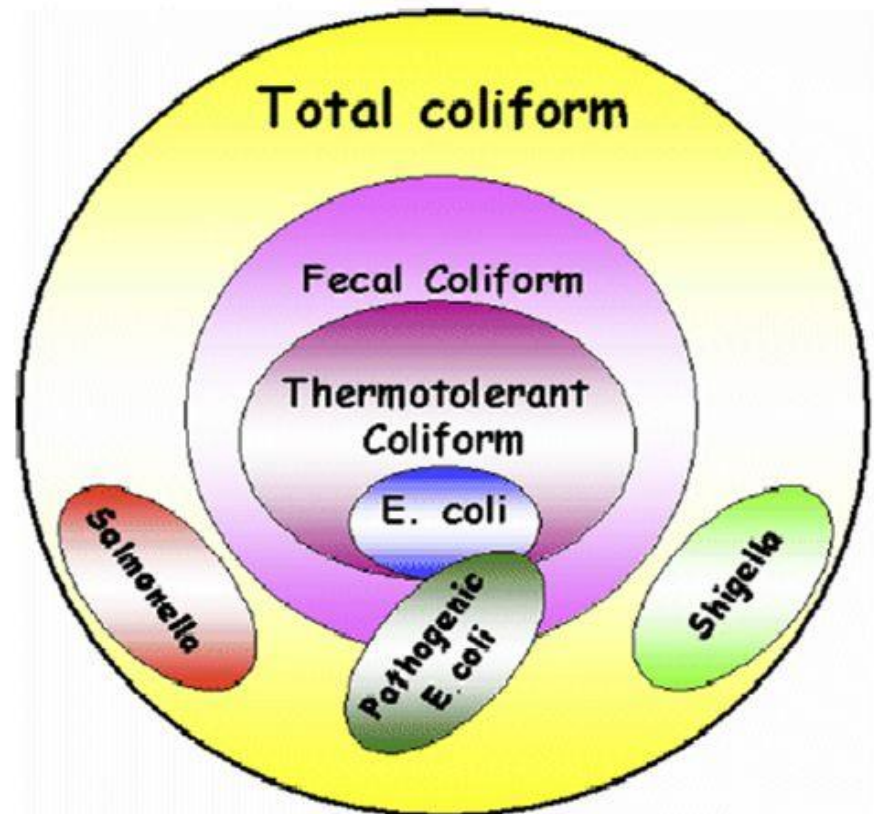
2. Some are saprophytes in water, soil and plants e.g. *Proteus* species.

Total Coliform, Fecal Coliform and E. coli



Fecal Coliform Bacteria

- are a sub-group of total coliform bacteria
- appear in great quantities in the intestines and feces of people and animals
- fecal coliform in a drinking water sample means that there is a **greater risk that other dangerous pathogens are present**



- **Medical importance of the family:**
 1. Indicator of fecal pollution of water.
 2. Responsible for 30% of cases of hospital acquired infections (nosocomial infection).
- **Based on clinical infections produced, Enterobacteriaceae are divided into two categories:**
- **Primary intestinal pathogens:** *Salmonella*, *Shigella*, *Yersinia* and certain strains of *E. coli*.
- **Opportunistic pathogens:** normally part of the usual intestinal flora that may produce infection outside the intestine e.g. Coliform bacilli.

Enterobacteriaceae divided into TWO main groups according to action on LACTOSE:

- **Lactose – fermenters** (rose pink colonies) include Coliform group: e.g. *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*.
- **Non-lactose fermenters** (pale yellow colonies) include: *Salmonella*, *Shigella*, *Yersinia*, and *Proteus*.



Escherichia coli



Enterobacter aerogenes



**Lactose
fermenting
colonies**

PINK



**Non-lactose
fermenting
colonies**

COLORLESS



Proteus vulgaris



Salmonella typhimurium



Staphylococcus aureus

MacConkey's Agar

- **Identification of Enterobacteriaceae:**
- **Microscopic:** using Gram staining method.
- **Cultural characteristics:** there are several selective and differential media used to isolates and distinguishes between LF & LNF, such as MacConkey agar.
- **Biochemical reaction:** there are several biochemical reaction used to Identification and distinguished member of Enterobacteriaceae, such as IMViC Tests.

- **GENUS: *Escherichia***

- It contains five species, the main species of medical importance is *Escherichia coli*.

- **Characteristics:**

- Gram-negative rod, Facultative anaerobe, motile with peritrichate flagella, non-capsulated usually, non-spore forming.

- Normal flora in human and animal gastrointestinal tract. Found in soil, water and vegetation.

Cultural characteristics:

- Aerobic and facultative anaerobic, optimum temperature for growth is 37°C, grow well on ordinary media
- On MacConkey agar, colonies are pink due to lactose ferment, smooth, glossy.
- On nutrient agar it produces large, smooth, and opaque.
- On Eosin methylene blue (EMB), it produce unique characteristic green metallic sheen colony.
- On blood agar: certain strains cause hemolysis

- **Biochemical tests:**

- Indole positive, Methyl red positive
- Voges-Proskauer negative, Citrate negative
- Ferment lactose and glucose with gas production.
- Hydrogen sulfide and urease negative

- **Diseases:**

- Urinary tract infection, Wound infection, Neonatal septicemia and meningitis, and diarrheal disease.

GENUS: *Klebsiella*

- It contains four major species, *K. pneumoniae* is mostly commonly isolated species.
- **Characteristics:**
 - Gram-negative bacilli, short, non-motile, capsulate and facultative anaerobes.
 - It possesses a polysaccharide capsule, which protects against phagocytosis and antibiotics, and makes the colonies moist and mucoid.
 - It has a distinctive “yeasty” odor.
 - It is found as a commensal in the intestinal tract.

- **Culture characteristics:**
- In nutrient agar, MacConkey agar colonies are similar to *E. coli* except that the colonies are large mucoid in EMB, it grow but without any sheen. On blood agar colonies are large, mucoid white to grey.
- **Biochemical tests:**
 - Motility (–), Indole (–), MR (–), VP (+), Citrate (+), Urease (+), H₂S (–), ferment lactose and glucose.
- *Klebsiella oxytoca*: It is indole positive.
- **Diseases:**
- Pneumonia, Urinary tract infection, Septicemia and meningitis (especially in neonates) and Wound infection.

Biochemical reactions of *E. coli* and *Klebsiella* species

	Indole	MR	VP	Citrate	Urease	H ₂ S	Motility	Glucose Fermentation	Lactose fermentation
<i>E. coli</i>	+	+	-	-	-	-	+	+	+
<i>K. pneumoniae</i>	-	-	+	+	+	-	-	+	+

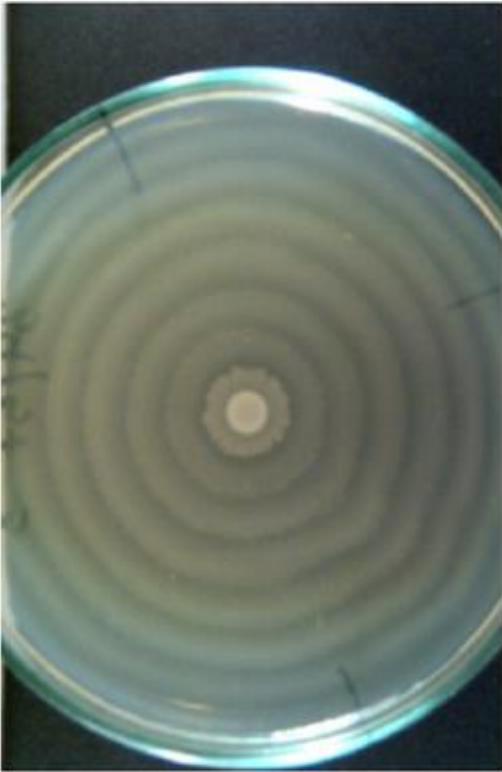
GENUS: *Proteus*

- **Characteristics:**
- Gram negative rods, Oxidase negative and Facultative anaerobes.
- *Proteus* does not ferment lactose and produce H₂S.
- Non capsulated and none spore forming.
- Actively motile and show swarming.
- Urease positive after 2-6 hrs (urea → NH₃+ CO₂)
- Grows well at alkaline pH

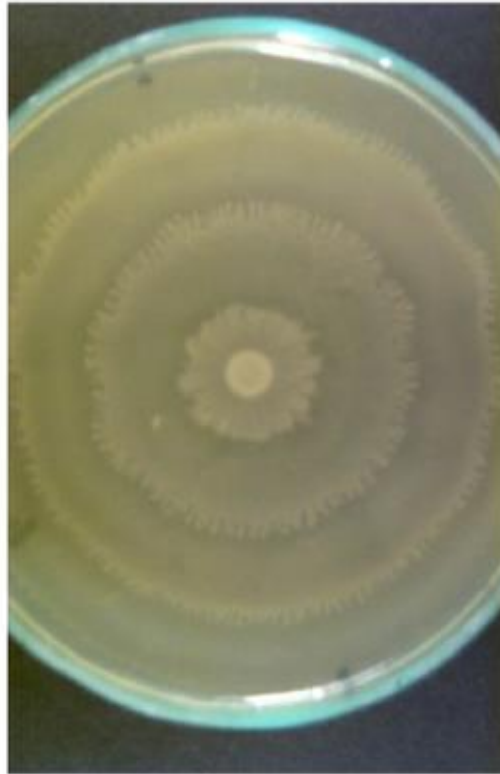
- Discontinuous swarming produces concentric circles around the point of inoculation.



A



B



C



- **Major pathogens** are *Proteus mirabilis* and *Proteus vulgaris*.
 - *P. mirabilis* causes urinary tract infections (UTIs): urease alkalinizes urine → precipitation of calcium and magnesium salts → stone formation → renal epithelium damage
 - *P. vulgaris* causes nosocomial infections (pneumonia, bacteremia) and UTIs.

Laboratory diagnosis:

- **Specimen:** Urine or Stool.
- **Cultural characteristics:**
 - On MacConkey agar they give pale yellow colonies.
 - On EMB agar they give colorless colonies.
 - On SS agar they give pale yellow colonies with black colour.
 - On ordinary media, such as nutrient agar, blood agar, show swarming (successive waves on the surface) due to high motility of *Proteus*. The suspected is picked up and examined by:
- **Gram stain:** Gram negative bacilli, motile, non-spore forming and non-capsulated.
- **Biochemical reactions:**
 - The biochemical reactions of *Proteus* are outlined in the following Table.

Biochemical Test	I	MR	VP	CIT	Urease	H ₂ S	Glucose F.	Lactose F	Motility
Proteus mirabilis	-	+	-	-	+ (2-6 h)	+	+	-	motile