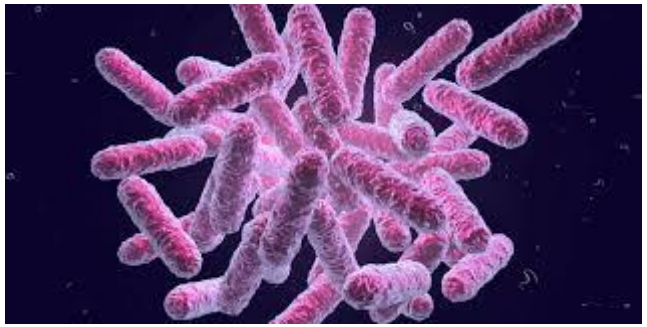
ENTEROBACTERALES

Assoc. Prof. Banu KASKATEPE

ENTEROBACTERIACEAE

Order: *Enterobacterales*

Family: *Enterobacteriaceae*



- Family *Enterobacteriaceae* often referred to as “enterics”
- Except for few, most are present in the intestinal tract of animals and humans as commensal flora; therefore, they are sometimes call “fecal coliforms”
- Some live in water, soil and sewage

Common characteristics of family *Enterobacteriaceae*

1. They are gram negative, short rods
2. They are non-sporulating,
3. Facultative anaerobes
4. These organisms have simple nutritional requirements and **MacConkey agar** is used to isolate and differentiate organisms of *Enterobacteriaceae* family (Pink colored colonies of lactose fermenter-coliforms and pale colored colonies of Non lactose fermenter)

ENTEROBACTERALES

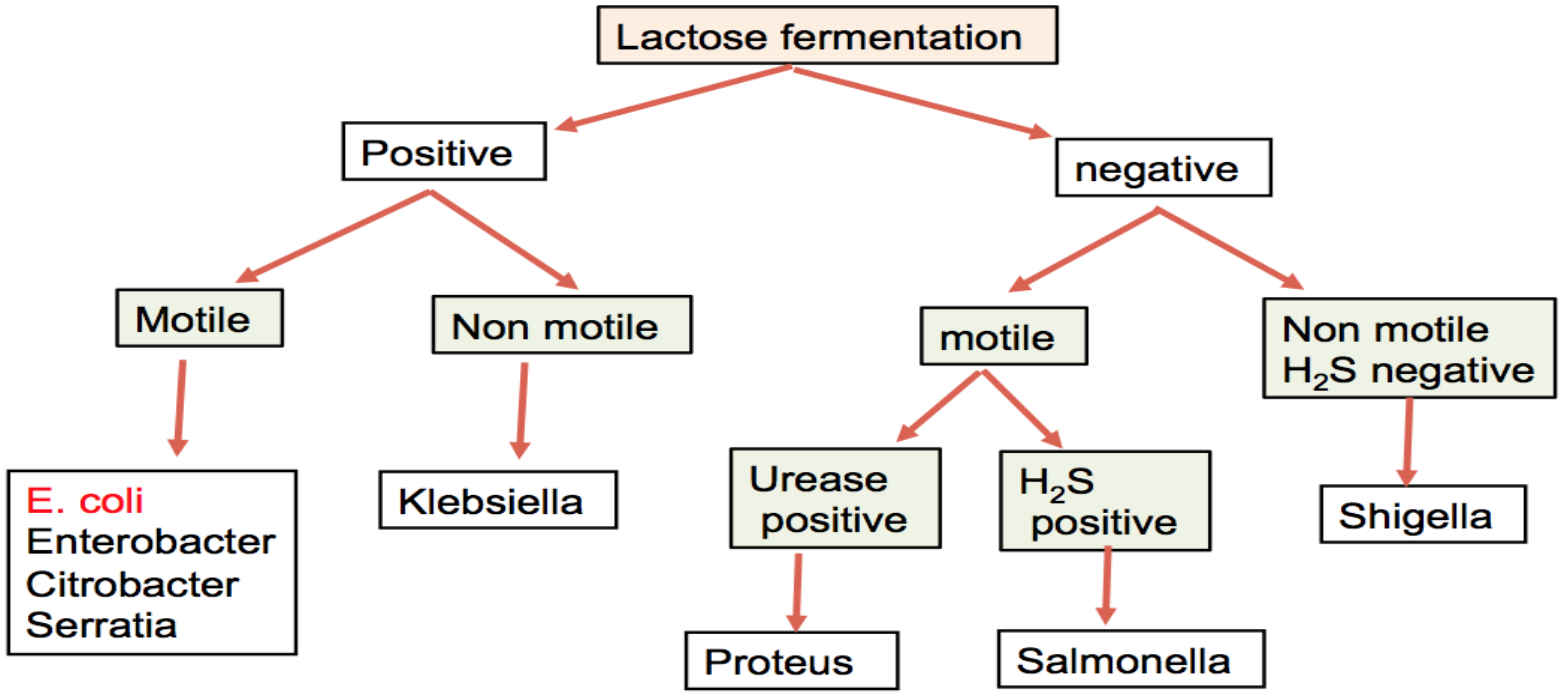
Lactose fermenters: (CEEK)

- *Citrobacter*
- *Escherichia*
- *Enterobacter*
- *Klebsiella*

Non lactose fermenters (ShYPS)

- *Shigella*
- *Yersinia (Yersiniaceae family)*
- *Proteus (Morganellaceae family)*
- *Salmonella*

Enterobacteriaceae:

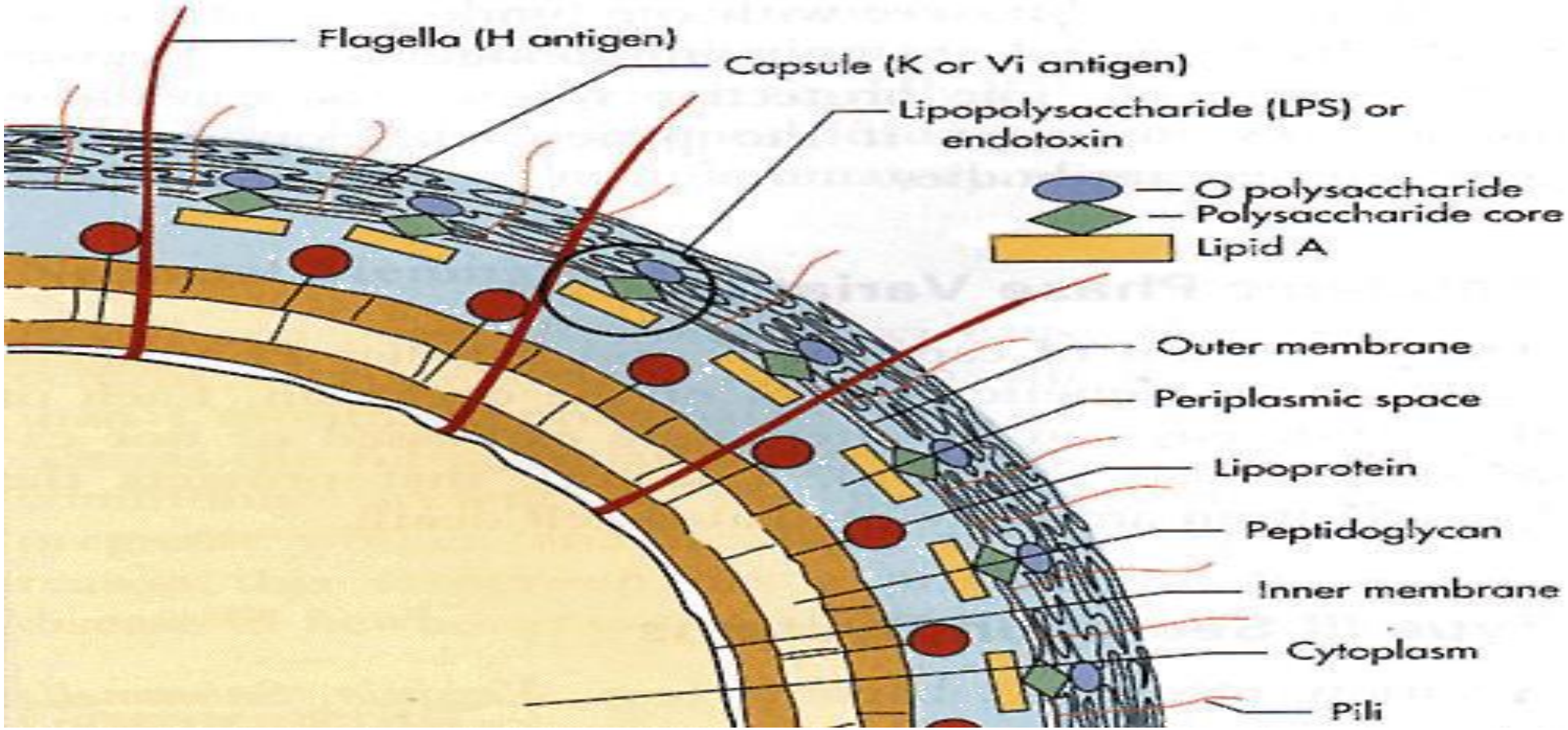


5. Most motile with peritrichous flagella (**Shigella and Klebsiella are non-motile)**
6. They are **catalase** positive
7. Cytochrome C oxidase negative
8. Usually reduces Nitrate to Nitrite
9. Contain a characteristic antigen, called the **enterobacterial common antigen** (ECA)
10. Produces acid from glucose; ability to ferment lactose- distinguishes enteric from obligately aerobic bacteria.

Antigens of *Enterobacteriaceae* are:

- O: (Outer membrane) somatic, heat-stable antigen located in the cell wall. Heat stable O antigen is often used to serotype
- H: flagellar, heat labile antigen
- K: Capsular, heat-labile antigen
- Vi: Capsule of Salmonella

Antigenic structure of *Enterobacteriaceae*



Classification of *Enterobacteriaceae*

Lactose fermenters

- *E.coli*
- Citrobacter
- Klebsiella
- Enterobacter

Non-lactose fermenters

- Salmonella
- Shigella
- Proteus
- Yersinia

There are several selective media used to distinguish these bacteria

- MacConkey agar
- Eosin Methylene Blue Agar
- Salmonella Shigella Agar
- Triple Sugar Iron Agar

Tests for identification of *Enterobacteriaceae* family

- Member of the *Enterobacteriaceae* family are identified based on their biochemical properties. Commonly used biochemical tests to identify them are ;

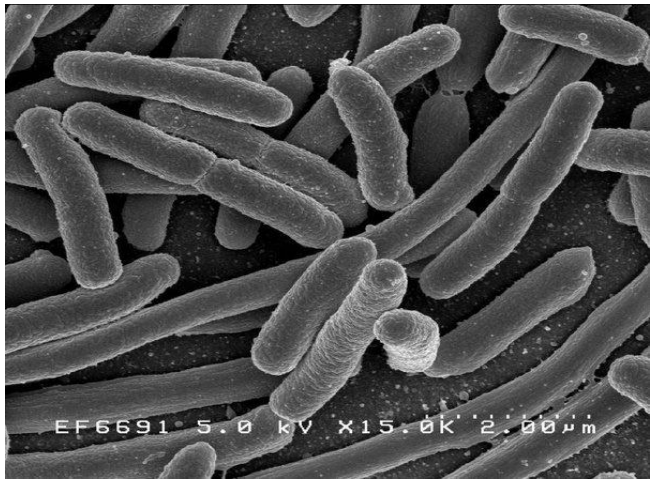
- Citrate utilization Test
- Indole Test
- Motility Test
- Methyl Red (MR) Test
- Voges–Proskauer (VP) Test
- Triple Sugar Iron (TSI) Agar Test
- Urease Test

Escherichia coli

- Named for Theodor Escherich German physician (ca. 1885)
- Demonstrated that particular strains were responsible for infant diarrhea, gastroenteritis and urinary tract infections

General Characteristics

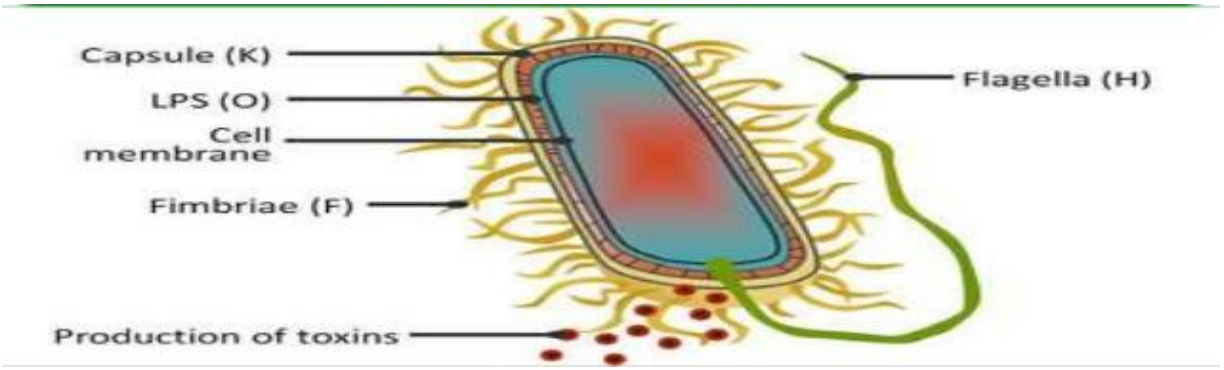
- *Escherichia co*li is a **Gram negative**, rod-shaped bacteria.



Source: Karen C. Carroll, Stephen A. Morse, Timothy Mietzner, Steve Miller: Jawetz, Melnick, & Adelberg's Medical Microbiology, 27th Edition.
www.accessmedicine.com

- Common isolate from colon flora
- It is a commensal that is found inhabiting the lower intestine of warm blooded animals.
- The harmless strains produce vitamin K and prevent colonization of the intestine by pathogenic bacteria.
- Lipopolysaccharide consists of outer somatic O polysaccharide, core polysaccharide (common antigen), and lipid A (endotoxin)

There are more than 700 different serotypes of *E. coli.* *E. coli* is classified into serotypes based on cell wall (O), capsular (K), fimbrial (F) and flagellar (H) antigens. Example *E. coli* O157:H7



O18ac:H7:K1

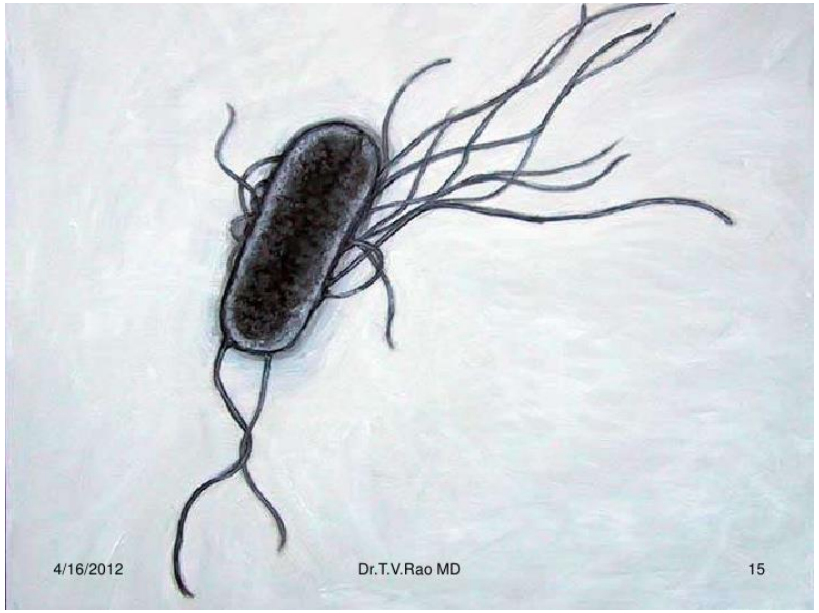
18th O antigen

7th H antigen

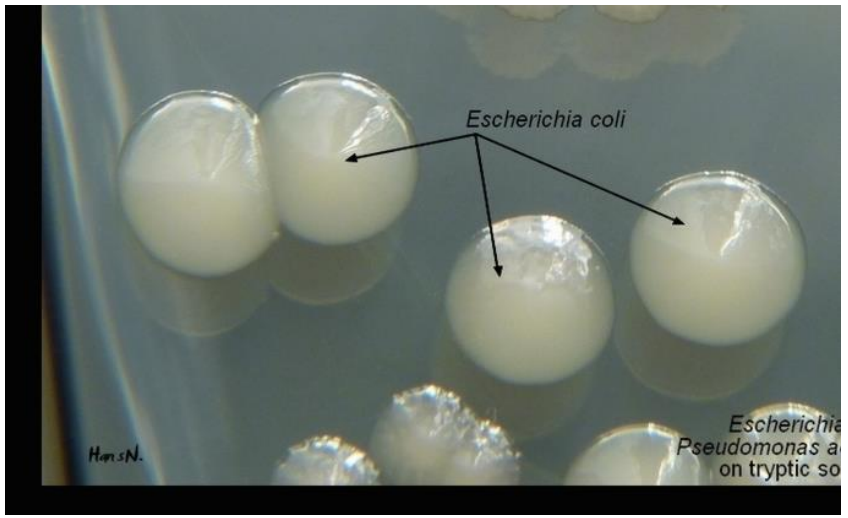
1st K antigen

- Motile by peritric flagella-Very few strains non motile
- Fermenter; oxidase negative
- Most significant species in the genus
- Important potential pathogen in humans
- Sepsis, UTIs, meningitis, gastroenteritis
- Detection of *E.coli* in water indicates pollution

and contamination with feces

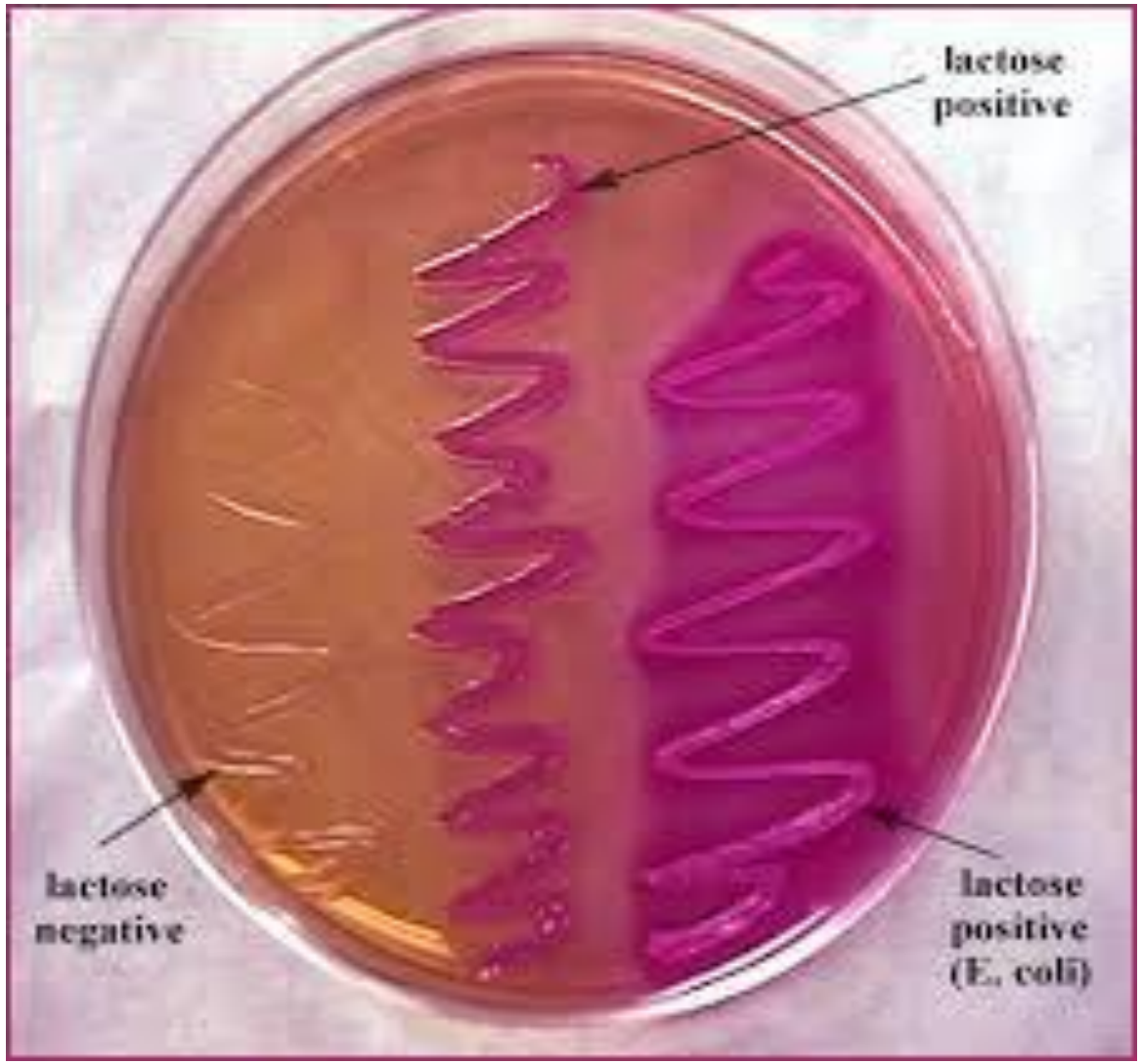


Cultural characteristics



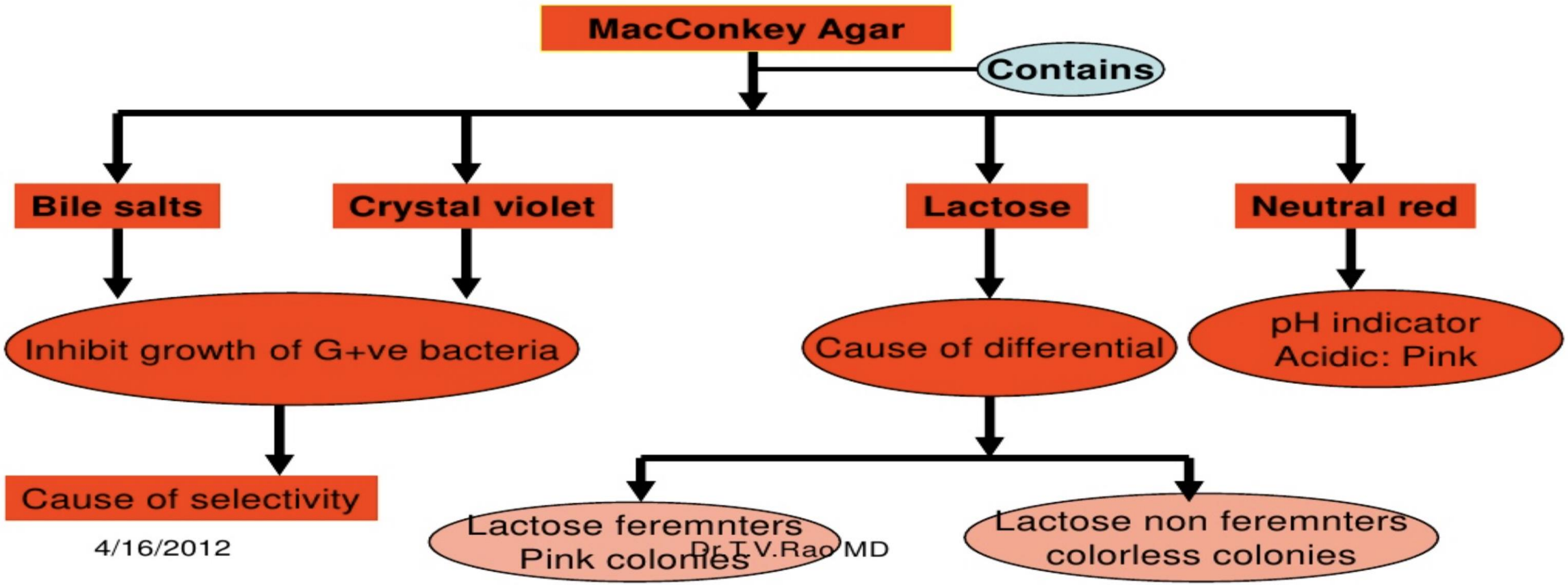
- Aerobic / Facultative anaerobic
- Grows between 10 – 40 ºC optimal at 37 ºC
- Grown in simple medium
- Produce large grayish ,thick white , moist, smooth, opaque colonies
- Some strains may contain capsule.

- Dry, pink (lactose positive) colony with surrounding pink area on MacConkey Agar.

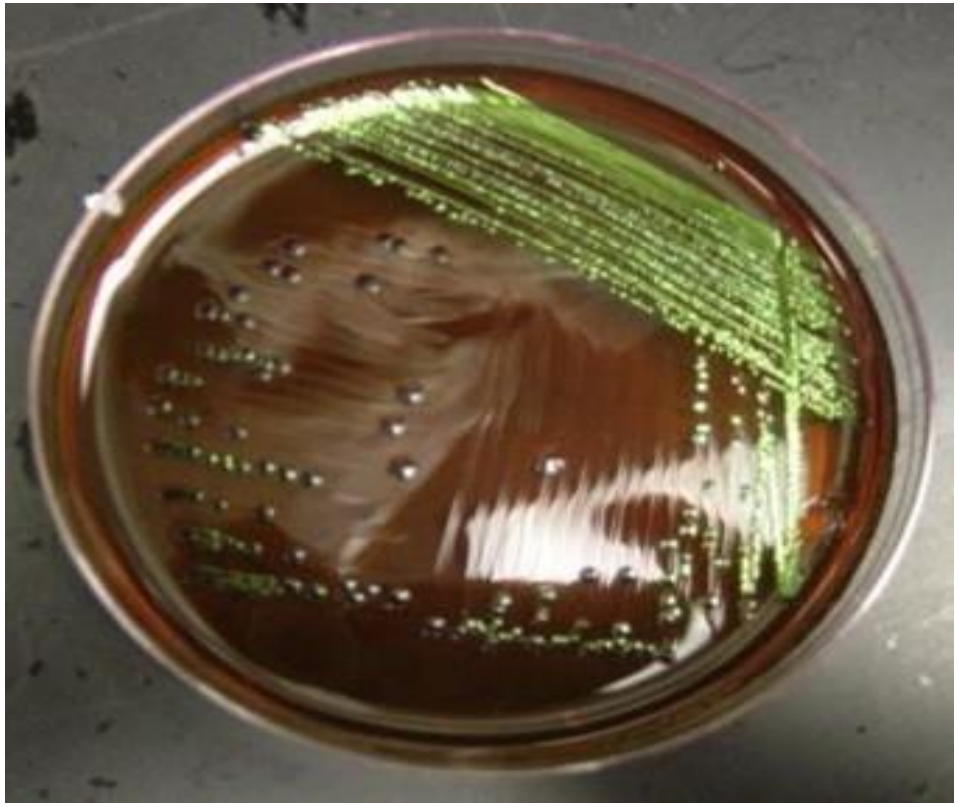


Differentiation between LF & NLF by Growth on MacConkey agar

- MacConkey agar is selective & differential medium for *Enterobacteriaceae*



- Blue-black colonies with a green Metallic sheen (due to lactose fermentation) on Eosin Methylene Blue (EMB) Agar

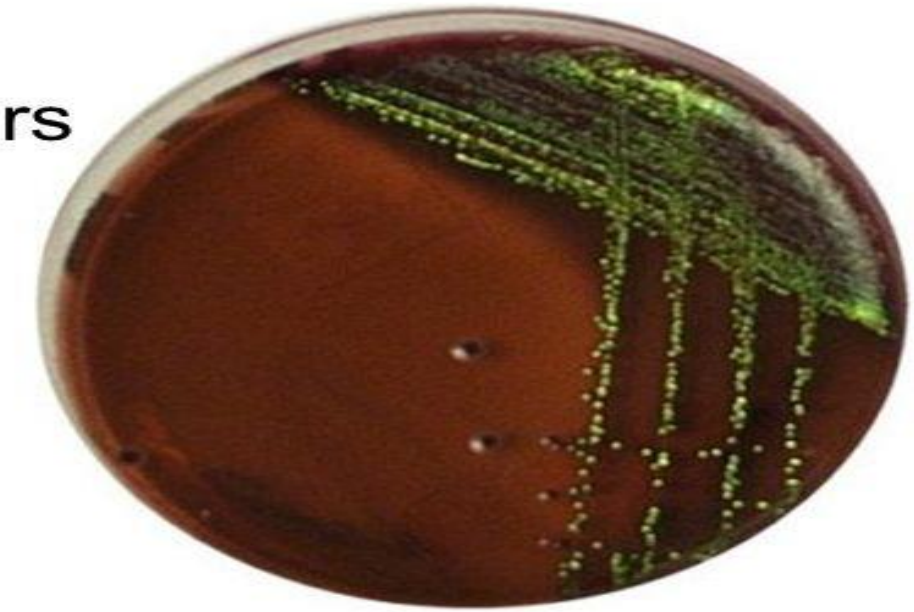


EMB – Eosin Methylene Blue Agar,
isolate Gram neg. bacteria
Selective – eosin; methylene blue; inhibitory to
Gram + organisms
Differential – lactose fermentation

Metallic green/blue black colonies- (+) vigorous lactose fermentors, acidic

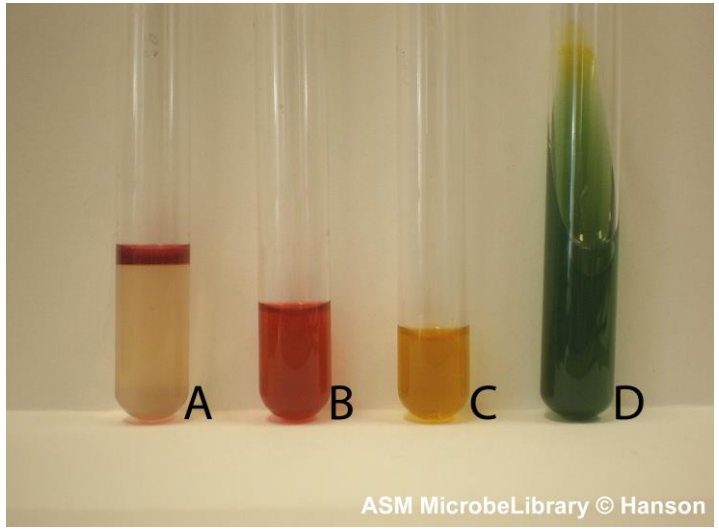
Dark Purple – (+) slower fermentors
acidic

Light pink/colorless –
(-) not fermenting

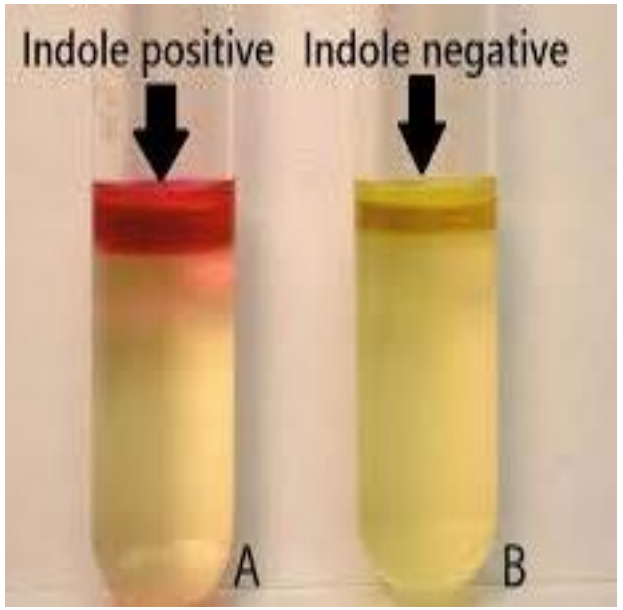


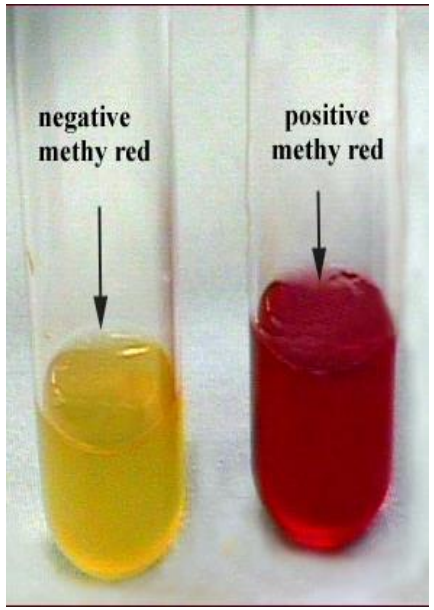
Biochemical characteristics

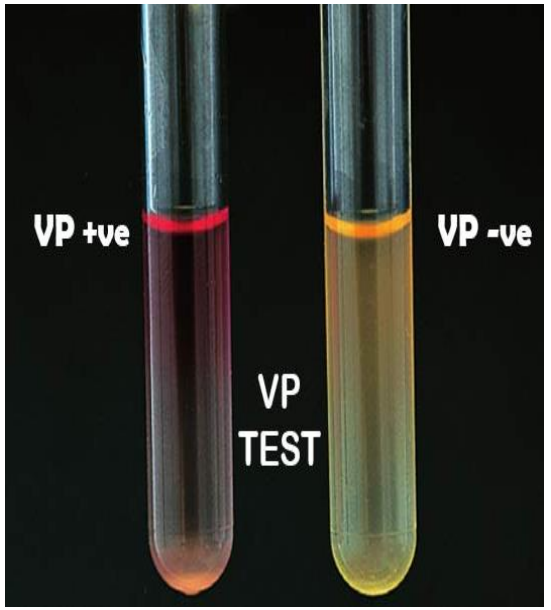
- Positive indole and methyl red tests
- Simmons citrate negative
- Voges-Proskauer test negative



Indole, Metil Red, Voges Proskauer- Citrate







For E.coli **IMVIC TESTS: +, +, -, -**

I = test for production of **indole** from tryptophan

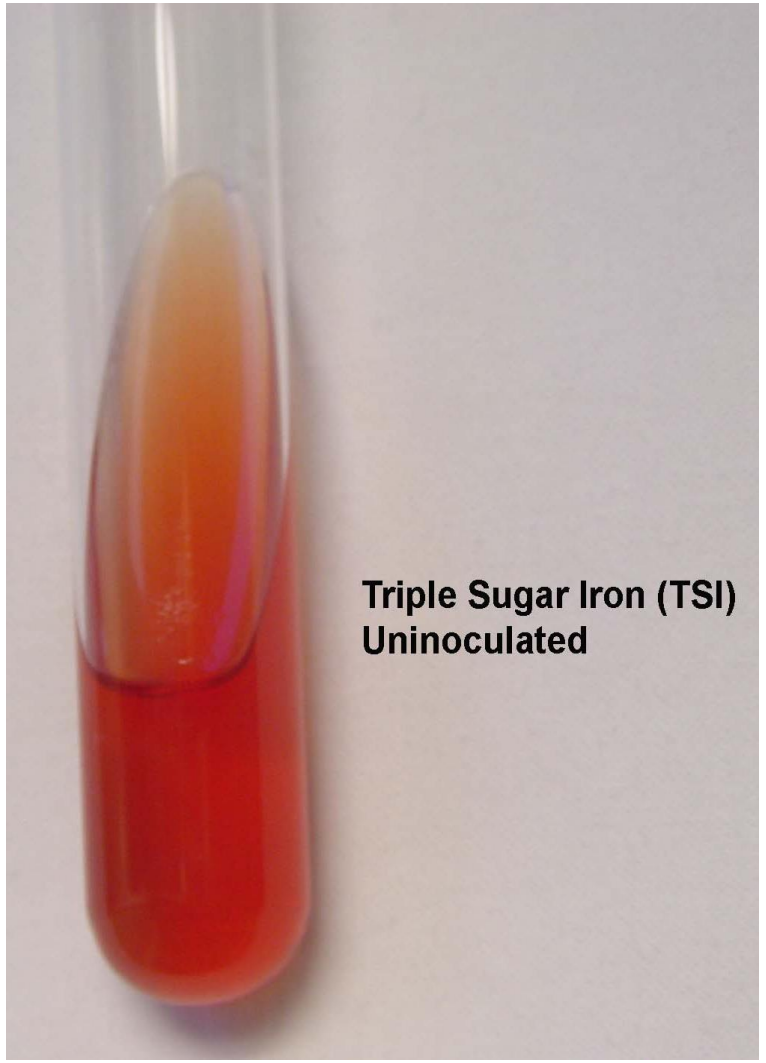
M = **methyl red** test for acid production from glucose

V = **Voges-Proskauer** test for production of acetoin from glucose

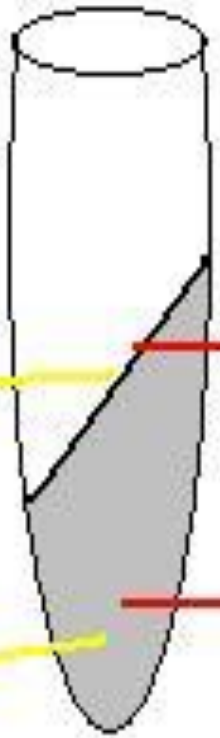
C = test for the use of **citrate** as the sole carbon source

TSI (Triple Sugar Iron Agar)

Purpose: To differentiate bacteria based on their ability to ferment glucose, lactose and/or sucrose, and to reduce sulfur to hydrogen sulfide.



Interpreting a Triple Sugar Iron (TSI) agar slant



Lactose/Sucrose fermented?
(then you'll see a yellow slant)

Glucose fermented?
(then you'll see a yellow deep)

**Lactose/Sucrose NOT fermented?
(then you'll see a red slant)**

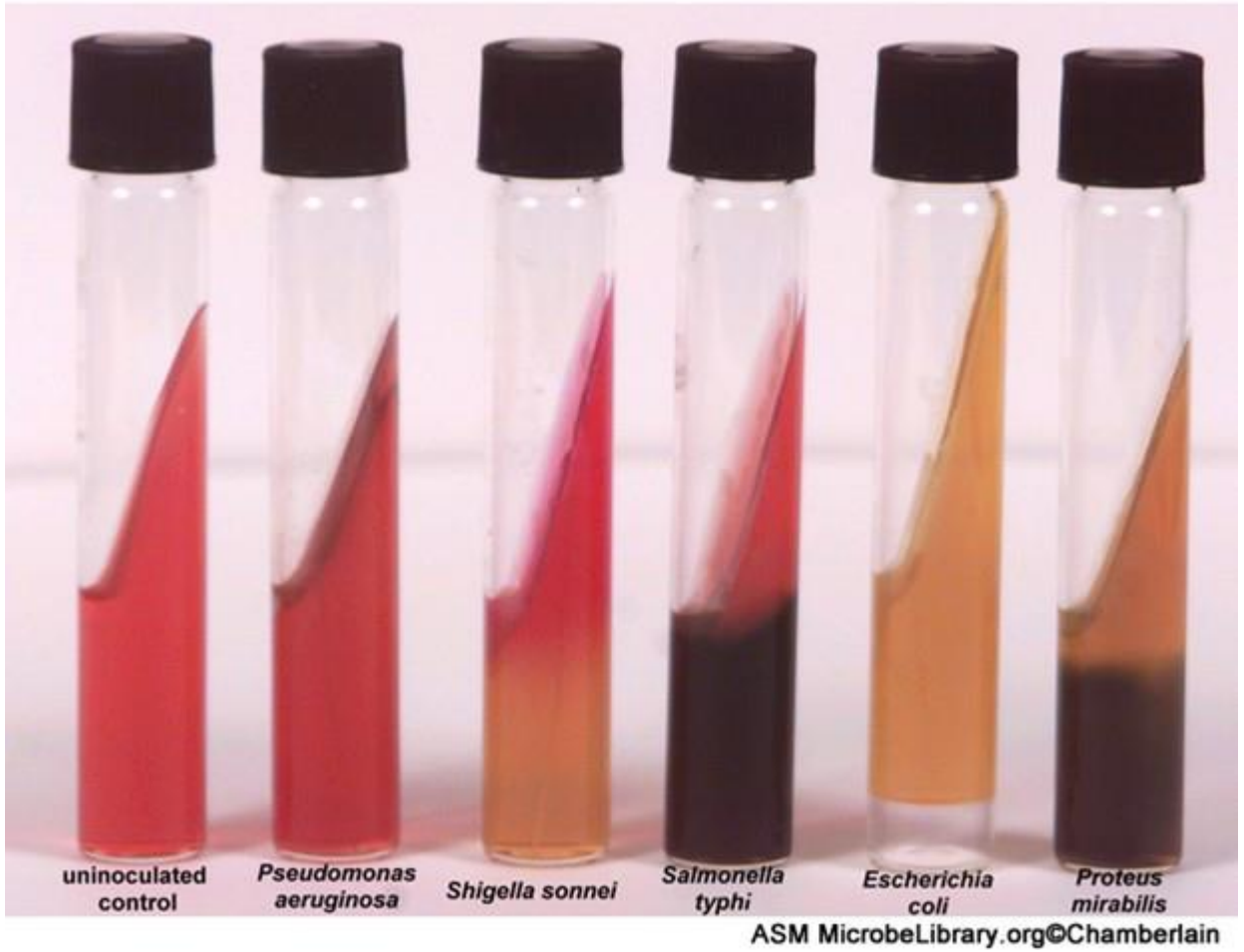
**Glucose NOT fermented?
(then you'll see a red deep)**

if you see a black precipitate then the organism produces H2S

if you notice that the medium is cracked or shifted, then the organism produces gas

***E.coli*:**

- Ferments glucose, lactose, sucrose
- Does NOT produce H_2S



TSI agar

Triple Sugar Iron Agar

0.1% dextrose

1.0% sucrose

1.0% lactose

(a) Red/red (no sugar fermentation)

(b) Control

(c) Red/yellow (Glucose fermented but lactose and sucrose not fermented)

(d) Yellow/yellow (Glucose fermented. Lactose and/or sucrose fermented)

(e) Red/yellow with H_2S

A B C D E

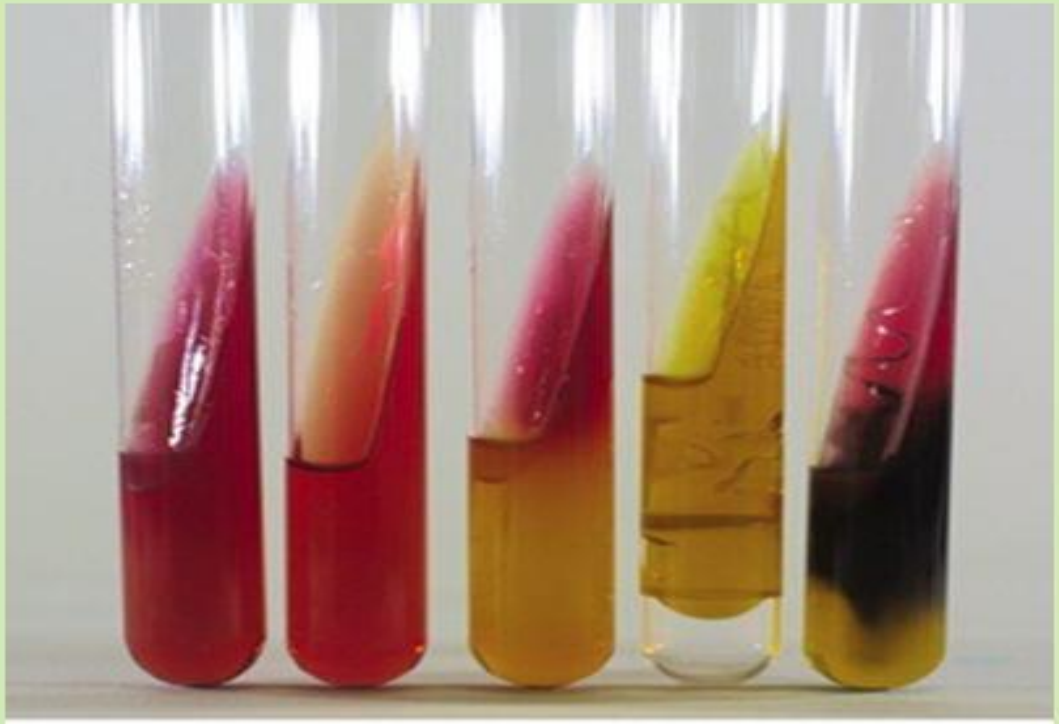


Figure 5-68

Antigenic structure of *E.coli*

- Somatic - 0 : more than 170
- Capsular K : more than 100
- Flagella H: more than 75 different antigens have been described
- Fimbria antigen (Type 1 fimbria can mediate adhesion to the cells)
- These antigenic structures are used for the identification of subspecies. *E. coli* 157:H7

Virulence factors;

- Endotoxin (blood clotting problems, shock, and death)
- Exotoxins
- Adhesins (colonization factor)
- Invasion capacity

- **Lipopolysaccharides/ Somatic or O antigen**
  - Heat stable
  - Most external in the cell wall
  - It is detected by bacterial agglutination
- **Capsular / K antigen:**
- **Flagella / H antigen:** heat and alcohol labile

Colicines / Bacteriocines

- Can be produced by many Gram –ve bacteria
- Active against some other bacteria of similar or closely related species.

Toxins of *E.coli*

- *E.coli* produce exotoxins
- Hemolysins, Enterotoxins causes diarrhea.
- Two types Enterotoxins
    - Heat labile (LT) and Heat stable (ST)

- **Enterotoxins** – produced by enterotoxigenic strains of *E. coli* (ETEC). Causes a movement of water and ions from the tissues to the bowel resulting in watery diarrhea. There are two types of enterotoxin: **Labile toxin** (LT) and **Stabile toxin** (ST).
- **LT – is heat labile** and binds to specific Gm1 gangliosides on the epithelial cells of the small intestine where it ADP-ribosylates Gs which **stimulates adenylate cyclase** to increase production of cAMP

Mechanism of Toxin

- Increased cAMP alters the activity of sodium and chloride transporters producing an ion imbalance that results in fluid transport into the bowel.
- *E.coli* labile toxin like Cholera toxin

Stabile Toxin

- Two units; ST A and ST B
- ST A Acts by activation of **Cyclic guanosine monophosphat**e(cGMP).
- Causes fluid accumulation in Intestine.
- *E.coli* (Some) produce Verocytotoxin causes cytotoxicity to Vero cells.
- Acts like Shigella dysentery toxin

DISEASES

Gastroenteritis: Most strains of the pathogenic *E. coli* are capable of pathology only within the **intestinal tract** (some exceptions)

- The other important infection outside the GIS;

Urinary tract infection: *E.coli* cause urinary tract infection.• Majority of UTI s are produce by *E.coli*.•

- Asymptomatic bacteriuria in pregnant women,• Pyelonephritis

Other infections:

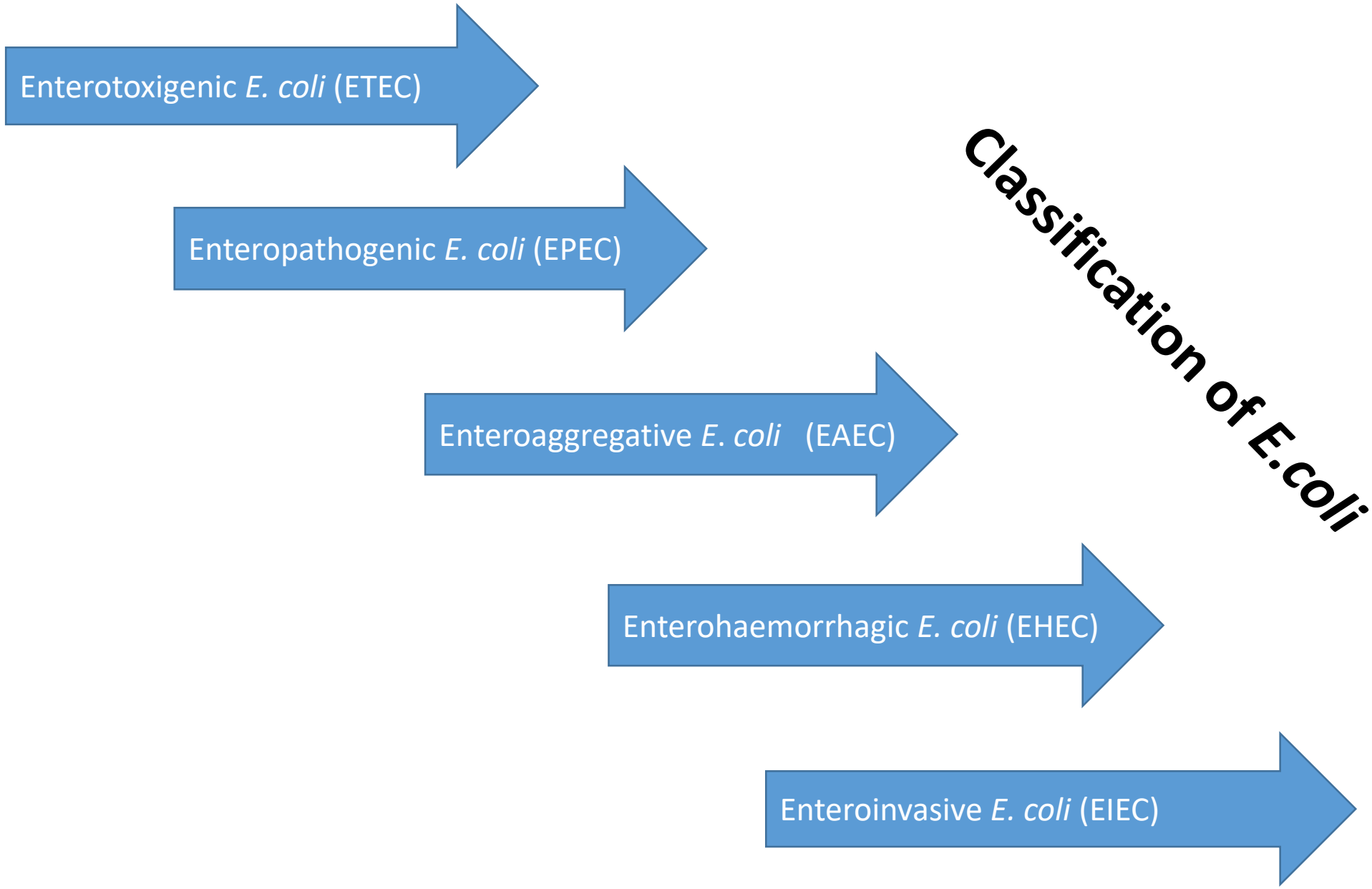
- Pyogenic infections
- Intraabdominal infections
- Peritonitis, Abscess
- Septicaemia

Classification of *E.coli*

The strains of *E. coli* that cause gastroenteritis are subdivided into following five major groups;

1. **Enterotoxigenic** **ETEC** (small bowel)
2. **Enteropathogenic** **EPEC** (small bowel)
3. **Enteroaggregative** **EAEC** (smallbowel)
4. **Enterohemorrhagic** **EHEC** (large bowel)
5. **Enteroinvasive** **EIEC** (large bowel)

Classification of *E.coli*



1. Enterotoxigenic *E.coli* (ETEC)

- Major cause of traveler's diarrhea in infant and adult in developing countries from contaminated food and water. (Traveler’s diarrhea-Weanling diarrhea)
- Adhere to intestinal mucosa by fimbriae

- ETEC produce two classes of enterotoxins: heat-labile toxin (LT) and heat-stable toxin (ST) each has two fragment (A and B)
  - Cause leakage of intestinal epithelial cells
    - Loss of electrolytes & water

- Symptoms :watery diarrhea, abdominal cramps and sometimes vomiting
- Laboratory Diagnosis

  Demonstration of Enterotoxin LT and ST

  Molecular methods

2. Enteropathogenic *E.coli* (EPEC)

- Associated with diarrhea in infants and children (Infantile diarrhea). Disease is rare in older children and adults, because they have developed protective immunity.
- Disease is characterized by bacterial attachment to epithelial cells of the small intestine, with subsequent **destruction of the microvillus**

- Low grade fever, malaise, vomiting and diarrhea.
- Mucous in stool but **not blood**.
- Do not produce enterotoxins.
- Not invasive.

- Since this is primarily a disease of the young (less the 6 months old), **fluid replacement is important**.
- intense vomiting - i.v. fluids are usually required
- disease self-limiting (antibiotics usually not required)
- breast feeding seems to have a strong protective effect

- Laboratory diagnosis: Stool specimens are plated on media such as MacConkey agar.
- Molecular method. (EPEC gene analyses)

3. Enteroaggregative *E. coli* (EAEC)

- Cause persistent watery diarrhea> 14 days (especially infants)
- Fimbriae allow for bacteria to stack up on each other.
- Stimulate mucous production called biofilm formation (bacterial community)
- Appear aggregated in a stacked brick formation on Hep-2 cells or glass.

- They have been associated with persistent diarrhea, especially in developing countries.
- They form a **heat stable enterotoxin** called EAST1 (enter aggregative heat stable enterotoxin-1).

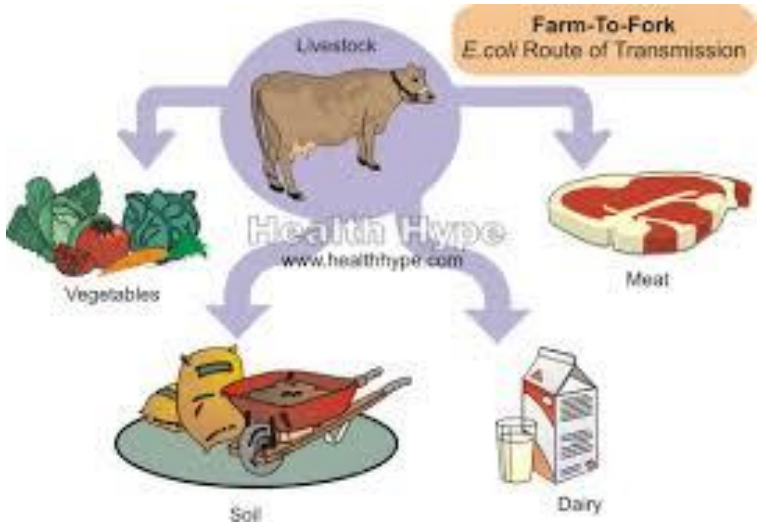
- Laboratory diagnosis: The pattern of adhesion to HEp-2 cell remain the key assay for detecting EAggEC.
- PCR

4. Enterohemorrhagic *E. coli* (EHEC)

- Produce **Verocytotoxin** (VT=shiga-like toxin=cytotoxin)
- The biological properties, physical characteristics and antigenicity of VT are very similar to those of Shiga toxin, produced by strains of *Sh. dysenteriae.*
- The diarrhea may range from mild and non bloody to stools that are virtually all blood.

- This category of diarrhea-causing *E. coli* was recognized in 1982 when an outbreak of hemorrhagic colitis occurred in the USA and was shown to be due to an unusual serotype, *E. coli* O157:H7, not previously incriminated as an enteric pathogen.
- Present in Human and Animal feces.
- **Salads, vegetables**, Proper cooking is important

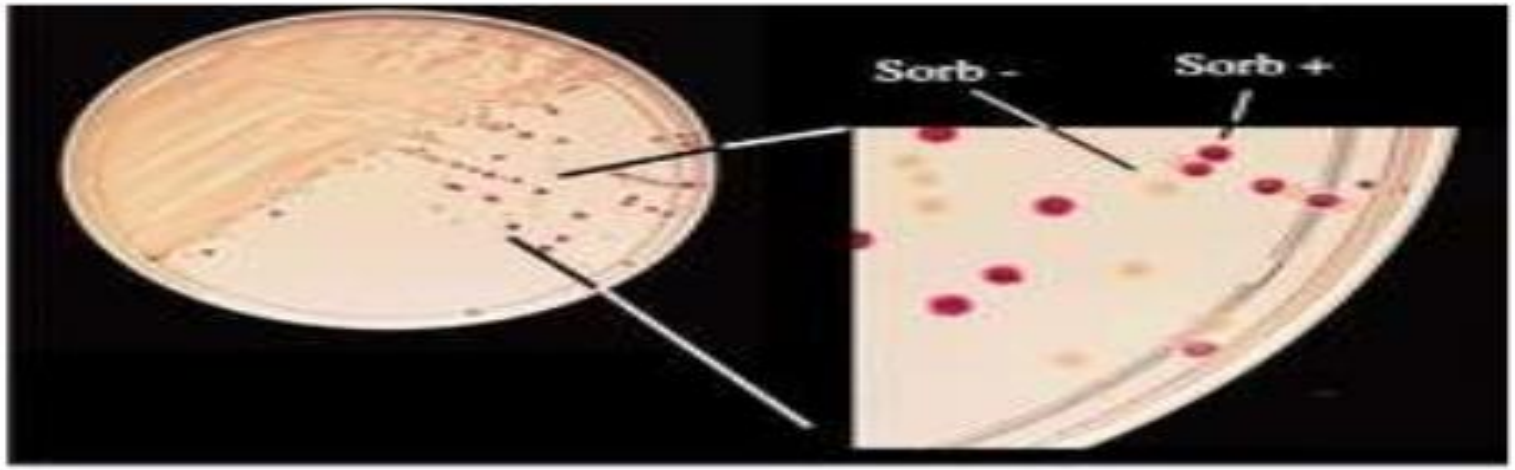
- Most cases of disease have been attributed to the consumption of undercooked ground beef or other meat products, water, unpasteurized milk or fruit juices (from contaminated fruit with feces from cattle), uncooked vegetables, and fruits.



- Lack of fever in most patients can help to differentiate this infection from that due to other enteric pathogens.
- The most severe clinical diseases of EHEC are **hemorrhagic colitis** and the **hemolytic uremic syndrome** (HUS: can cause kidney damage and failure).

- **Initially, a nonbloody diarrhea with abdominal pain** develops in patients after 3 to 4 days of incubation.
- **Vomiting** is observed in approximately half of the patients.
- Within 2 days of onset, disease in **30% to 65%** of patients progresses to a **bloody diarrhea** with severe abdominal pain.
- Complete resolution of symptoms typically occurs after 4 to 10 days in most untreated patients; however, **HUS** is a serious complication, particularly in young children.
- Death can occur in 3% to 5% of patients with HUS.

- O157:H7, can be identified in stool cultures on sorbitol-MacConkey media by their inability to ferment sorbitol.



O157:H7 sorbitol negative strain.

- Infection with O157: H7 results in high levels of serum antibodies to the O157 LPS antigens.
- Molecular methods are used for detecting antibodies.
- Latex agglutination can be used as a rapid test

Enterohemorrhagic bacteria Escherichia coli (EHEC)

Most Escherichia coli (E.coli) strains are harmless.

But some, like enterohemorrhagic **E. coli (EHEC)**, are a hazard to human health and life.

Incubation period:
three to eight days

E. coli (EHEC), once in the human stomach, begins producing toxins that cause serious illnesses

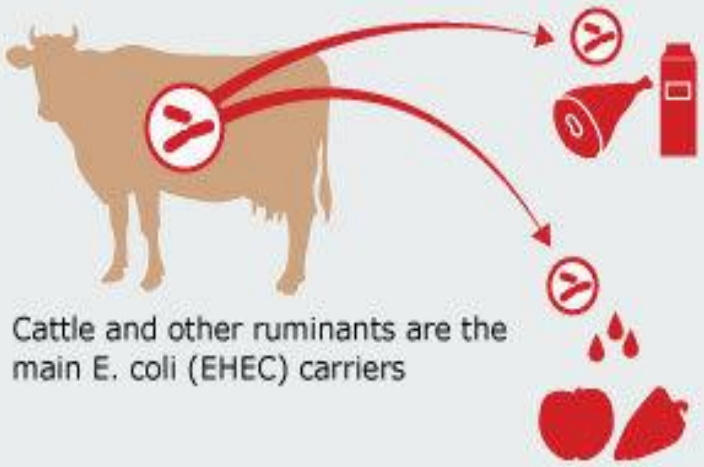
Symptoms caused by E. coli (EHEC)

- Stomach muscle spasms
- Diarrhea (sometimes bloody diarrhea)
- Fever
- Vomiting

Complications:
hemolytic uremic syndrome (HUS)

Death rate: **3-5 %**

INFECTION SOURCES



Cattle and other ruminants are the main E. coli (EHEC) carriers

Uncooked meat and raw milk

The bacteria die when food is exposed to heat (70°C and higher)

Fruit and vegetables (droppings of sick animals find their way into water bodies that in turn feed the soil)

www.rian.ru

5. Enteroinvasive *E. coli* (EIEC)

- Resembles Shigella in many respects.
- Biochemically, genetically, and pathogenetically closely related to *Shigella* spp.
- The bacteria are able to invade and destroy the colonic epithelium, producing a disease characterized initially by watery diarrhea(Cause watery diarrhea to dysentery similar to Shigellosis)

- **Laboratory diagnosis of EIEC**; The original **sereny test**, in which the bacteria are tested for the ability to cause conjunctivitis in guinea-pig, has been superseded by tissue culture methods that exploit the ability of the bacteria to invade monolayers of Hep-2 or HeLa cells.
- Molecular methods

Treatment – *E.coli* (Gastrointestinal disease)

- Fluid and electrolyte replacement therapy
- Antibiotics – not used usually unless systemic infections prevails (e.g. Hemolytic uremia syndrome)

For the other infections firstly the agent is isolated then treatment is applied according to the antibiotic sensitivity test results.

KEY POINTS

1. *E. coli* forms a consistent component of the normal intestinal microbiota
2. *E. coli* is the commonest cause of urinary tract infection.
3. Cause gastroenteritis with five types.
4. **ETEC** causes a chlorea-like illness (traveler's diarrhea).
5. **EHEC** esp. 0157: H7 are a major cause of (HUS) kidney failure in young children

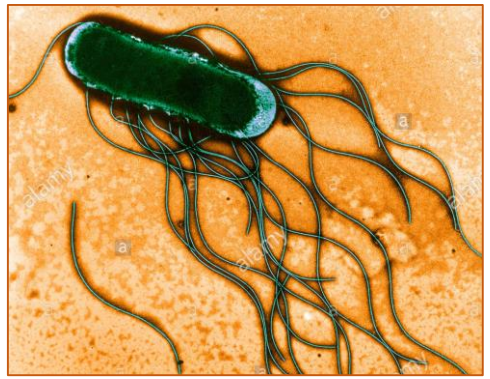
6. **EPEC** cause infantile enteritis

7. Infection with **EIEC** resembles infection that caused by *Shigella dysanteria* type 1

8. **EAggEC** cause chronic diarrheal illness.

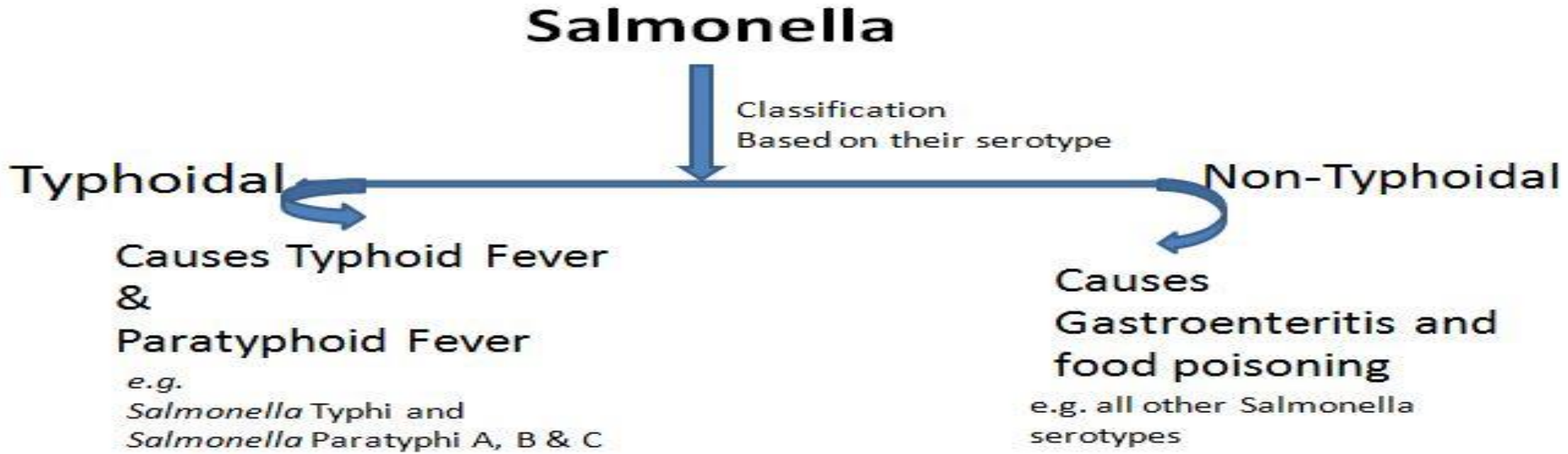
9. Antibiotic treatment is appropriate in UTI and serios sepsis, but most enteric infections are managed conservatively.

SALMONELLA



- It is a bacterium that causes Salmonellosis.
- Second most common bacterial food borne illness.
- The most commonly reported cause of enteric disease.
- In humans; the cause of two diseases called **salmonellosis**:
  - **enteric fever (typhoid),** resulting from bacterial invasion of the bloodstream, and
  - **acute gastroenteritis,** resulting from a foodborne infection/intoxication

- A very complex group contains more 2000 ssp.
- Divided into two groups
  - Enteric fever group
  - Food poisoning group- Septicemias



Enteric fever group

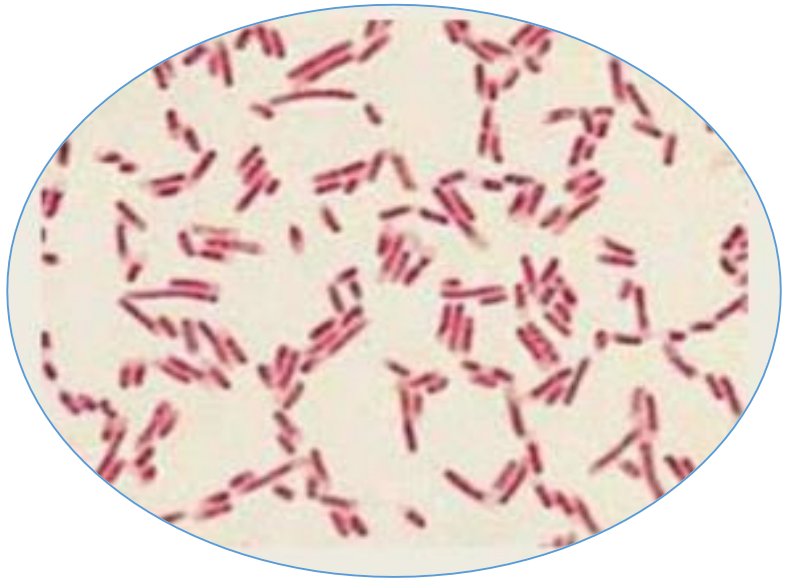
- Enteric fever caused by Salmonella Typhi and other groups called as Paratyphi A, B, C
- Salmonella Typhi causes typhoid, S. Paratyphi A, B, C cause paratyphoid fevers.

Food poison group

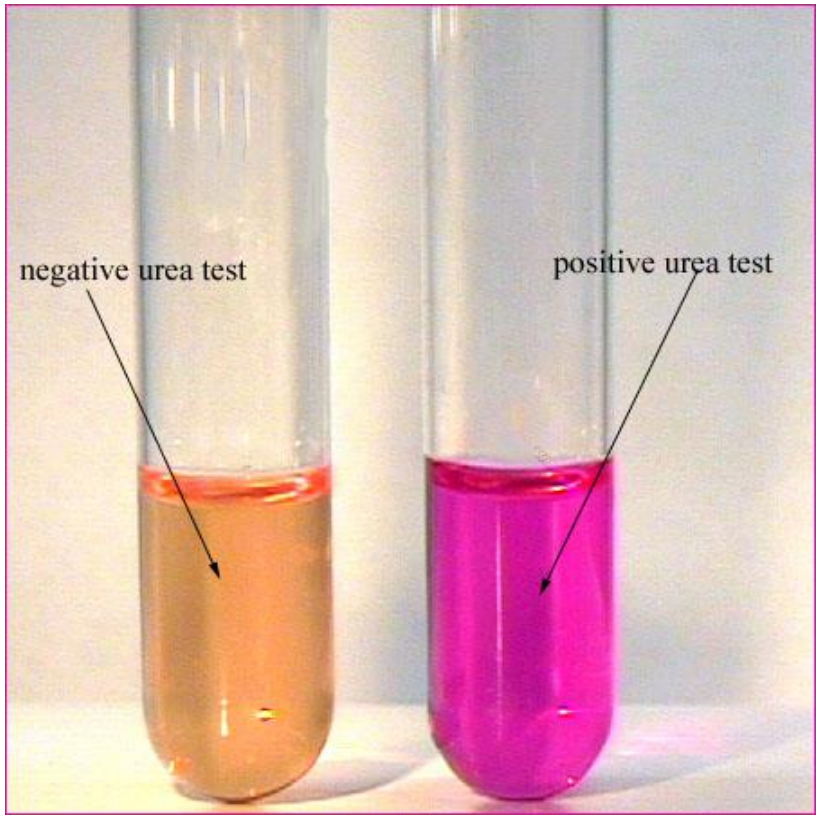
- Spread from animals-humans
- Causes gastroenteritis- Septicemias, localized infection

General Characteristics

- Gram negative, rod-shaped bacilli
- Aerobic/ Facultative anaerobic
- Lactose negative
- Peritrichous flagella (ensure motility)
- Motile (except *S. gallinarum* and *S. pullorum*)
- Uncapsulated (except S. Typhi)



- H_2S production by *S. typhi*
- Temp 15- 41 ºC/ opt 37 ºC
- Colonies appear as large 2-3 mm, circular, low convex
- On MacConkey medium appear colorless (NLF)
- Enrichment medium: Selenite F medium; It is used for isolation of Salmonella from contaminated specimens (stool specimens)
- Urea**:** negative



- IMVIC: **(-, + , - , +)**
- Resistance of Salmonella
  - 55º C – 1 hour
  - 60º C – 15 MT
- Boiling ,Chlorination, Pasteurization Destroy the Bacilli

Selective Broth and Media

- **Selective Enrichment Broth**
  - Selenite F
  - Tetrathionate enrichment Broth (Tetrathionate reductase enxyme)
- **Selective Media**
  - Brillant green agar
  - MacConkey Agar
  - XLD4 (Xylose Lysine Deoxycholate) Agar

On MacConkey Agar

- Salmonella gives colorless colonies

- On Brillant Greeen Agar

Salmonella colonies are red

- On XLD4 Agar

Colonies are red with a black center due to H_2S production

Antigenic structure of Salmonella

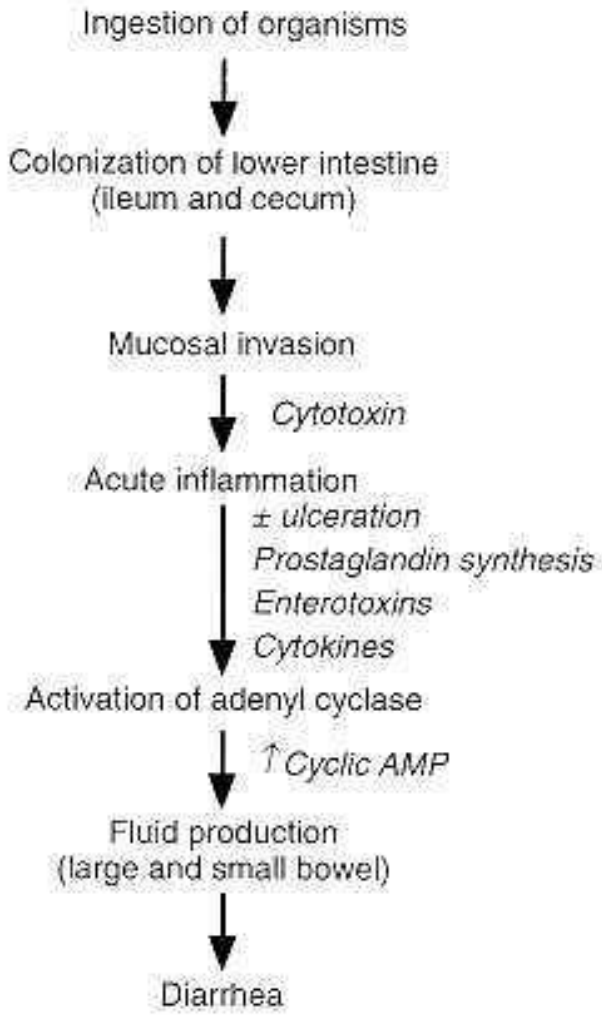
1. Somatic or O antigens: forms integral part of cell wall (LPS)
2. Flagellar or H antigens
3. Vi surface antigen:
   - Covers O antigen,
   - Acts as virulence factor, protects the bacilli against phagocytosis
   - Poorly immunogenic

Clinical Syndromes

- Although Salmonella can cause a wide spectrum of clinical illness there are three major syndromes,
  - Enteric fever
  - Gastroenteritis
  - Bacteremia

A) Enteritis

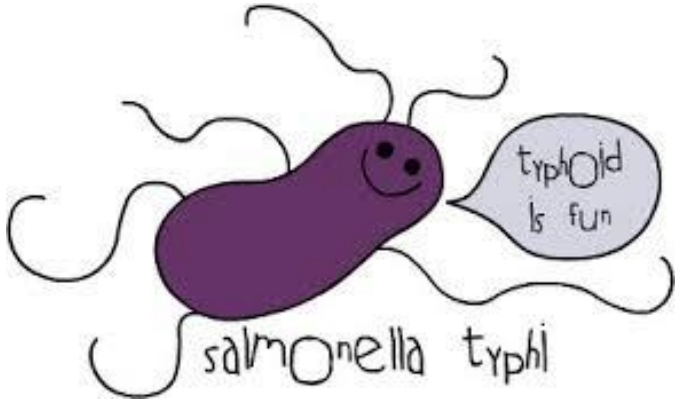
- Salmonella Enteritidis
- Salmonella Typhimurium
- Most serotypes cause enteritis, an infection that is limited to the terminal ileum. The salmonellae invade the intestinal wall and produce enterotoxins that cause **nausea, vomiting and diarrhea.** Bacteria rarely spread beyond the gastrointestinal wall.



- In many countries Salmonella Enteritidis is the third most commonly reported form of “food poisoning”.
- The infection is zoonotic, and the poultry is the source of infection.
- Other sources of infection include milk products, food and water contaminated with animal feces or urine.

- 8-48 hours after the ingestion of food or drink contaminated with Salmonella, enterocolitis begins with **nausea, vomiting, abdominal pain, diarrhea** which can vary from mild to severe.
- In some cases manifestation include fever, headache and chills.
- Salmonella enteritis last about 5 days, but severe loss of fluids and electrolytes may be life threatening in infants and elderly patients.

B)Enteric Fever



- Two serotypes typhi and paratyphi can cause typhoid.
- The salmonella invade the wall of the terminal ileum and than spread to the intestinal lymphatics, where they are phagocytosed by PMNs and macrophages.
- Salmonella phagocytosed by PMNs are killed, but those phagocytosed by macrophages survive and multiply within phagocytic vacuoles.
- Wandering macrophages that contain salmonellae, deliver salmonellae to various reticuloendothelial tissues.
- Infected macrophages are eventually destroyed and salmonellae released from lysed macrophages cause septicemia.

- Ingested bacteria are first taken by invasion in Peyer's plaques during the incubation period of 10-14 days and enter the mesentery lymph nodes and then into the circulation. Bacteria multiply in macrophages in the liver, spleen and bone marrow, where they reach with primary bacteremia. From here, they reach the blood again and again to the intestine via bile.

Diagnosis

- The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens (blood, stool, urine).
- Laboratory identification of the genus Salmonella is done by biochemical tests; the serologic type is confirmed by serologic testing.

(Gruber Vidal test): Tube agglutination test, detect O and H antigens

- Diagnosis of thyphoid and paratyphoid
- First week negative because titers raise in second week , increase of titers is diagnostic
- But nowadays ELISA test and immunoblotting are used for detecting O, H, and Vi antigens.

- But nowadays ELISA test and immunoblotting are used for detecting O, H, and Vi antigens.
- The laboratory diagnosis of bacterial food poisoning depends on isolation of the casual organism from samples of faeces or suscepted foodstuffs.

Prophylaxis

- TAB vaccine: S.Typhi, S. Paratyphi A,B)
- TY21a
- The vaccine prepared Vi antigen

Treatment

- Ceftazidime, ceftriaxone, cefotaxime
- Fluoroquinolones (Ciprofloxacin)
- Co-tromoxazole

KEY POINTS FOR SALMONELLA

- *There are more than 2000 different antigenic types of Salmonella; those pathogenic to human are serotypes of S. enterica.*
- *Most serotypes of. S. enterica cause food-borne gastro-enteritis and have animal reservoirs.*
- *S. enterica serotypes typhi and paratyphi cause typhoid fever.*

- *Typhoid and other systemic salmonella infections are treated with, Ceftazidime, ceftriaxone, cefotaxime, Fluoroquinolones (Ciprofloxacin), Co-tromoxazole*
- *Antibiotics have no place for gastro-enteritis treatments.*
- *Clean water, sanitation, and hygienic handling of foodstuffs are the keys to prevention.*

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