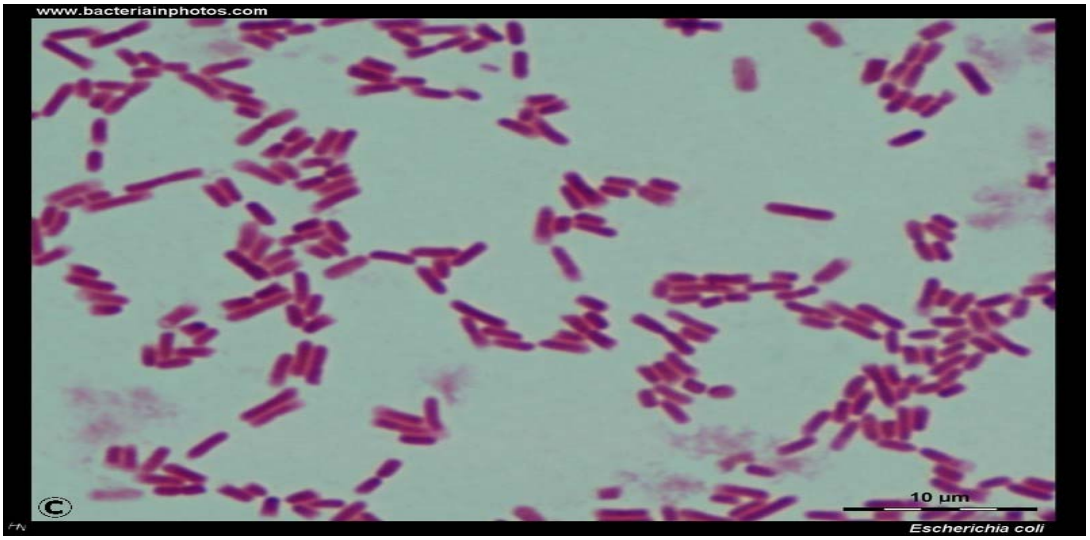
ENTEROBACTERIACEAE

E. coli and *Klebsiella*



Instructor

Dr. Maytham Ihsan

Ph.D Vet Microbiology

Enterobacteriaceae

General Characteristics

- **Enterobacteriaceae or Coliform bacteria***,* are Gram-negative rods up to 3 µm in length
- Ferment glucose and a wide range of other sugars and are oxidase-negative.
- They are catalasepositive, non-spore-forming facultative anaerobes which grow well on **MacConkey (MAC)** agar because they are **not** inhibited by the bile salts in the medium. These enteric organisms reduce nitrates to nitrites, and some species, notably *Escherichia coli*, ferment lactose.
- The motile enterobacteria have peritrichous flagella.
- The family contains more than 40 genera and over 180 species. Less than half of the genera are of veterinary importance. Most strains are normal commensals of man & animals bowel. May contaminates water, soil & vegetations.
- The term 'coliform', formerly only used to describe enterobacteria capable of fermenting lactose, is now sometimes used to describe other members of the family.
- Can be grouped into three cateogories :

1- Major enteric and systemic pathogens:

– *Escherichia coli* – *Salmonella* serotypes – *Yersinia* species

2- Opportunistic pathogens:

– *Proteus* species – *Enterobacter* species – *Klebsiella* species

3- Non-Pathognens

- *Hafnia* - *Erwinia (* isolated from feces & environment lead to contamination of clinical specimens*)*

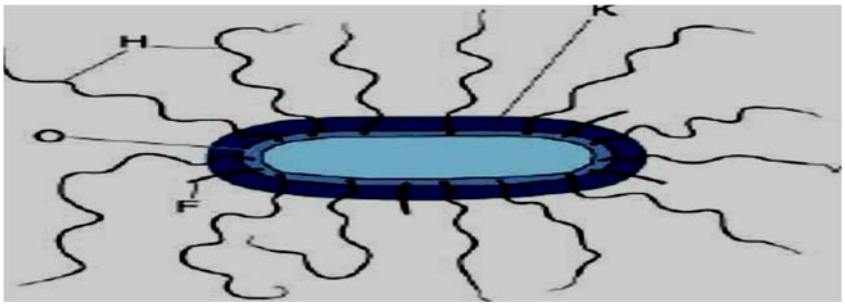
Differentiation of the *Enterobacteriaceae*

- Reactions on selective/indicator media
- Lactose fermentation in MacConkey agar (E.coli & Kelbisella spp.) are lactose fermenters with pink discoloration.

- Salmonella-Shigella (SS) Agar, Brilliant Green (BG) agar & xylose–lysine–deoxycholate (XLD) agar. On BG agar *Salmonella* colonies and the surrounding medium show a red alkaline reaction. On XLD medium the colonies of most *Salmonella* serotypes are red (alkaline reaction) with black centres due to hydrogen sulphide (H2S) production.
- Chromogenic agars like: **Eosin Methylene Blue (EMB)** especially for *E. coli* which revealed **green metallic sheen**.
- Colonial morphology:

- Mucoid colonies are typical of *Klebsiella* and *Enterobacter* species
- *Proteus* species produce characteristic swarming on non-inhibitory media such as blood agar.
- Biochemical tests:
- Reactions in triple sugar iron (TSI) agar: Contains 0.1% glucose, 1% lactose and 1% sucrose and chemicals to indicate H2S production as well as to Phenol red is used as pH indicator.
- Urease production
- The **IMViC** (Indole production, Methyl red test, Voges-Proskauer test, Citrate utilization) tests are a group of biochemical reactions used to differentiate *E. coli* from other lactose fermenters.
- Tests for motility allow differentiation of *Klebsiella* species (non-motile) from *Enterobacter* species (motile). Both species produce similar mucoid colonies which are difficult to distinguish visually.
- Commercial biochemical tests: RapID ONE System.
- Serotyping of *E. coli & Salmonella:*
- Slide agglutination tests with antisera are used to detect **O (somatic)** and **H (flagellar)** antigens and sometimes detection of **K (capsular)** antigens as well as to **F (fimbrial)** antigens.
- Molecular techniques: Polymerase Chain Reaction (PCR)
- Molecular typing techniques: By Pulse Field Gel Electrophoresis (PGFE) & recently gene sequencing.

K, H, O and F antigens



Schematic diagram of a typical member of the Enterobacteriaceae indicating the K (capsular), O (somatic), F (fimbrial) and H (flagellar) antigens used for serotyping isolates

Escherichia coli General Characteristics

- ***Escherichia coli,*** gram negative plump rods motile usually motile with peritrichous flagella.
- Lactose fermenter produces pink colonies on MacConkey agar and has characteristic biochemical reactions in **IMViC** tests **(+ + - -**).
- Some strains produce colonies with a metallic sheen when grown on eosin–methylene blue (EMB) agar.
- Haemolytic activity on blood agar is a characteristic of certain strains of *E. coli*.
- **Somatic** (**O**), **flagellar** (**H**) and sometimes **capsular** (**K**) antigens are used for **serotyping** *E. coli*.
- In recent years, *E. coli* O157:H7 and other enterohaemorrhagic serotypes have emerged as major food-borne, zoonotic pathogens in humans, responsible for the haemorrhagic colitis–haemolytic uraemic syndrome (HUS).

Virulence Factors of Pathogenic *E. coli*

- Capsular polysaccharides.
- Endotoxin, a lipopolysaccharide (LPS) component of the cell wall of Gram-negative organisms, is released on death of the bacteria. It is composed of a lipid A moiety, core polysaccharide and specific side chains.

➢ The **role of LPS** in disease production includes: **1-** pyrogenic activity, **2-** endothelial damage leading to disseminated intravascular coagulation, and **3-** endotoxic shock. These effects are of greatest significance in septicaemic disease.

- Fimbrial adhesins which are present on many strains of *E. coli* allow attachment to mucosal surfaces in the small intestine and in the lower urinary tract.

Examples: strains like K99 (F5) and F41 isolated from calves & K99 from lambs.

- Adhesin termed **intimin** is associated with attaching and effacing *E. coli* (**AEEC**). Is one of the products of genes encoded in a **pathogenicity island.** Intimin binds to the **translocated intimin receptor (Tir)** and is translocated into the host cell by the bacterium where it forms a receptor to which intimin can bind.
- Alpha & Beta Hemolysins, produced by certain strains.
- **Siderophores,** iron-binding molecules such as **aerobactin** and **enterobactin**.
- Heat-labile (**LT**) and heat-stable (**ST**) **Enterotoxins**, produced by many Enterotoxigenic ***E. coli*** (ETEC).

Types of Pathogenic *E. coli* - Enteric Disease

1) **Enterotoxigenic *E. coli* (ETEC)**, like K99 cause neonatal colibacillosis
2) **Enteropathogenic *E. coli* (EPEC)**, cause enteritis & diarrhoea.
3) **Enteroinvasive *E. coli* (EIEC)**, Invade enterocytes & deep layer of intestinal mucosa responsible for colisepticaemia.
4) **Attaching & Effacing *E. coli* (AEEC)**, Shiga-like toxins (ST) or Verotoxins (VT) destroy the microvilli, isolated from calves & rabbit with enteric disease.

Extra-intestinal disease

- **Avian pathogenic *E. coli***
- **Septicaemic *E. coli***
- **Uropathogenic *E. coli***
- **Strains causing localised infections**

Diseases Caused by *E. coli*

- Cows & Ewes: Coliform mastitis.
- Calves & Lambs: White Scour “colibacillosis” (up to 1 week of age), colisepticaemia.
- Calves: Joint ill
- Lambs: Watery mouth.
- Canine: Urinary Tract Infection (UTI) & Pyometra in bitches. Colisepticaemia in pups.
- Poultry: Chick omphalitis. All ages – Colisepticaemia & Coligranuloma.

Diagnosis

- Specimens: blood, mastatic milk, cervical swabs & mid-stream urine. To be examined for direct microscopy & culturing onto selective media like MAC agar & EMB.
- Haemolysis on blood agar.
- Biochemical identification kits as well as to IMViC test.
- Detection of enterotoxins ST & LT from culture filtrate or supernatants by using serological rapid tests.
- Serotyping of E. coli strains depending on O,H,K & F Ags. Certain rapid kits used like for K99 strains.
- ELISA used for identification of certain strains like O157:H7
- Molecular techniques: Escpecially PCR or with using specific DNA probes hybidization.
- Antimicrobial Susceptibility test.

The clinical relevance, growth characteristics and biochemical reactions of members of the Enterobacteriaceae which are of veterinary importance.

	Escherichia coli	*Salmonella* serotypes	*Yersinia* species	*Proteus* species	*Enterobacter aerogenes*	*Klebsiella pneumoniae*
Clinical importance	Major pathogen	Major pathogens	Major pathogens	Opportunistic pathogens	Opportunistic pathogen	Opportunistic pathogen
Cultural characteristics	Some strains haemolytic	–	–	Swarming growth[a]	Mucoid	Mucoid
Motility at 30°C	Motile	Motile	Motile[b]	Motile	Motile	Non-motile
Lactose fermentation	+	–	–	–	+	+
IMViC tests						
Indole production	+	–	v	±[c]	–	–
Methyl red test	+	+	+	+	–	–
Voges-Proskauer	–	–	–	v	+	+
Citrate utilization test	–	+	–	v	+	+
H_2S production in TSI agar	–	+	–	+	–	–
Lysine decarboxylase	+	+	–	–	+	+
Urease activity	–	–	+[b]	+	–	+

Klebsiella species

- Important members are:
- *Klebsiella pneumoniae* and *Kl. aerogenes*
- Normal commensals of respiratory tract of healthy animals & man.
- Are gram negative thick rods, non-motile & non-sporeforming.
- Grow well on MAC agar & ferment lactose yielding pink discoloration of the agar.
- Produce polysaccharide capsular materials in both host tissues & laboratories media with a characteristics large mucoid glistening smooth colonies.

- They have smooth (O), Rough (R) and Capsular (K) antigens.
- IMViC reaction (**- - + +**)
- Induce localized pyogenic lesions or generalized infections.

Diseases Caused by *Klebsiella* spp.

- Bovine Mastitis
- Metritis in mares.
- Pneumonia in foals And calves.
- Airsacculitis in poultry.
- UTI in dogs.

GOOD LUCK