AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 3.

Microbiology diagnosis of intestinal bacterial diseases

(escherichiosis, enteric fever, salmonellosis)

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Morpho-biological characteristics of intestinal bacteria. Antigen structure, serological classification and serotypes.

2. Intestinal bacteria as a representative of the normal microflora of the human body and as a typical opportunistic bacteria

3. Groups of enteropathogenic intestinal bacteria

4. Pathogenesis and clinical manifestations of diseases caused by intestinal bacteria,

5. Antibiotic-resistant forms of intestinal bacteria. Extended-spectrum beta-lactamase (ESBL) resistant bacteria.

6. Microbiological diagnosis of diseases caused by intestinal bacteria

7. General characteristics of Salmonella, intestinal bed and paratyphoid agents, their morpho-biological characteristics. Antigen properties and classification.

8. Pathogenesis of typhus fever.

9. Microbiological diagnostics of typhus fever: bacteriological and serological (Vidal reaction and ELISA). Determination of bacterial transportability

10. Specific treatment and prevention of typhus fever.

11. Salmonella as the causative agent of food toxicoinfection and nosocomial infections.

12. Microbiological diagnosis of salmonellosis

Purpose of the lesson:

- To inform students with the main morpho-biological characteristics of intestinal bacteria and salmonella and the diseases they cause, and to teach them the principles of microbiological diagnosis, specific treatment and prevention of escherichia, typhus and salmonellosis. To inform them about broad-spectrum beta-lactamase-resistant bacteria.

ENTEROBACTERİACEA family

- The family Enterobacteriaceae, or Enterobacteriaceae, includes more than 20 genera that are similar in morphological, tinctorial and cultural properties. The genus includes numerous pathogenic and opportunistic bacterial genera.
- Representatives of the genus ***Escherichia, Shigella, Salmonella*** of the family Enterobacteriaceae are the causative agents of acute intestinal infections in humans. *Yersinia pestis causes plague, Y.pseudotuberculosis and Y.enterocolitica are the causative agents of pseudotuberculosis and intestinal yersiniosis, respectively.*
- Enterobacteriaceae are Gram-negative, non-spore-forming, mostly motile, some non-motile rod-shaped bacteria. Some form a capsule. They are facultative anaerobes and grow easily in normal nutrient media. Metabolism is oxidative and fermentative. They break down glucose only to produce acid, sometimes acid and gas. Nitrates are reduced to nitrites. They are catalase positive and oxidase negative.

ENTEROBACTERİACEA - Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Gammaproteobacteria
- (Order): Enterobacterales
- (Family): Enterobacteriaceae
- (Genus): ----------

Enterobacteriaceae:

- Biostraticola (2008)
- Buttiauxella (1982)
- Cedecea (1981)
- **Citrobacter (1932)**
- Cronobacter (2008)
- Enterobacillus (2015)
- **Enterobacter (1960)**
- **Escherichia (1919)**
- Franconibacter (2014)
- Gibbsiella (2011)
- Izhakiella (2016)
- **Klebsiella (1885)**
- Kluyvera (1981)
- Kosakonia (2013)
- Leclercia (1987)
- Lelliottia (2013)
- Limnobaculum (2018)
- Mangrovibacter (2010)
- Metakosakonia (2017)
- Phytobacter (2017)
- Pluralibacter (2013)
- Pseudescherichia (2017)
- Pseudocitrobacter (2014)
- Raoultella (2001)
- Rosenbergiella (2013)
- Saccharobacter (1990)
- **Salmonella (1900)**
- Scandinavium (2020)
- **Shigella (1919)**
- Shimwellia (2010)
- Siccibacter (2014)
- Trabulsiella (1992)
- Yokenella (1985)

Enterobacteriaceae

Opportunistic pathogens

Escherichia coli

Klebsiella pneumoniae

Enterobacter aerogenes

Serratia marcescens

Proteus spp.

Providencia spp.

Citrobacter spp.

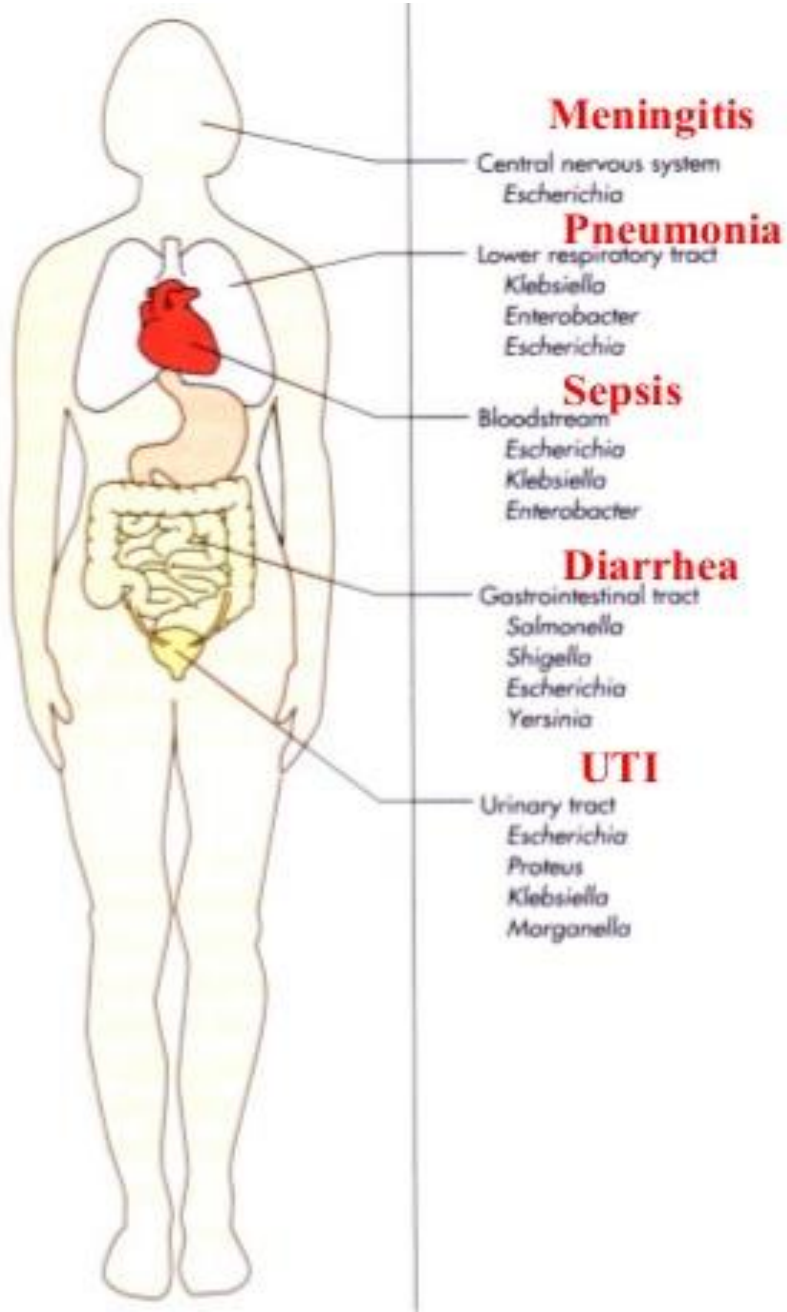
Obligate pathogens

Salmonella spp.

Shigella spp.

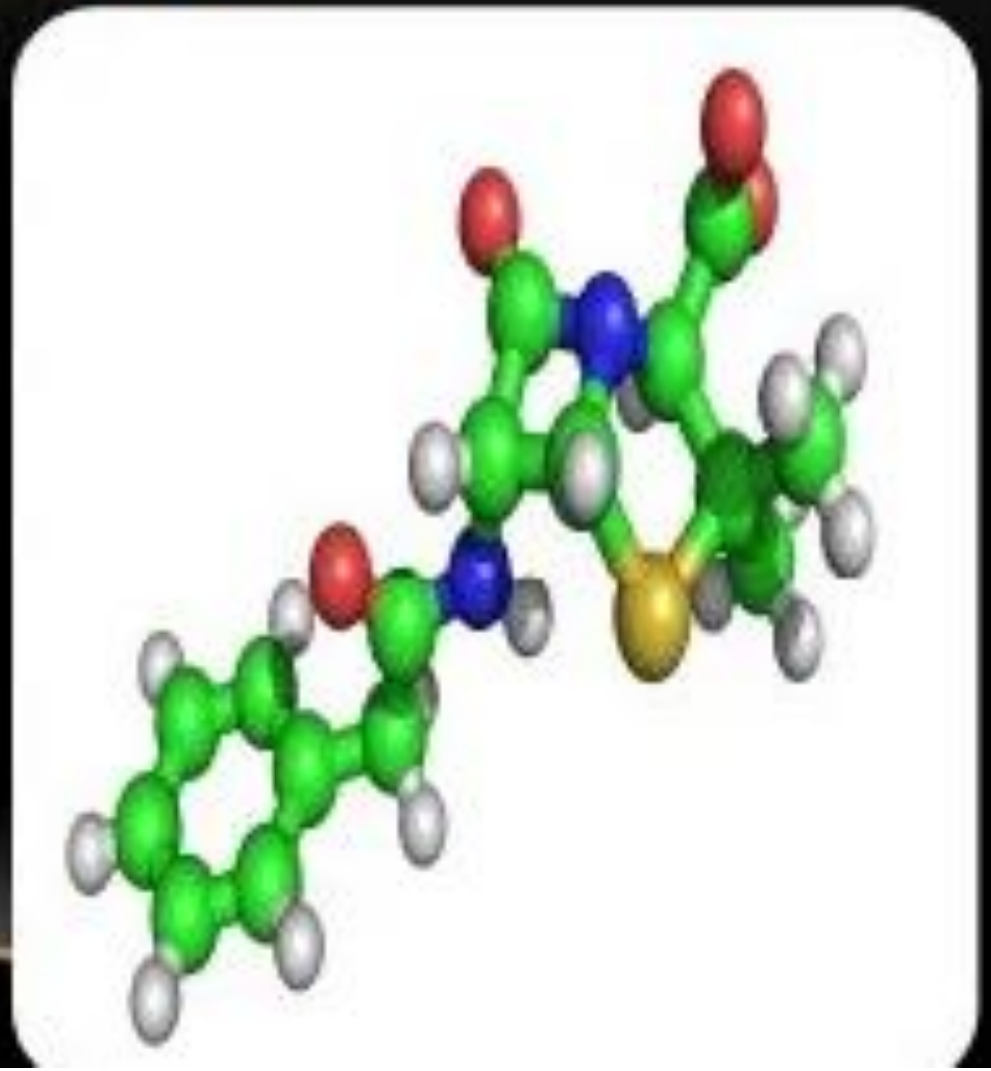
Yersinia spp.

Some *E. coli* strains



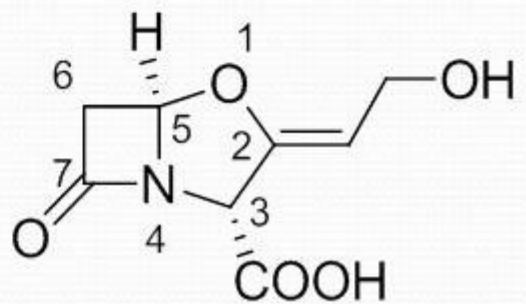
WHAT ARE EXTENDED-SPECTRUM B-LACTAMASES?

- ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and Cefotetan) or carbapenems (e.g., meropenem or imipenem).



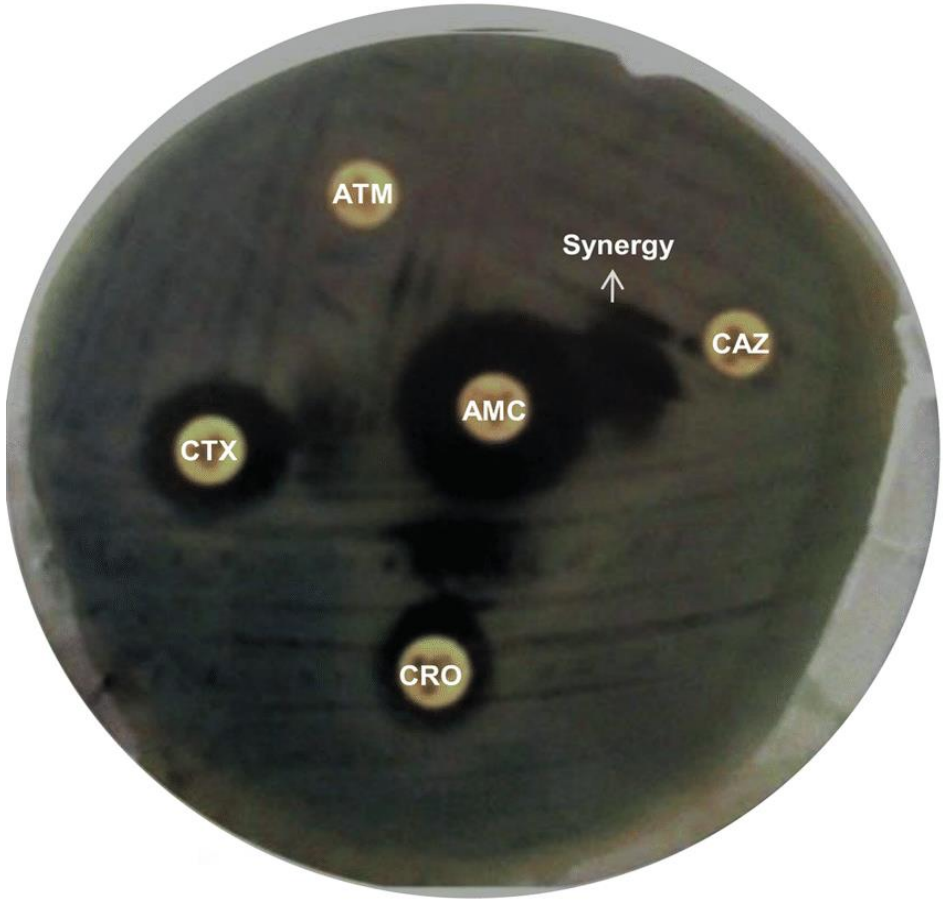
β-lactamase inhibitors

- Almost all have weak antibacterial activity.
- Important in combination with penicillins sensitive to β-lactamase degradation.
- Clavulanic acid is the first one of this class.
  - Natural product from streptomyces.
  - Has a powerful and irreversible inhibition of β-lactamase enzymes because it will covalently bind to two positions in the active site.
  - Normally used in combination with amoxicillin and other β-lactamase sensitive penicillins

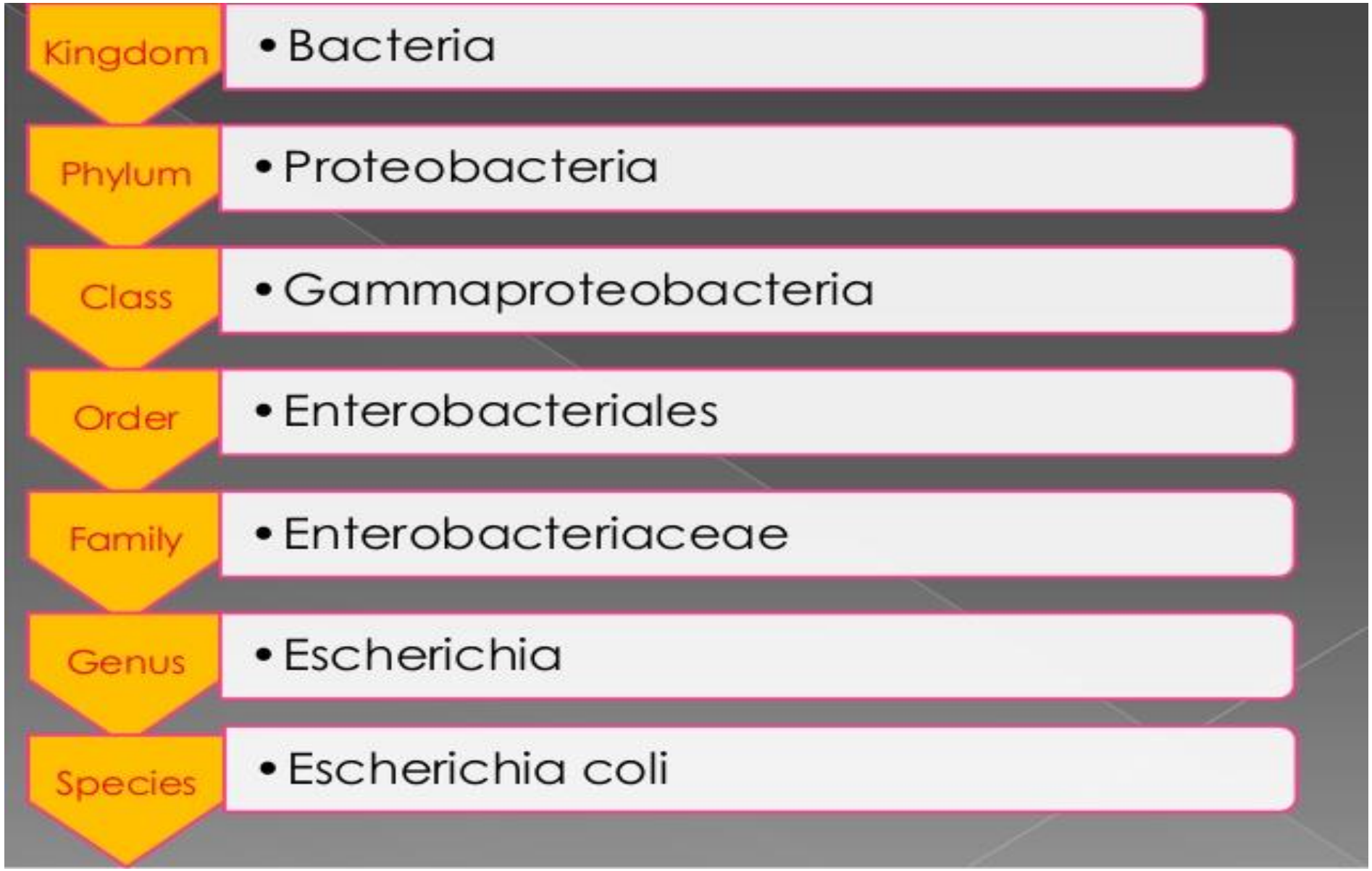


Beta-Lactamase Inhibitors

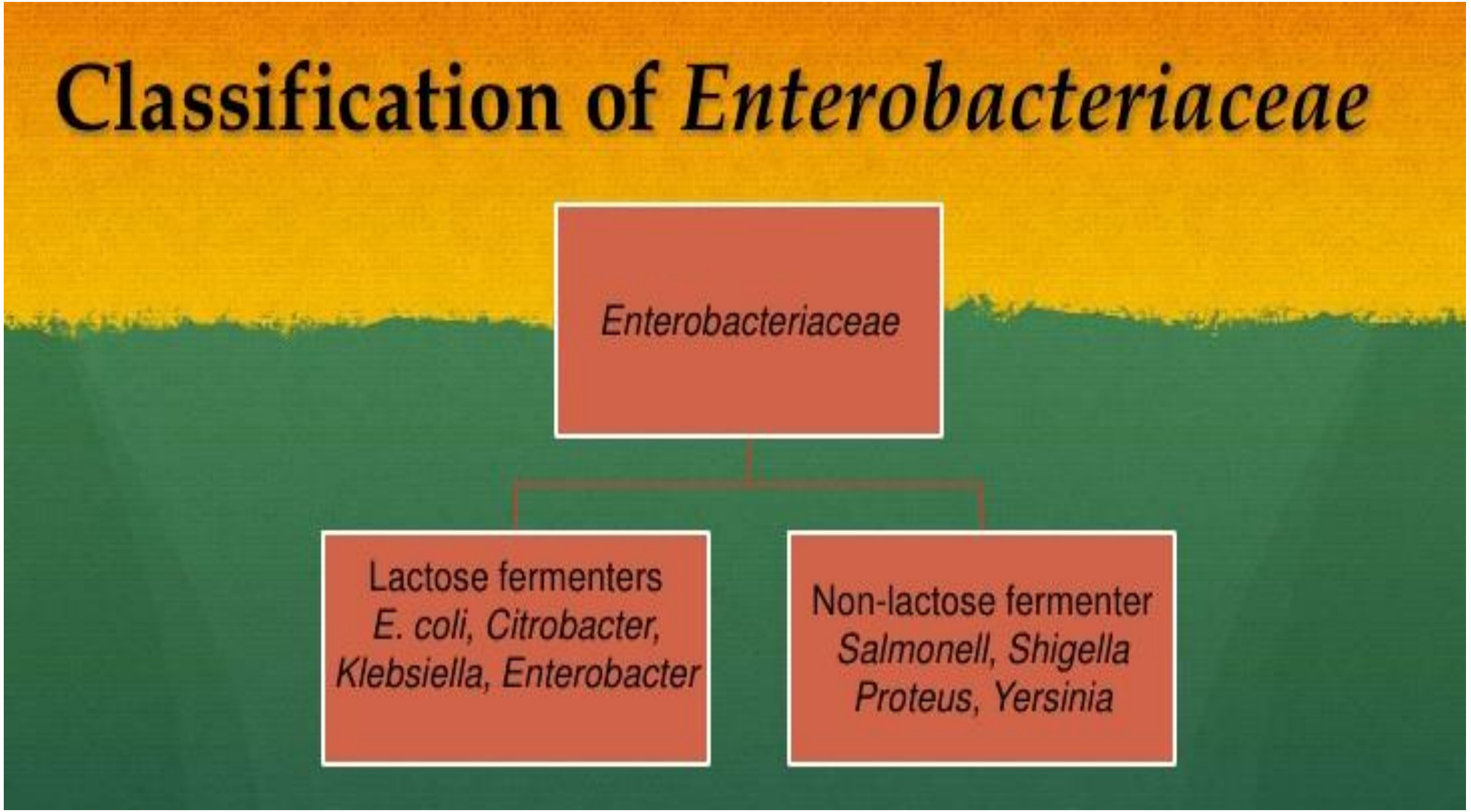
- Clavulanic acid, Tazobactam, Sulbactam
- **Drug Class:** inhibitors of beta-lactamase
- **Trade Names:**
  - Augmentin ® (Amoxicillin + Clavulanate)
  - Zosyn ® (Piperacillin + Tazobactam)
  - Timentin ® (Ticarcillin + Clavulanate)
- **Mechanism of Action:**
  - these three substances resemble β-lactam molecules & are **potent inhibitors of "most" plasmid-mediated beta-lactamases**.
  - **Sulbactam has intrinsic activity against Acinetobacter & may be used against MDR strains.**
- **Indications:**
  - used in fixed combination with specific penicillins: ampicillin, amoxicillin or ticarcillin
  - **penicillin-β-lactamase inhibitor combinations are used for empirical therapy against a wide range of potential pathogens including treatment of aerobic & anaerobic infections (e.g. intra-abdominal infections)**.
  - The β-lactam inhibitor merely extends the activity of the combined penicillin



Phenotypic detection of ESBL producing E. coli. The AMC (amoxicillin/clavulanic acid) disc was placed at the center of the agar plate, while the discs of ATM (aztreonam), CAZ (ceftazidime), CRO (ceftriaxone), and CTX (cefotaxime) were placed in close proximity. The resistance of the E. coli isolate to all cephalosporin and aztreonam, and the synergy between AMC and CAZ, phenotypically confirmed the ESBL production.


Kingdom
• Bacteria
Phylum
• Proteobacteria
Class
• Gammaproteobacteria
Order
• Enterobacteriales
Family
• Enterobacteriaceae
Genus
• Escherichia
Species
• Escherichia coli

Classification of *Enterobacteriaceae*



E.coli

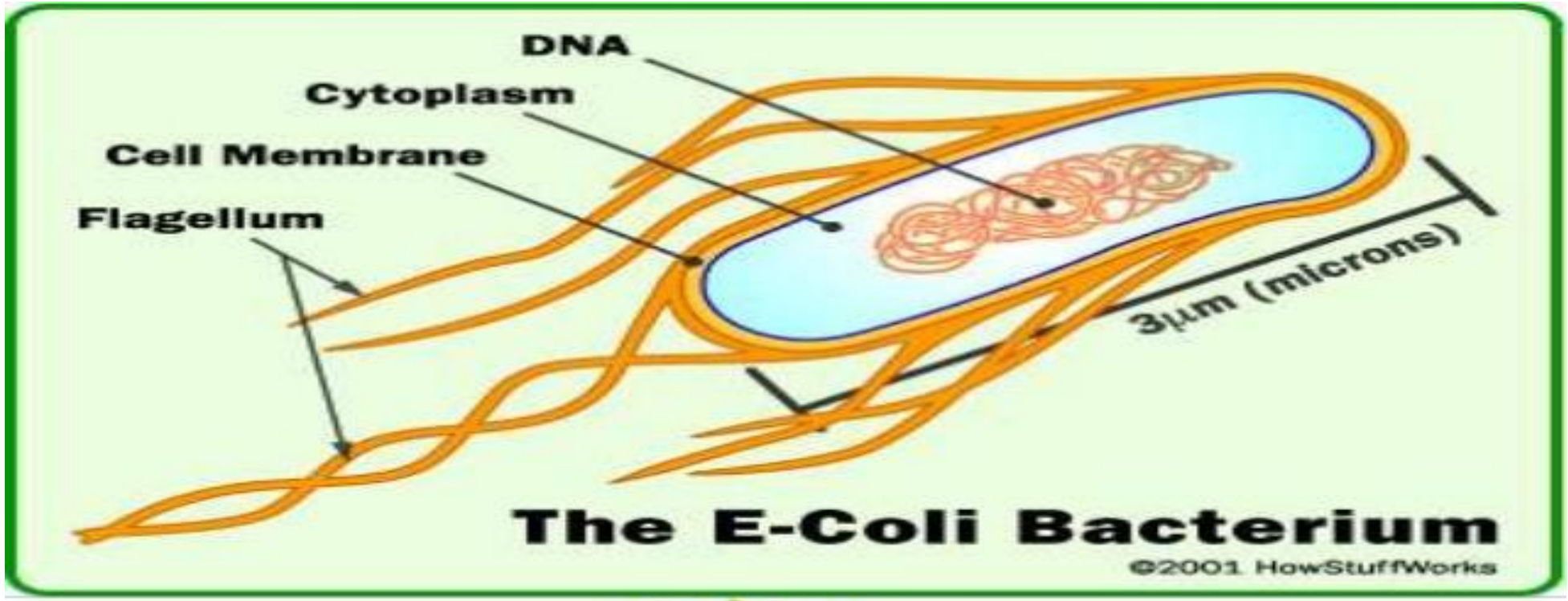
- Morphology Gram - ve Straight rods,
- 1-3 X 0.4 -0.7 microns,
- Appear in singles or in pairs,
- Motile by peritrichate flagella.
- Very few strains non motile
- Not spore forming, Non acid fast.

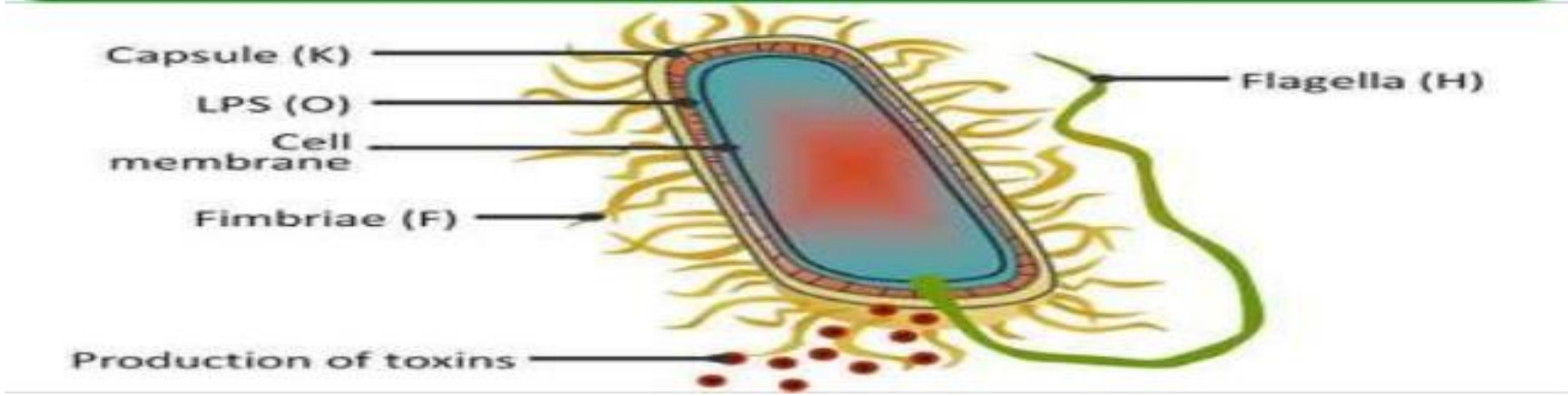
- commonly found in the lower intestine.
- Most *E. coli* strains are harmless.
- fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease.

E.coli

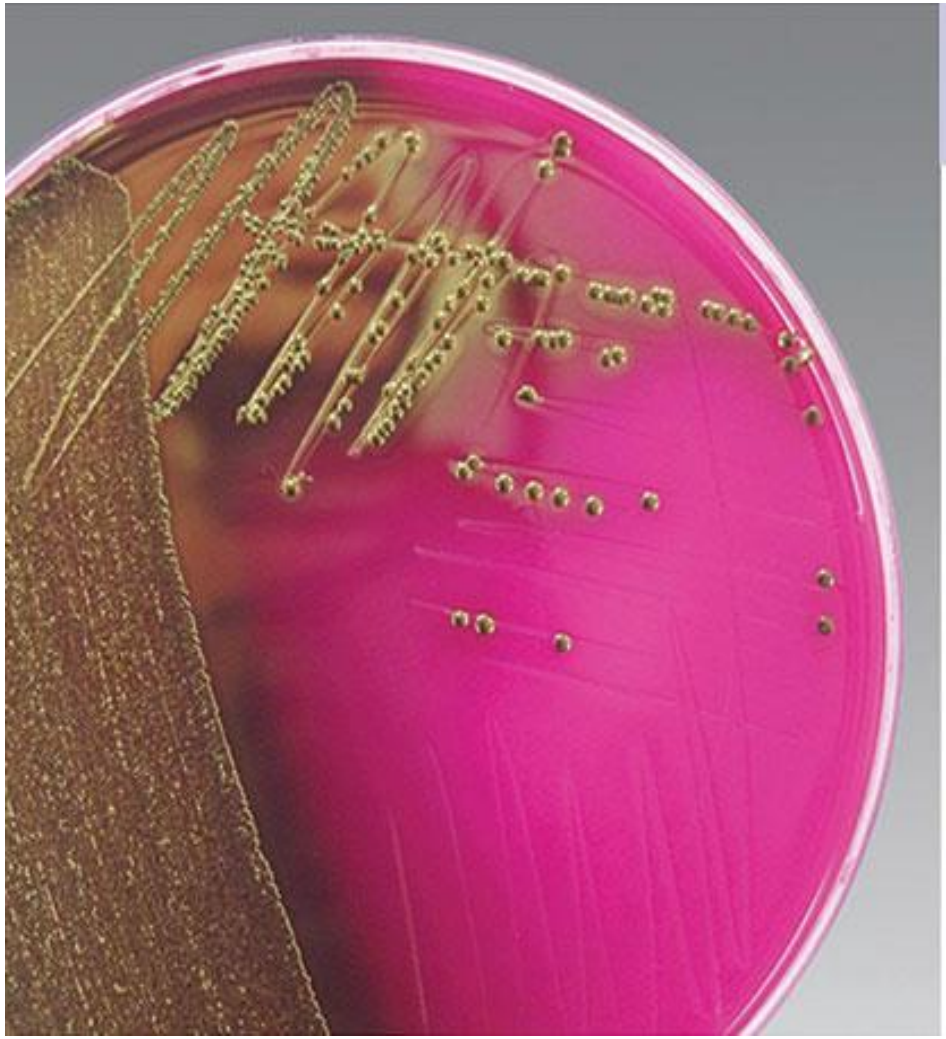
Cultural characters

- Aerobic / Facultative Anaerobic
- Grows between 10 – 40 c optimal at 37 c
- Grown in simple medium
- Produce Large grayish ,Thick white , moist smooth opaque colonies
- May contain capsule.
- On MacConkey medium Produce Bright pink Lactose fermenters.


DNA
Cytoplasm
Cell Membrane
Flagellum
3μm (microns)
The E-Coli Bacterium
©2001 HowStuffWorks

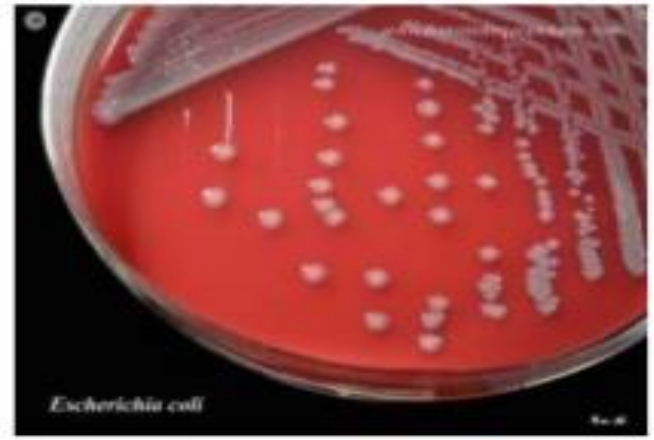

Capsule (K)
LPS (O)
Cell membrane
Fimbriae (F)
Production of toxins
Flagella (H)

Endo Agar



On blood agar

- *E. coli* appear as medium sized, smooth, round, grayish-white colonies on blood agar.



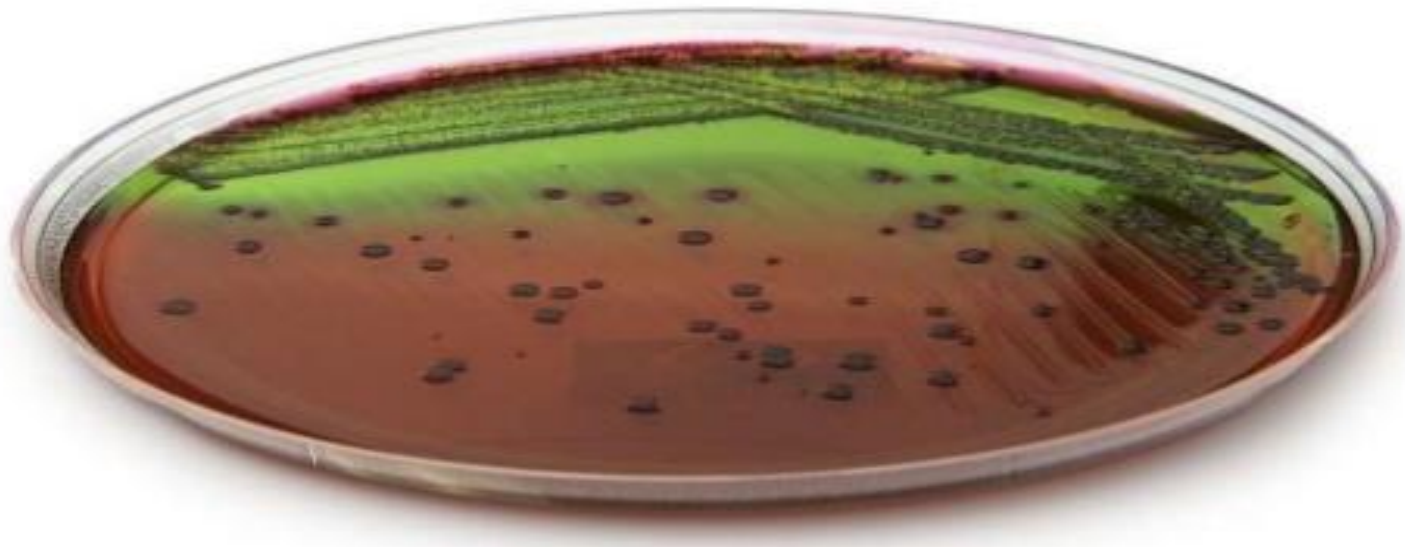
- Some strains produce beta hemolysis.

On macConkey agar

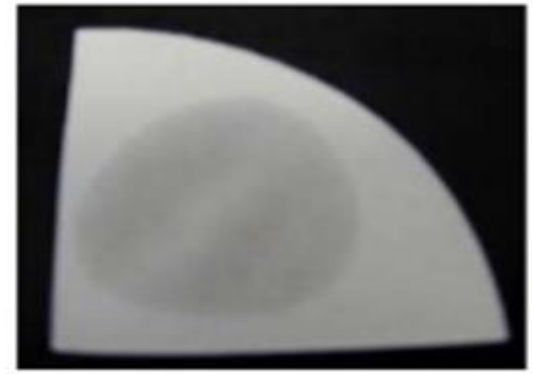
- the red color of the bacterial growth indicates *E. coli* is able to ferment lactose.

On EMB

- Ferment lactose.
- blue-black colonies with a greenish metallic sheen.

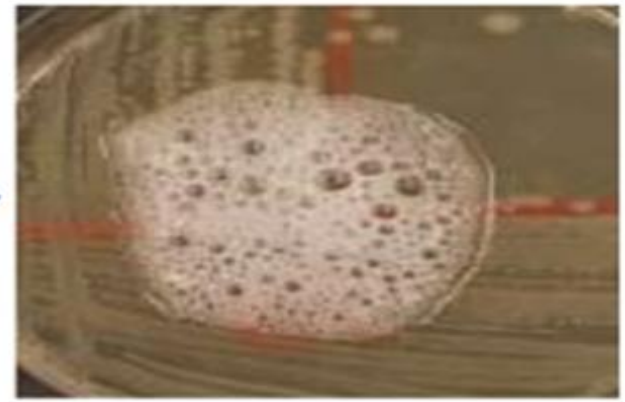


- **Oxidase test**
- Oxidase –negative



- **Catalase test**
- positive

  Bubble formation

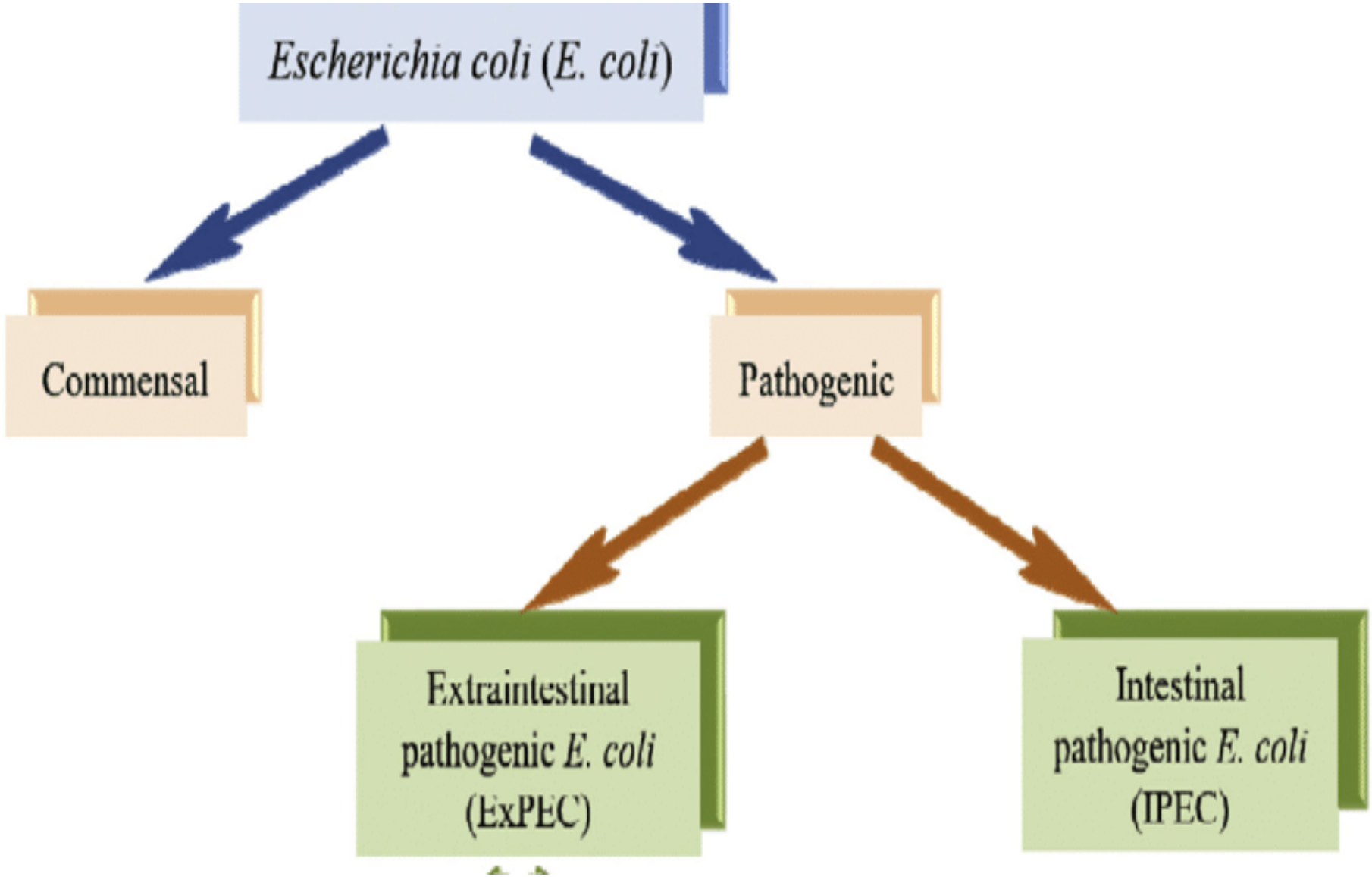


Motility test

- *Escherichia coli* is highly motile and will show turbidity throughout the tube.

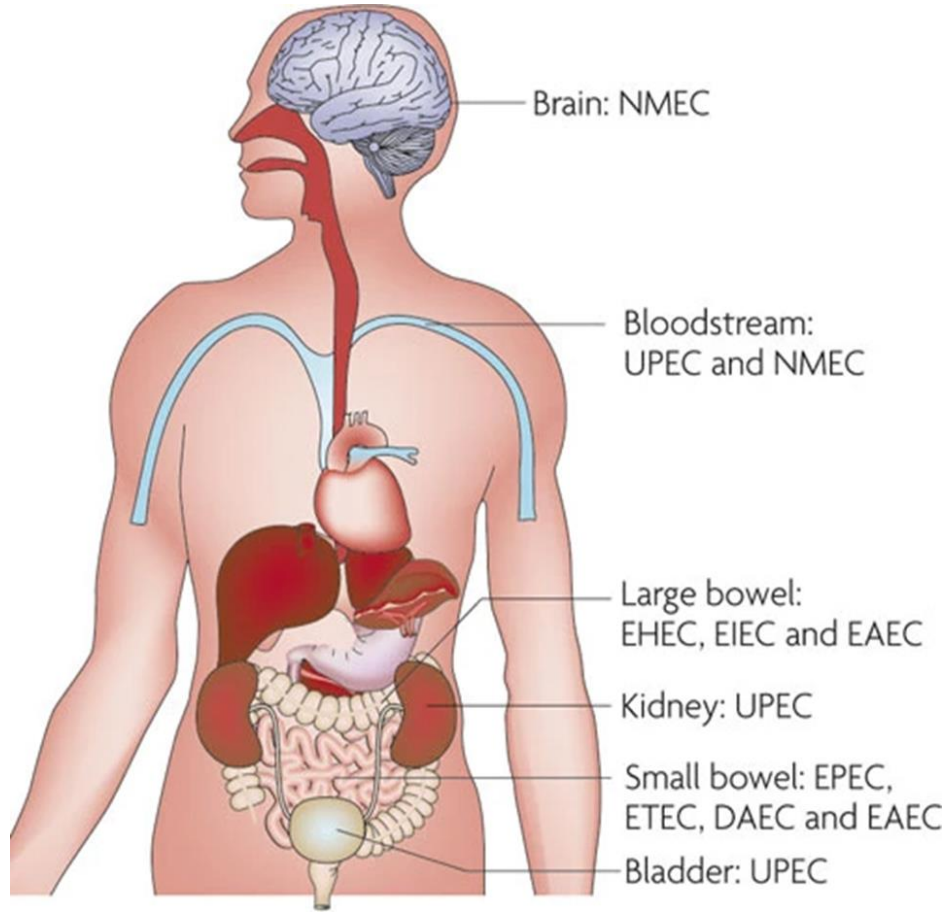
Klebsiella pneumoniae
Non motile

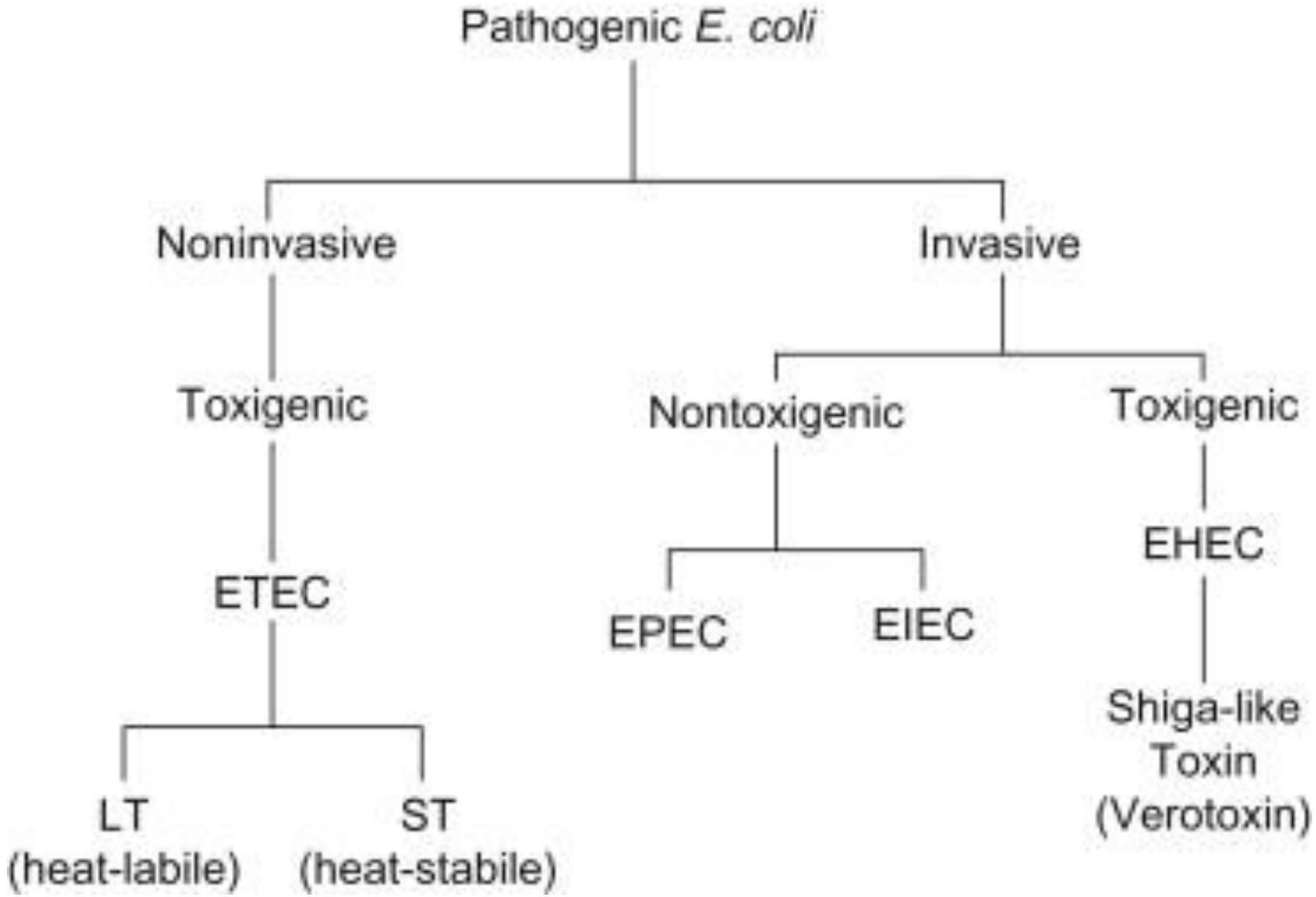
Escherichia coli
motile


Escherichia coli (E. coli)
Commensal
Pathogenic
Extraintestinal pathogenic E. coli (ExPEC)
Intestinal pathogenic E. coli (IPEC)

Pathogenic *E. coli*

- **Intestinal pathogenic *E. coli* InPEC**
  - **Enterotoxigenic *E. coli* ETEC**
  - **Enteropathogenic *E. coli* EPEC**
  - **Enteroinvasive *E. coli* EIEC**
  - **Enterohemorragic *E. coli* EHEC**
  - **Enteroaggregative *E. coli* EAEC**
  - **Adherent invasive *E. coli* AIEC**
  - **Diffusely adherent *E. coli* DAEC**
- **Extraintestinal pathogenic *E. coli* ExPEC**
  - **Uropathogenic *E. coli* UPEC**
  - **Sepsis-associated *E. coli* SEPEC**
  - **Neonatal meningitis-associated *E. coli* NMEC**
  - **Avian pathogenic *E. coli* APEC**
  - **Mammary pathogenic *E. coli* MPEC**

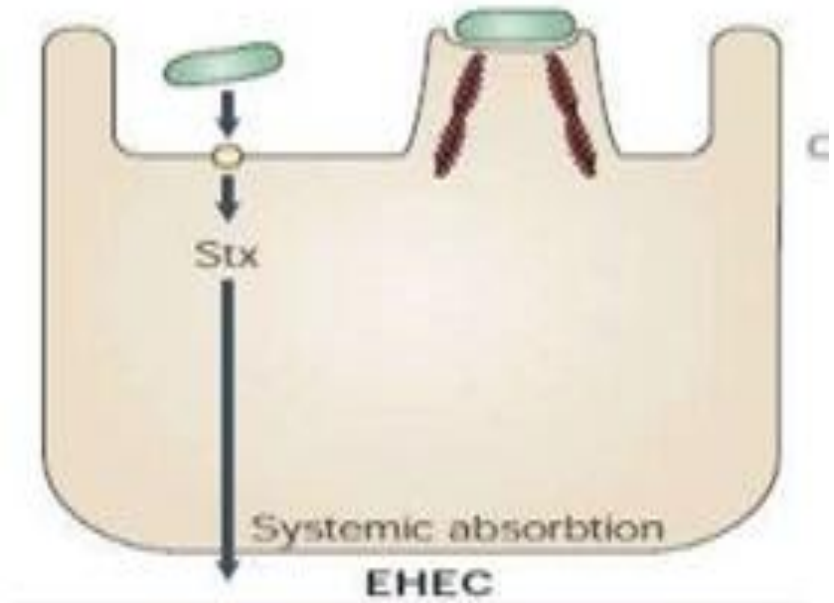



Pathogenic E. coli
Noninvasive
Invasive
Toxigenic
Nontoxigenic
Toxigenic
ETEC
EPEC
EIEC
EHEC
LT
(heat-labile)
ST
(heat-stabile)
Shiga-like
Toxin
(Verotoxin)

Pathogenesis

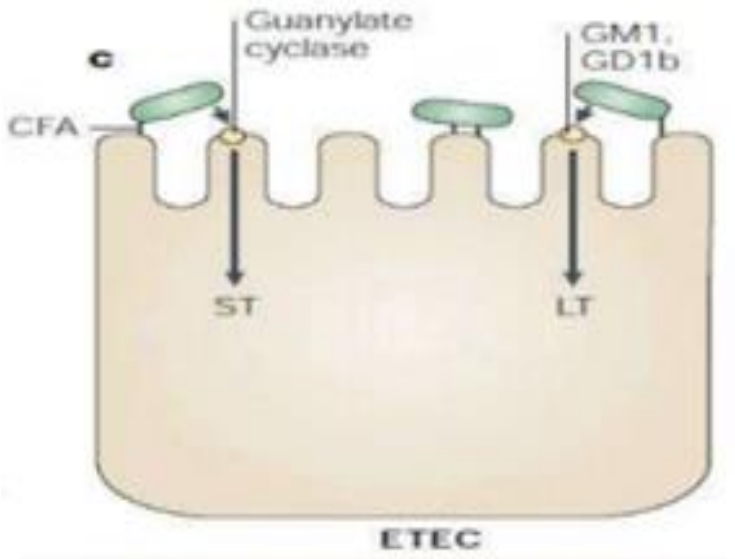
Enterohaemorrhagic E. coli

- This is a strain of E. coli that produces cytotoxins that disrupt protein synthesis within host cells.
- These toxins are also called verocytotoxins or Shiga-like toxins.
- Enterohaemorrhagic E. coli are pathogenic to humans.
- They produce verocytotoxins that form attaching and effacing lesions on epithelial cells.
- Infection occurs via the faecal-oral route.
- Symptoms range from mild diarrhoea to severe bloody diarrhoea.
- Complications include haemolytic uremic syndrome (HUS) which can lead to death if untreated.
- Common serotype E. coli O157:H7



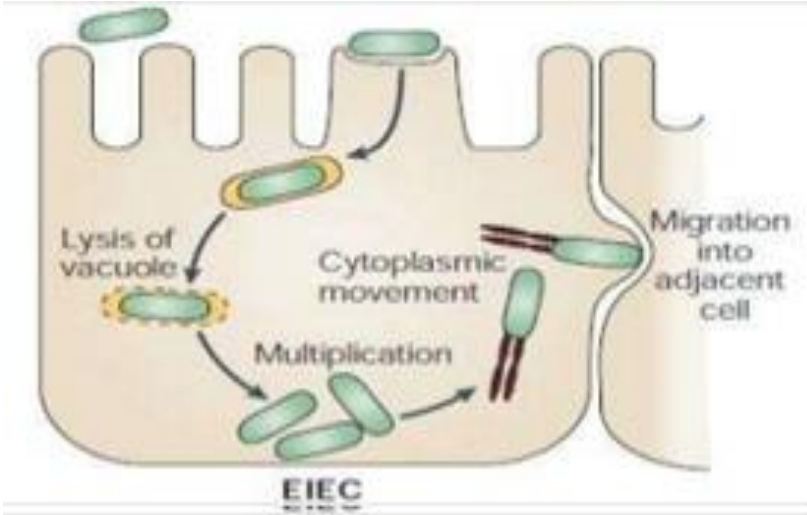
Enterotoxigenic E. coli

- Also known as traveler's diarrhoea.
- Infection leads to watery diarrhoea which may last up to a week.
- Symptoms include abdominal cramps, sometimes nausea and headache.
- It establishes itself by adhering to the epithelium of the small intestine via colonization factor antigens (CFA).
- This is followed by expression of heat stable (ST) or heat labile (LT) enterotoxins.
- These toxins increase adenylate cyclase> ⬆ CAMP levels> ⬆ secretion of chloride ions and water.



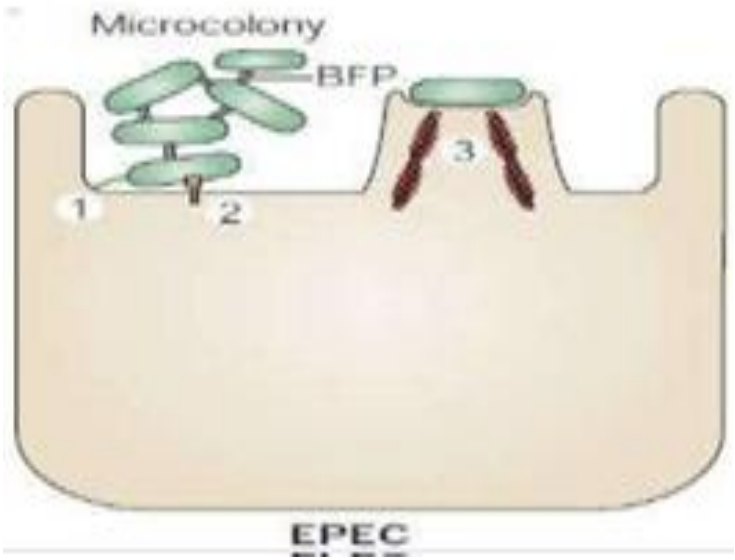
Enteroinvasive E. coli

- Transmitted through faecal-oral route.
- Following ingestion, organisms invade epithelial cells of the intestine resulting in a mild form of dysentery.
- Illness is characterized by presence of blood and mucus in stools of infected individuals.
- Characteristic features of EIEC are their ability to induce entry into epithelial cells and disseminate from cell to cell.
- EIEC infection can occur through contaminated food or water or through mechanical factors such as flies.
- The genes required for entry is clustered on a virulence-associated invasion plasmid in EIEC strains.



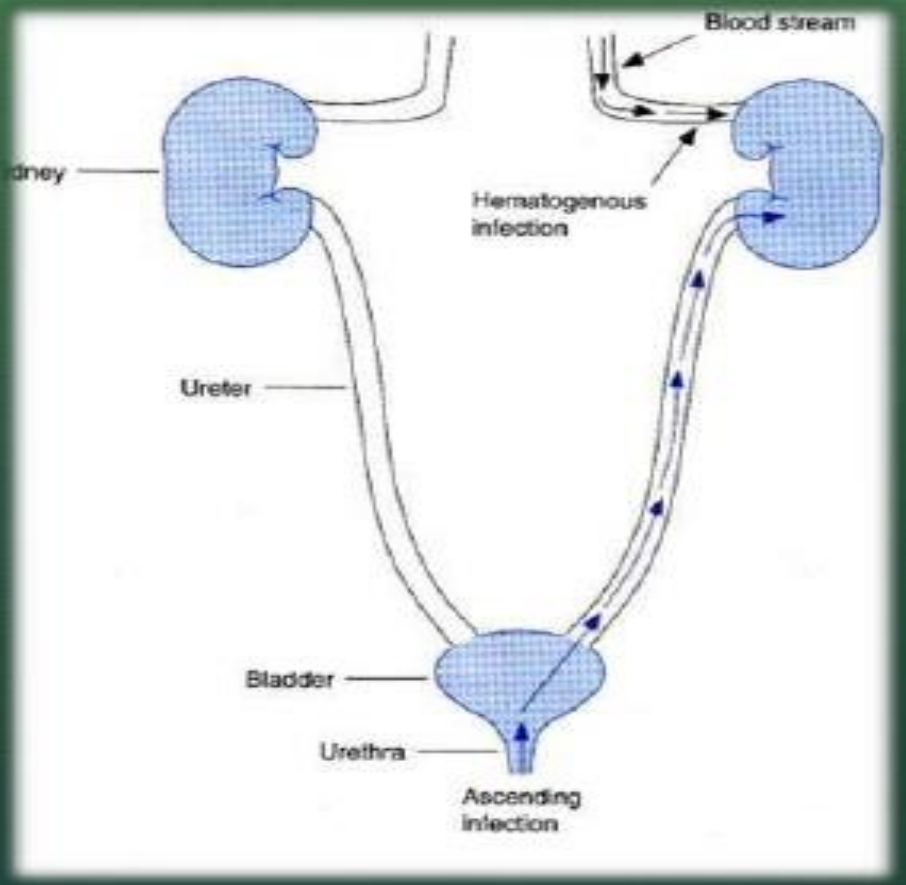
Enteropathogenic E. coli

- Following ingestion, organisms adhere to the epithelial cells of the intestine causing watery or bloody diarrhoea.
- Adherence is mediated by EPEC adherence factor (EAF) and intimin- a non-fimbrial adhesin.
- EPEC attach to and alter the integrity of the intestine.
- Bloody diarrhoea is associated with attachment and an acute tissue-destructive process.
- EPEC do not produce toxins.
- Their virulence mechanism involves the formation of attaching and effacing lesions followed by interference with host cell signal transduction.
- This strain is most commonly associated with paediatrics/kids.

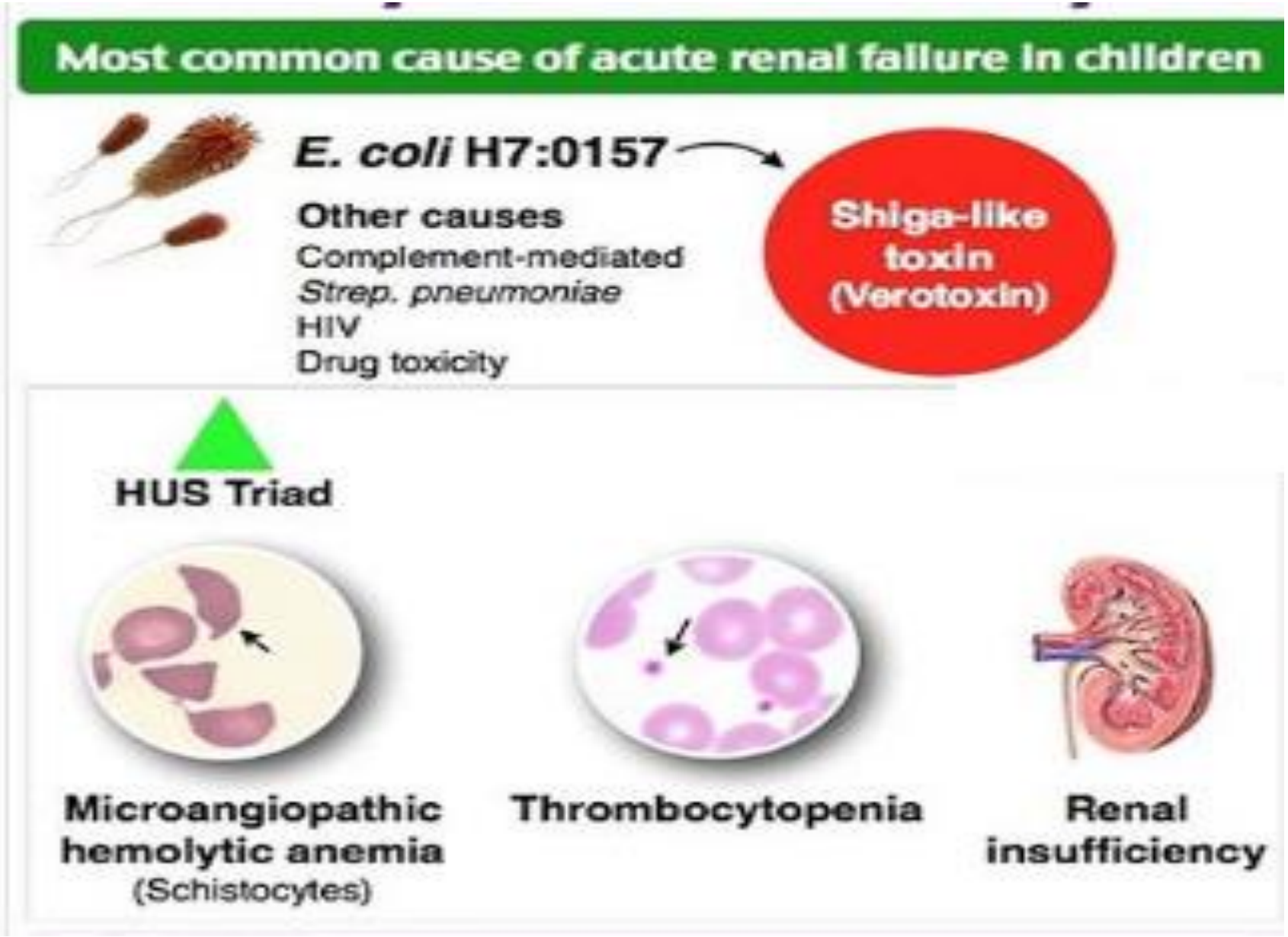


E. Coli leading cause of UTI

- **Clinical significance**
  - Is the leading cause of urinary tract infections which can lead to acute cystitis (bladder infection) and pyelonephritis (kidney infection).



EHEC – hemolytic uremic syndrome (HUS)



Other infection with E.coli

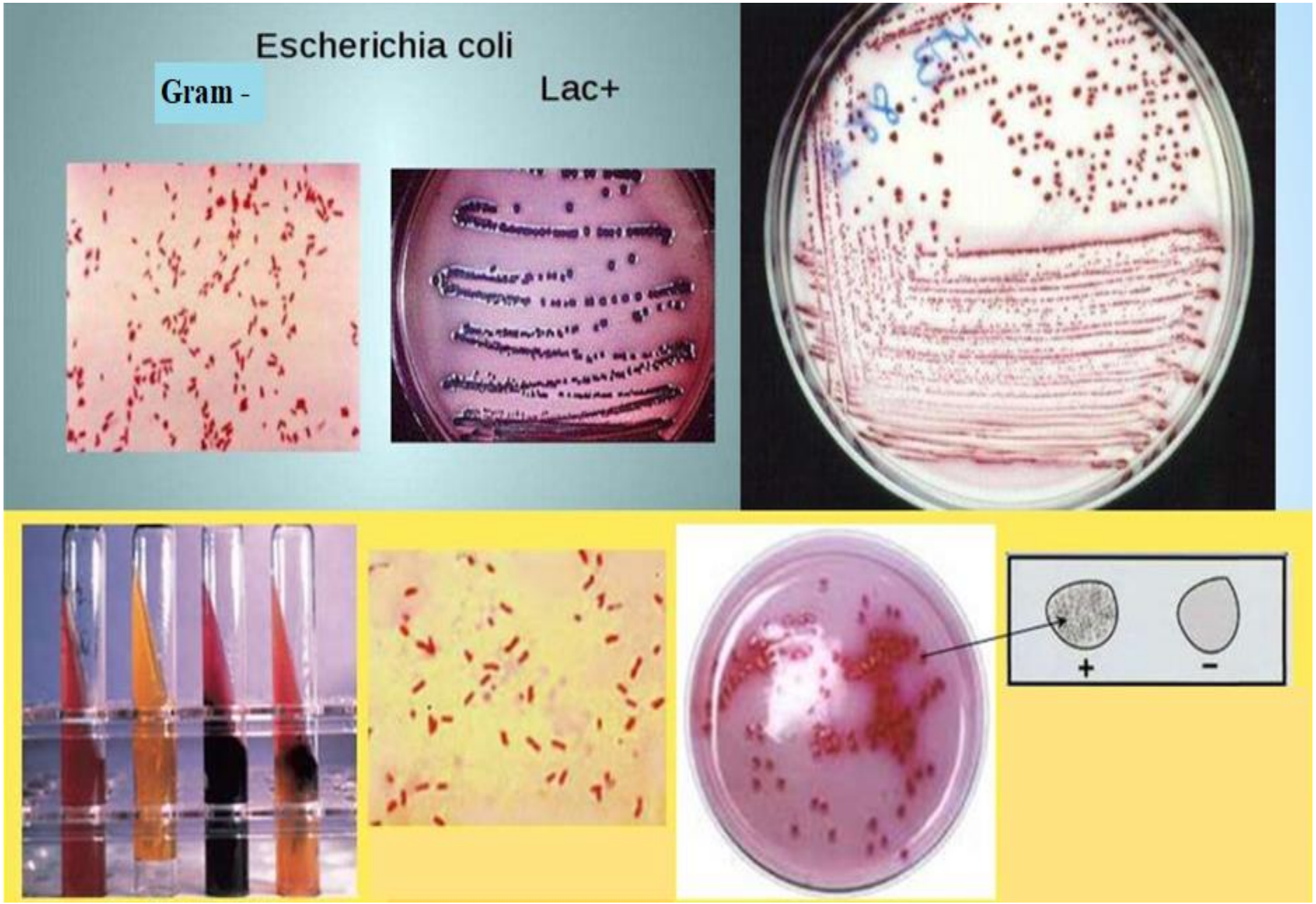
- Pyogenic infections.
- Intraabdominal infections
- Peritonitis. Abscess.
- Septicemias
- Produce Drug resistant infections.

Laboratory diagnosis:

Specimen:

- Urine.
- pus
- blood
- stool
- CSF
- sputum

Microbiological diagnosis - cultural method:

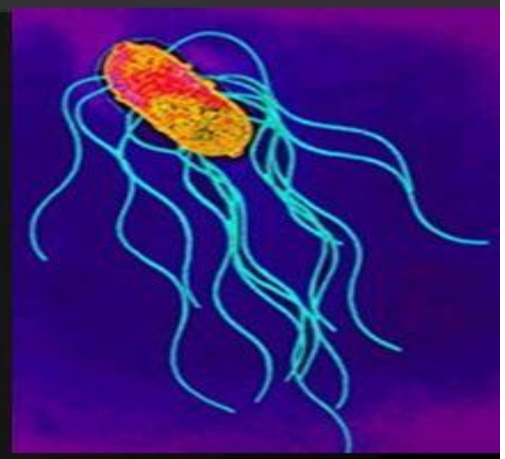


Lab Diagnosis

Tests for identification of E. coli:

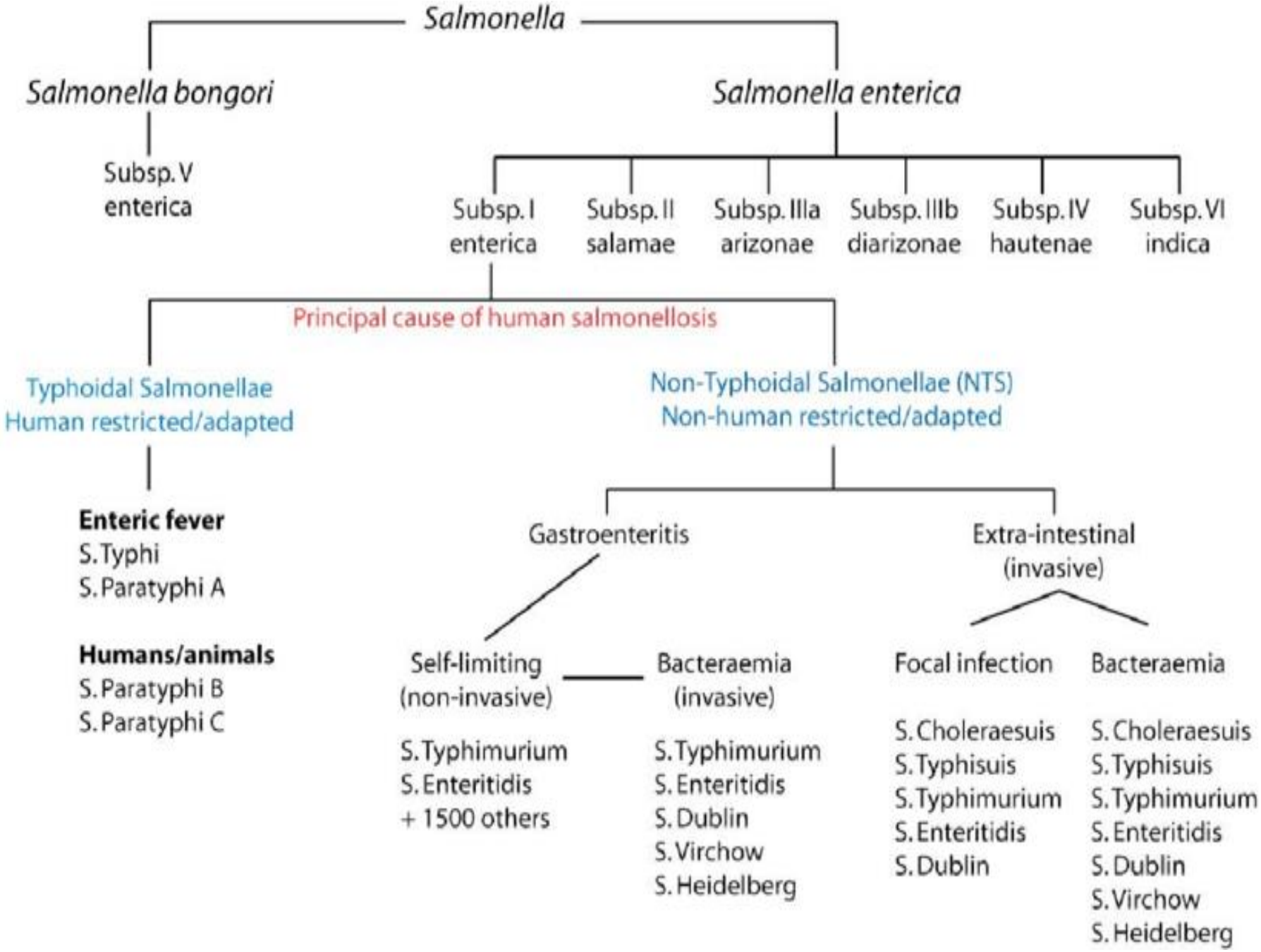
- MacConkey agar- positive
- Indole- positive
- Methyl red- positive
- Citrate- negative
- TSI (H_2S)- negative
- Lysine decarboxylase- positive
- Motility (36□ C)positive
- Acid/gas production- positive
- Lactose fermenter
- Oxidase- negative

SALMONELLA



Salmonella - Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Gammaproteobacteria
- (Order): Enterobacterales
- (Family): Enterobacteriaceae
- (Genus): Salmonella


Salmonella
Salmonella bongori
Subsp. V enterica
Salmonella enterica
Subsp. I enterica
Subsp. II salamae
Subsp. IIIa arizonae
Subsp. IIIb diarizonae
Subsp. IV hautenae
Subsp. VI indica
Principal cause of human salmonellosis
Typhoidal Salmonellae
Human restricted/adapted
Non-Typhoidal Salmonellae (NTS)
Non-human restricted/adapted
Enteric fever
S. Typhi
S. Paratyphi A
Humans/animals
S. Paratyphi B
S. Paratyphi C
Gastroenteritis
Extra-intestinal (invasive)
Self-limiting (non-invasive)
Bacteraemia (invasive)
S. Typhimurium
S. Enteritidis
+ 1500 others
S. Typhimurium
S. Enteritidis
S. Dublin
S. Virchow
S. Heidelberg
Focal infection
Bacteraemia
S. Choleraesuis
S. Typhisuis
S. Typhimurium
S. Enteritidis
S. Dublin
S. Choleraesuis
S. Typhisuis
S. Typhimurium
S. Enteritidis
S. Dublin
S. Virchow
S. Heidelberg

Classification

- Based on DNA-DNA hybridization: 2 species

a. *Salmonella enterica* and
b. *Salmonella bongori*

Salmonella enterica comprises 6 sub species

S. enterica subspecies *enterica*
subspecies *salamae*
subspecies *arizonae*
subspecies *diarizonae*
subspecies *houtanae*
subspecies *indica*

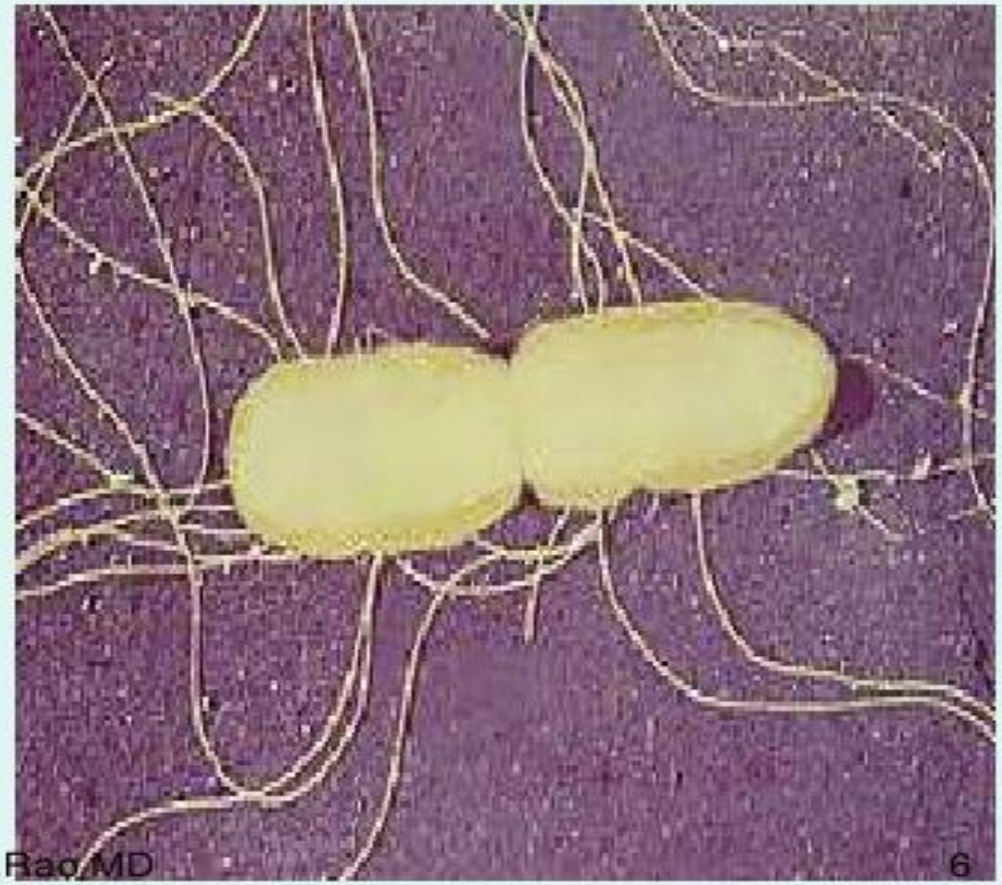
Morphology

- Gram-negative rods
- Motile
- Nonsporing, noncapsulated meas. 2-4 x0.6 micron

- Family: Enterobacteriaceae
- Gram-negative rods
- Motile except *Salmonella* Gallinarum and *S.* Pullorum
- Aerobic and facultatively anaerobic
- Catalase positive; oxidase negative
- Attack sugars by fermentation and produces gas
- Citrate utilization usually positive except *S.* Typhi and *S.* Paratyphi A
- Lysine decarboxylase usually positive except *S.* Paratyphi A
- G+C content 50-53 mol%

Morphology of Salmonella

- Gram negative bacilli
- 1-3 / 0.5 microns,
- Motile by peritrichous flagella



Cultural Characters

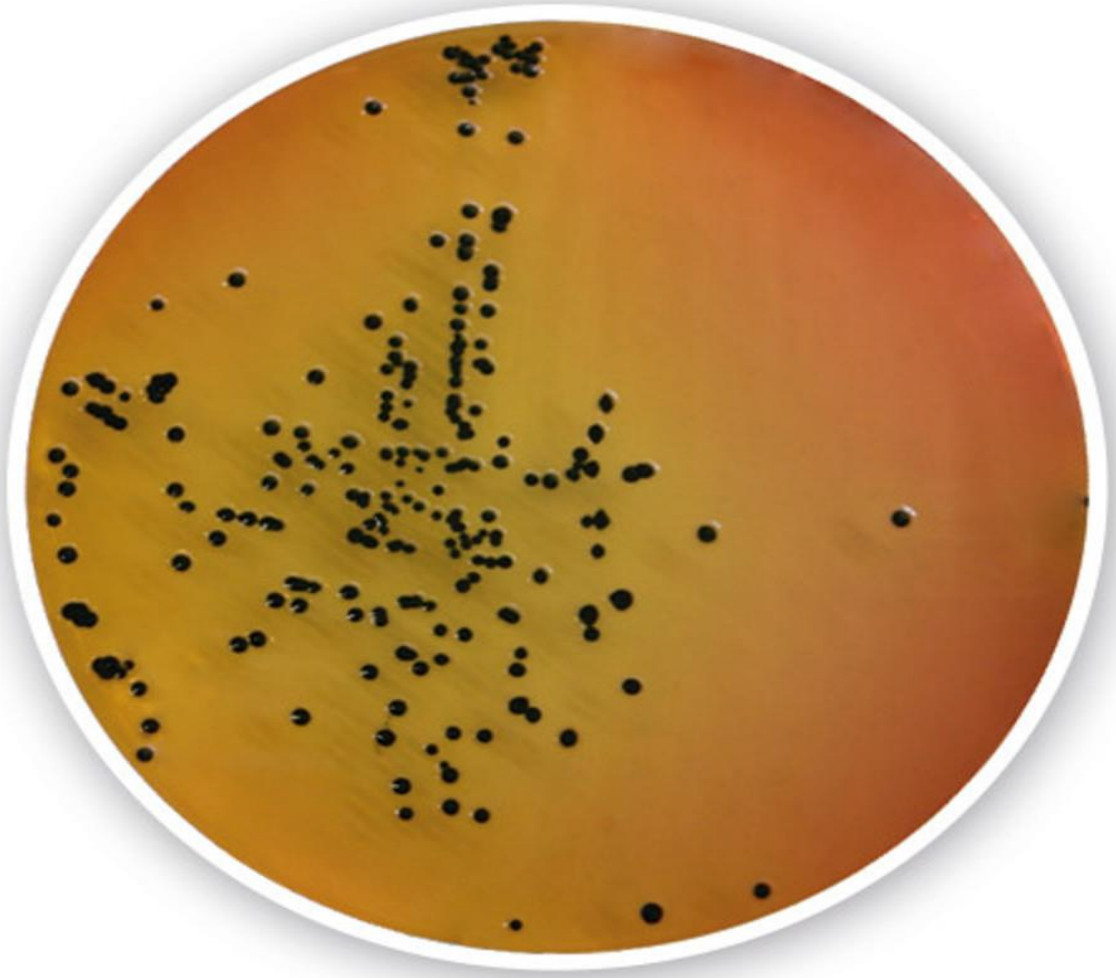
- Aerobic / Facultatively anaerobic
- Grows on simple media - Nutrient agar,
- Temp 15 - 41°c / 37° c
- Colonies appear as large 2 -3 mm, circular, low convex,
- On MacConkey medium appear

  Colorless (NLF)

Selective Medium - Wilson Blair Bismuth sulphide medium. Produce Jet black colonies

H_2S produced by Salmonella typhi

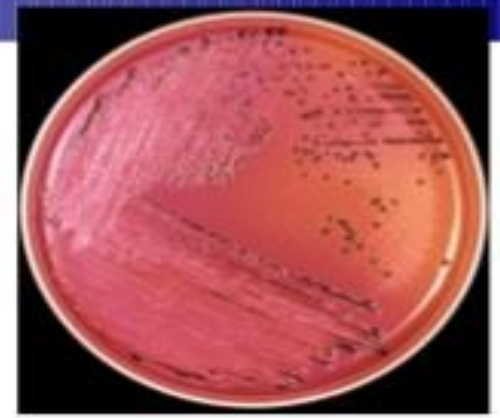
Salmonella spp.
SS (salmonella-shigella) agar



Salmonella spp.
Bismuth Sulfite agar

Salmonella on Mac Conkey's agar

Cultural character



- Grow on ordinary culture media
- In MacConkey agar and DCA: Small, circular, translucent, nonlactose fermenting colonies.
- In Wilson and Blair Bismuth sulfite medium: Black colonies with metallic sheen due to production of H_2S
- Selenite F and tetrathionate broth (enrichment media for stool specimen culture)

Biochemical Characters

- Glucose ,Mannitol ,Maltose produce A/G
- Salmonella typhi do not produce gas
- Lactose/Salicin/sucrose not fermented.
- Indole -
- Methyl Red +
- V P -
- Citrate +
- Urea -
- H_2S - produced by Salmonella typhi
- Paratyphi A do not produce H_2S

- Salmonella can cause

3. Enteric fever
4. Gastroenteritis
5. Septicemia with or without focal suppuration
6. Carrier state

Pathogenicity

- Enteric Fever-
2. Typhoid fever- S. typhi
3. Paratyphoid fever – S.paratyphi A,B,C

- Salmonella typhi—typhoid fever
- Eberth-Gaffky bacillus/Eberthella typhi

- Practically salmonella has been divided into

6. The enteric fever group-typhoid,paratyphoid bacilli
7. The food poisoning group

Clinical Manifestations

- Typhoidal salmonella – Enteric fever
- Non typhoidal salmonella – Gastroenteritis

- Bacteremia
- Osteomyelitis
- Localised infections
- Carriers

Clinical manifestations

- Head ache, malise,anorexia ,coated tongue
- Abdominal discomfort,
- Constipation / Diarrhea
- Step ladder type fever,
- Relative bradycardia,
- A soft palpable spleen
- Hepatomegaly
- Rose spots appear

PATHOPHYSIOLOGY

Ingest contaminated food

↓

Ingested bacilli invade small intestinal mucosa

↓

Taken up by macrophage & transported to regional lymph node

↓

S.typhi multiply in the intestinal lymphoid tissue

↓

Intact with enterocytes & M cells(ileal Peyer's pathches) during the 1-3 week of incubation period (Diarrhoea)

↓

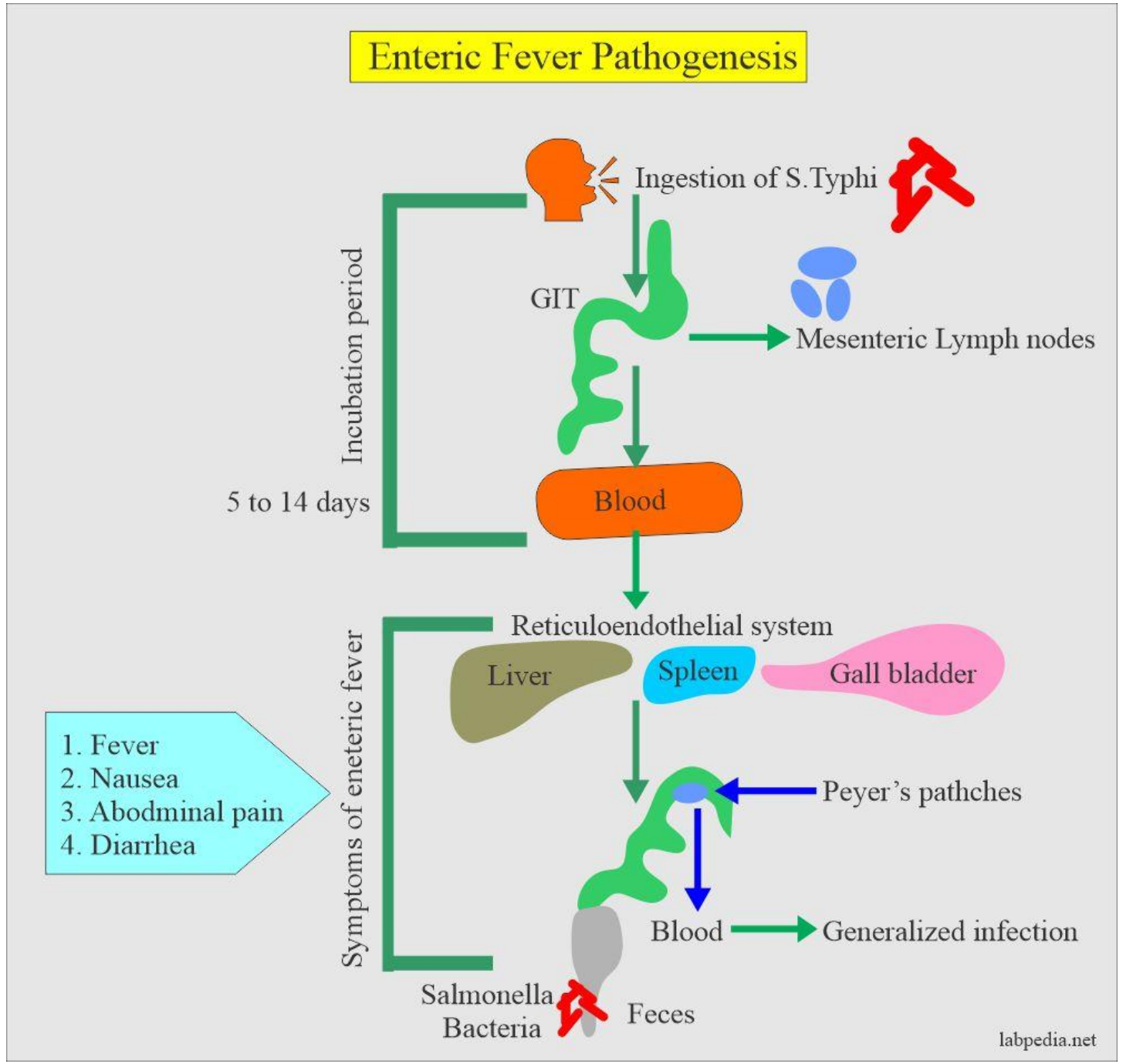
End of incubation period, bacilli enter bloodstream(Bacteraemia phase)

↓ (onset of typhoid fever)

Bacteria invade the gallbladder, biliary system and lymphatic tissue of the bowel and multiply in high number

↓

Then pass into the intestinal tract (stool)


Enteric Fever Pathogenesis
Ingestion of S.Typhi
GIT
Mesenteric Lymph nodes
Incubation period
5 to 14 days
Blood
Reticuloendothelial system
Liver
Spleen
Gall bladder
1. Fever
2. Nausea
3. Abodminal pain
4. Diarrhea
Symptoms of eneteric fever
Peyer's pathches
Blood
Generalized infection
Salmonella
Bacteria
Feces
labpedia.net

CLINICAL FEATURES

Stage 1 (1ST WEEK)

- Slowly rising (stepladder fashion) of temperature for 4-5 days
- Abdominal pain & myalgia
- Malaise
- Headache
- Constipation
- Relative bradycardia

Stage 2 (2ND WEEK)

- Signs and symptoms of 1st week progress

End of 2ND WEEK

- Delirium, complications, then coma & death (if untreated)

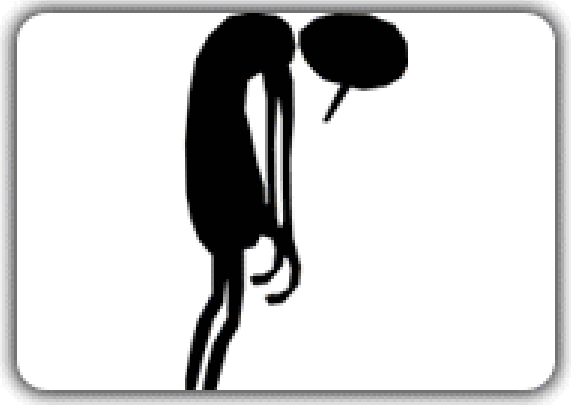
End of 1ST WEEK

- Rose spots may appear on the upper abdomen & on the back of sparse
- Cough
- Splenomegaly
- Abdominal distension with tenderness
- Diarrhea

Typhoid fever symptoms

High fever.

Headache



Weakness

Dry cough

Stomach pain

Constipation

Rashes

Laboratory diagnosis of Enteric fever

- Typhoid fever + Paratyphoid fever

- Typhoid fever – S.Typhi
- Paratyphoid fever – S.Paratyphi A, B, and C

Specimen collection

Blood
Serum
Urine
Feces
BoneMarrow
Bile
Pus
CSF
Sputum
Gall bladder
Liver
Spleen
Mesentric lymph nodes

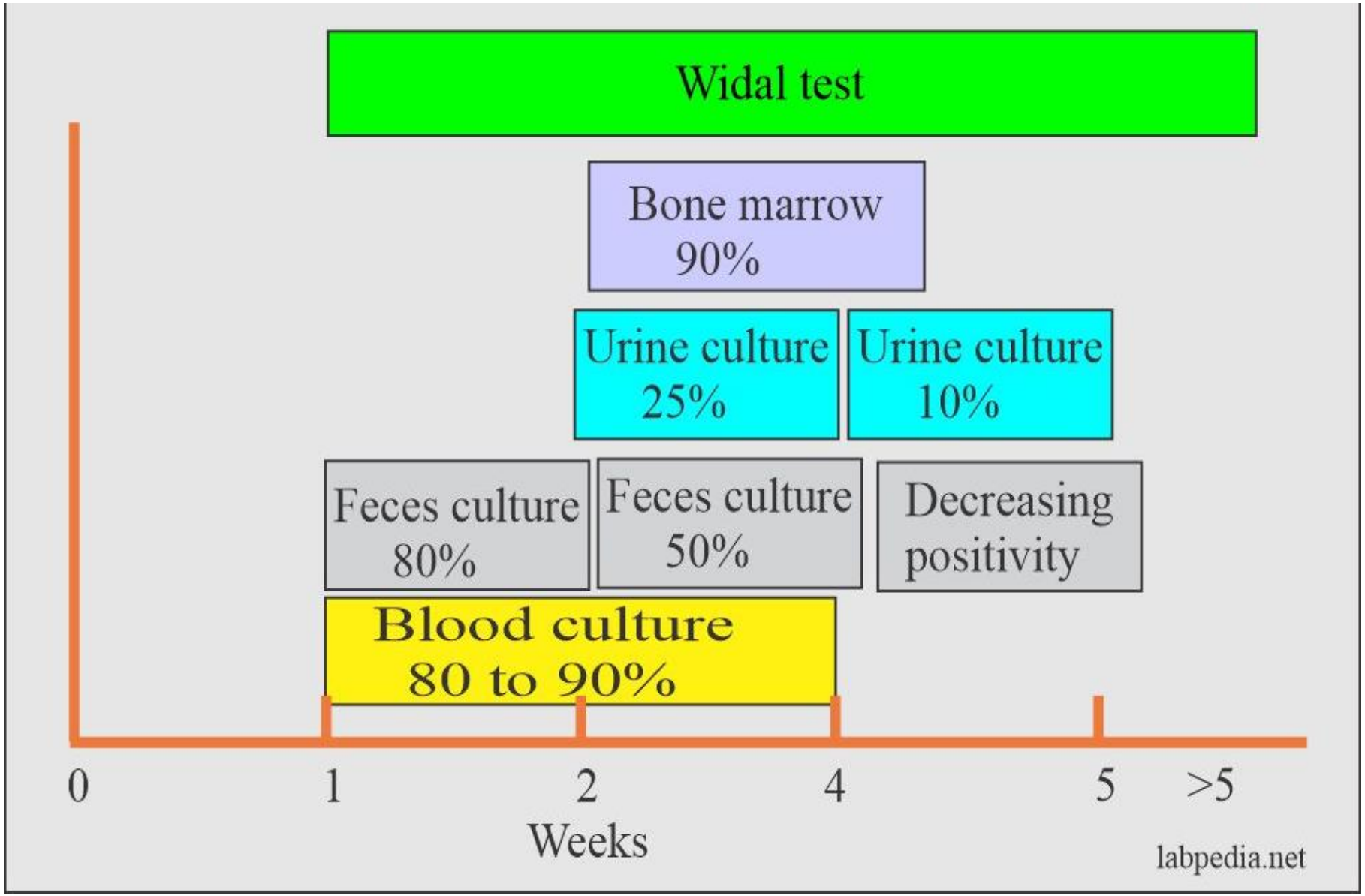
Ideal specimen

First week	Blood (culture)
Second week	Serum (Antibodies)
Third week	Stool
Fourth week	Urine

Chance of isolation

Specimens	First week	Third week
Blood	50 to 80%	30%
Feces	40 to 50%	80%
Urine	-	25%

Effectiveness of microbiological diagnostic methods



Blood culture

- Volume of blood :

  10 to 15 ml from adults and adolescents , 2 to 4 ml in children
- Ratio of blood to bile broth: 1:10
- Or add saponin to BHI broth with 0.05% SPS
- Inoculate the blood immediately
- Transport immediately, never store under 15degC
- Incubate as soon as possible

Diagnosis of Enteric Fever

Widal test

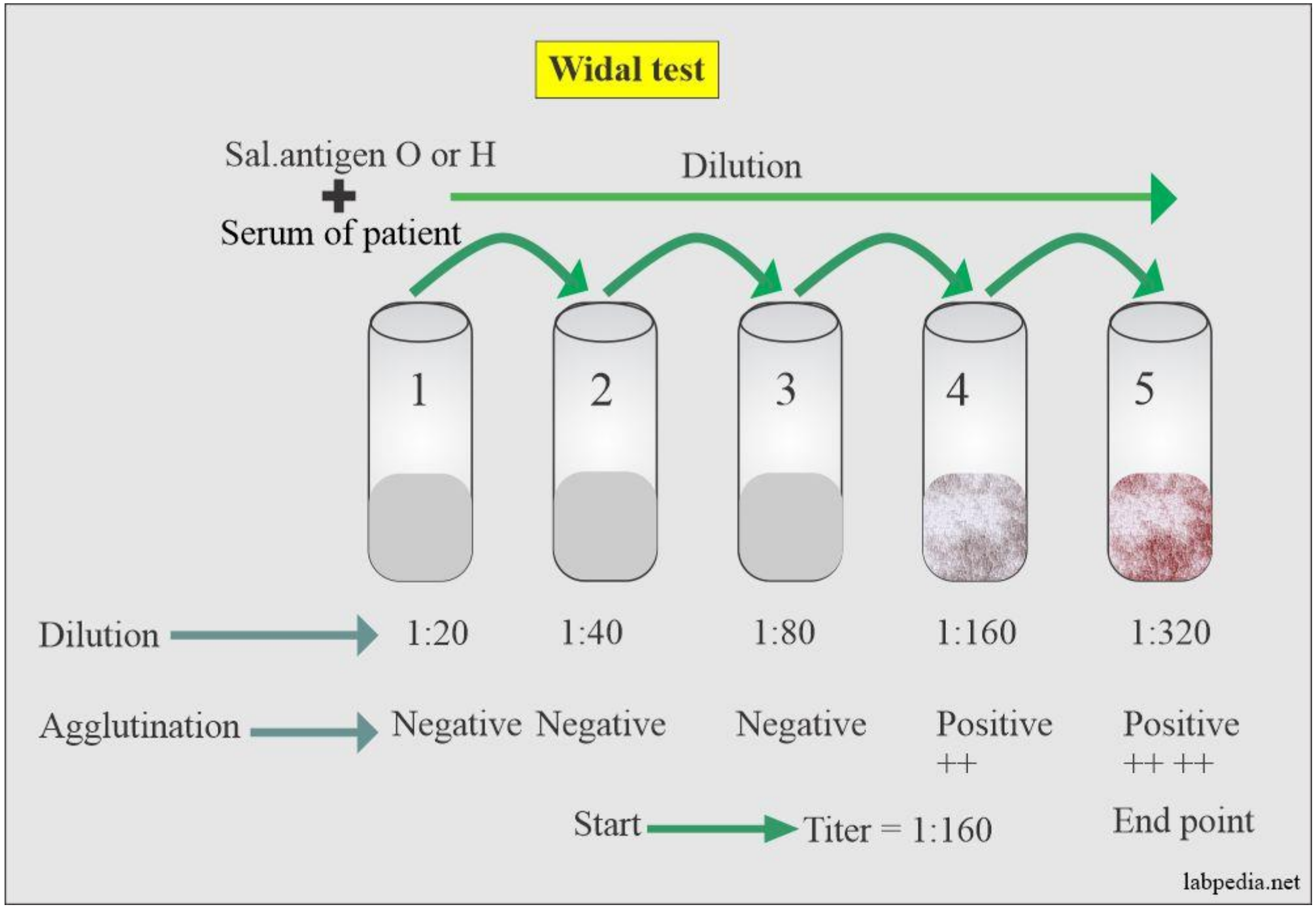
- Serum agglutinins raise abruptly during the 2nd or 3rd week
- The Widal test detects antibodies against O and H antigens
- Two serum specimens obtained at intervals of 7 – 10 days to read the raise of antibodies.
- Serial dilutions on unknown sera are tested against the antigens for respective Salmonella
- False positives and False negative limits the utility of the test
- The interpretative criteria when single serum specimens are tested vary
- Cross reactions limits the specificity

Widal Test

- Single test not diagnostic.
- Paired samples tests
- Diagnostic.
  - O > 1 in 80
  - H > 1in 160

H agglutinins appear first

False positives in
- Unapparent infection,
- Immunization
- Previously infected


Widal test
Sal.antigen O or H
+
Serum of patient
Dilution
1
2
3
4
5
Dilution
1:20
1:40
1:80
1:160
1:320
Agglutination
Negative
Negative
Negative
Positive
++
Positive
++ ++
Start
Titer = 1:160
End point
labpedia.net

Antimicrobial susceptibility testing

Drugs :

1. Amoxycillin
2. Co-amoxiclav
3. Cefuroxime
4. Cotrimoxazole
5. Ciprofloxacin
6. Chloramphenicol

Vaccines

- An Injectable vaccine Typhium Vi
- Contains purified Vi polysaccharide antigen from S.typhi strain Ty2
- A single dose, subcutaneous route
- Given to children > 5 years
- Immunity lasts for 2- 3 years.
- Follow a booster

Salmonellosis

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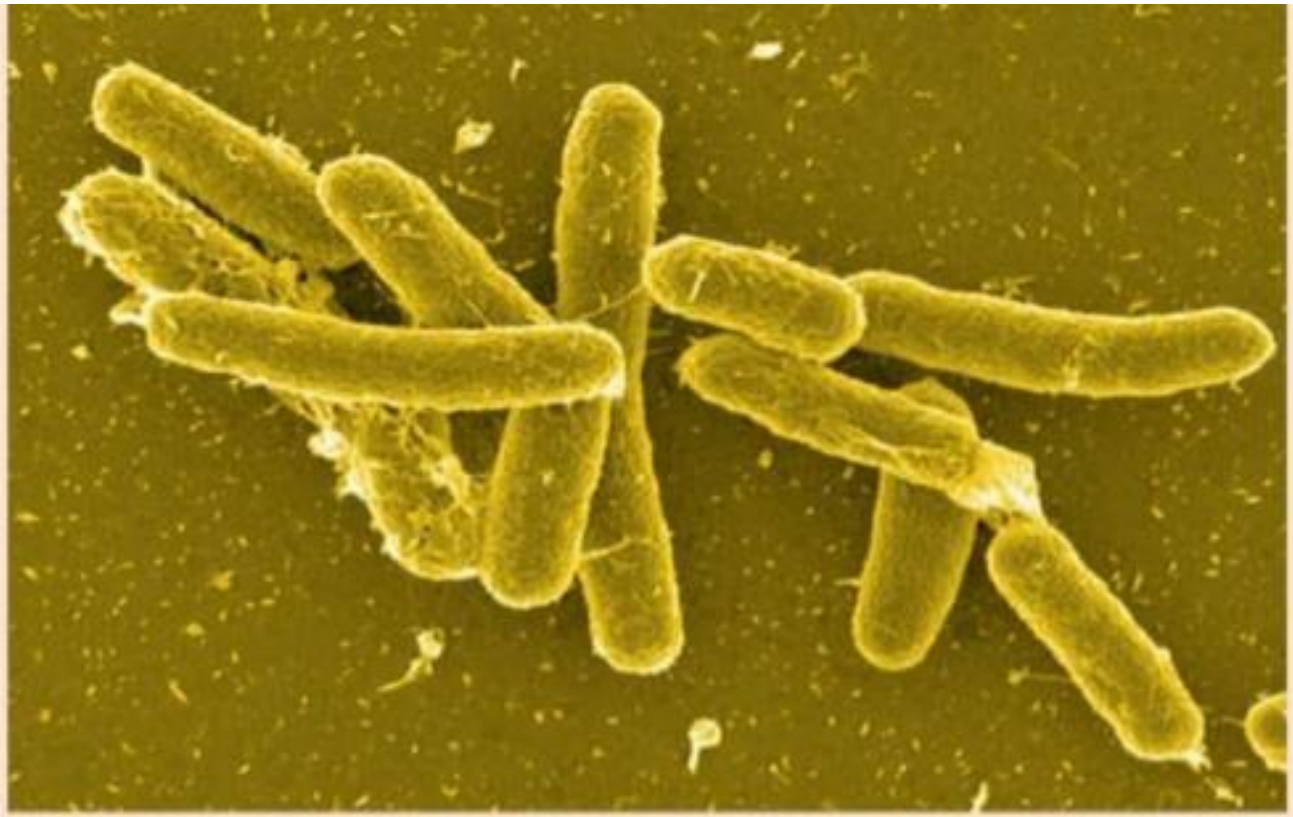


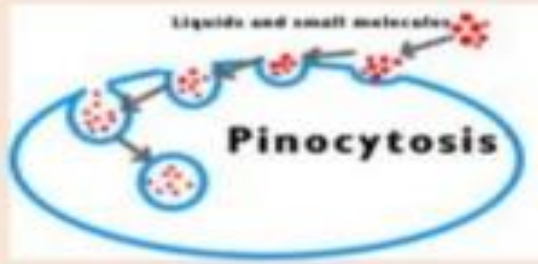
- Gram Negative Bacteria
- Species:
  - S. Bongori
  - S. Enterica
  - Six sub species
- More than 2500 Serovars/Serotypes
- Many are zoonotic

Human Transmission

- Fecal-oral: direct or indirect
- Commonly contaminated items
  - Meat, eggs, water
- Fecal material from:
  - Reptiles
  - Chicks
  - Ducklings
  - Livestock, dogs, cats, adult poultry

Pathophysiology

- Bacteria colonizes in the ilium – the end part of small intestine
- They invade intestinal epithelium
- They expand within the epithelium and lymphoid cavities
- The invasion happens when organisms start to ruffle
- This way they stimulate the pinocytosis



- They multiply & spread through mesenteric lymph nodes
- After reaching intestines they create an acute inflammation

CLINICAL PRESENTATION

Incubation period: 6–72 hours

but illness usually occurs within 12–36 hours after exposure

Symptoms:

- acute diarrhea
- abdominal pain
- fever
- Vomiting
- Headache, fever, chills, myalgia
- Severe dehydration: infants, elderly

- The illness usually lasts 4–7 days, and most people recover without treatment.

- Rates of invasive infections and death are generally higher among infants, older adults, and people with immunosuppressive conditions (including HIV), hemoglobinopathies, and malignant neoplasms.

DIAGNOSIS

- Diagnosis is based on isolation of Salmonella organisms
- About 90% of isolates are obtained from routine stool culture, but isolates are also obtained from blood, urine, and material from sites of infection
- Isolates of salmonellae are needed for serotyping and antimicrobial susceptibility testing
- PCR

Antibiotic Therapy for Nontyphoidal Salmonella Infection in Adults

Indication	Agent	Dosage (route)	Duration, days
Preemptive Treatment			
	Ciprofloxacin	500 mg bid (PO)	2–3
Severe Gastroenteritis			
	Ciprofloxacin	500 mg bid (PO) or 400 mg q12h (IV)	3–7
	Trimethoprim-sulfamethoxazole	160/800 mg bid (PO)	
	Amoxicillin	1 g tid (PO)	
	Ceftriaxone	1–2 g/d (IV)	