Enteric Gram-Negative Rods (Enterobacteriaceae)

Dr Mohammed R. Mohaisen

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INTRODUCTION

The Enterobacteriaceae are a largest, most heterogeneous collection of medically important gram-negative rods, 48 genera, whose natural habitat is the intestinal tract of humans and animals.

The family includes many genera (*Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus,* and others).

- *Escherichia coli,* are part of the normal flora and incidentally cause disease

- Salmonellae and shigellae, are regularly pathogenic for humans.

The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a **complex antigenic structure**, and produce a variety of toxins and other virulence factors.

Enterobacteriaceae or it could be named enteric gram-negative rods (enteric bacteria), or (coliforms).

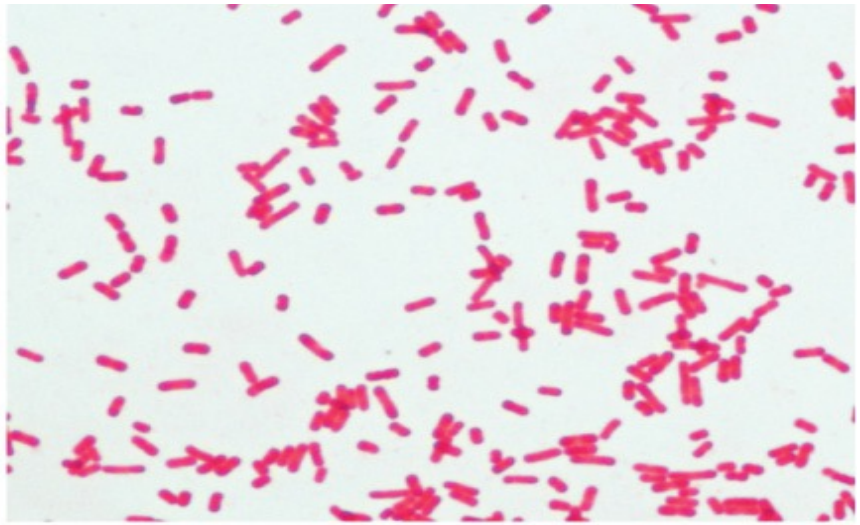
- There are four major features:

-The Enterobacteriaceae are short gram-negative rods (Figure1).

-Typical morphology is seen in growth on solid media in vitro,

- morphology is highly variable in clinical specimens.

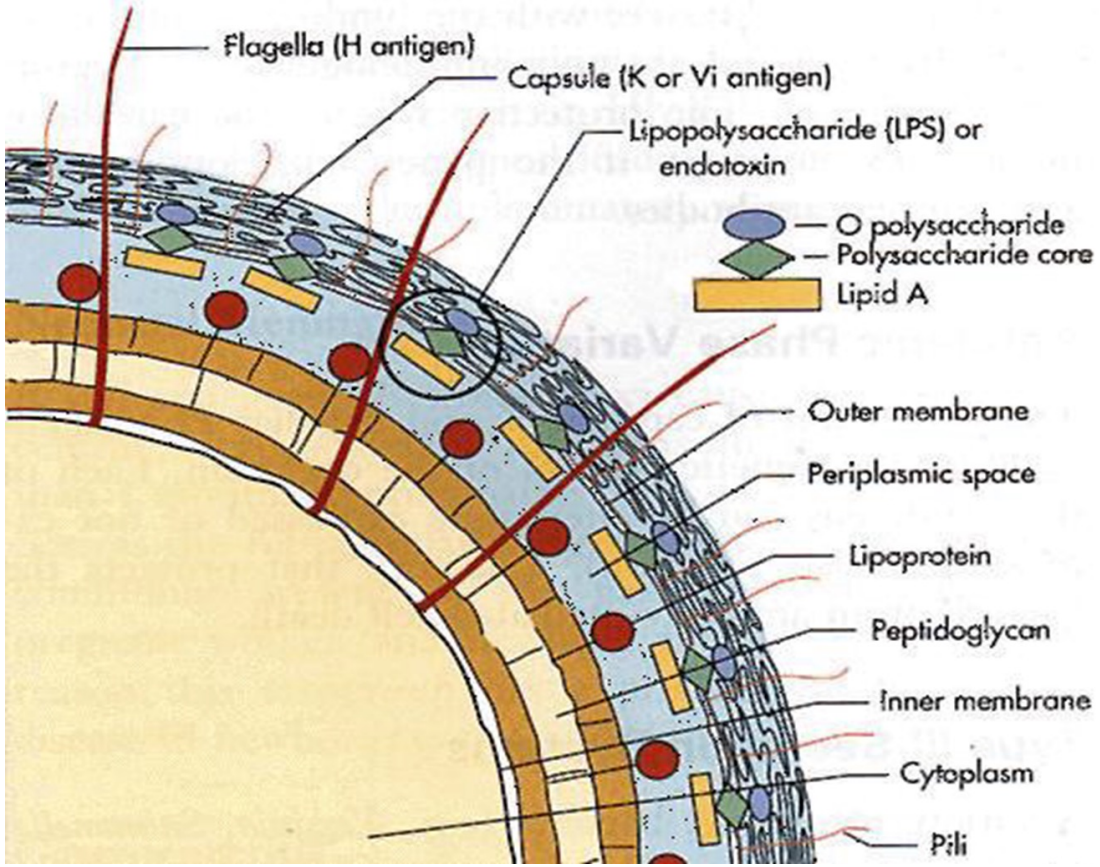
-Capsules are large and regular in *Klebsiella*, less so in *Enterobacter*, and uncommon in the other species.



Antigenic Structure :

Enterobacteriaceae have a complex antigenic structure. They are classified by:

- -More than 150 different heat-stable somatic O (lipopolysaccharide) antigens.
- - More than 100 heat-labile K (capsular) antigens. In *Salmonella typhi,* the capsular antigens are called Vi antigens.
- - More than 50 H (flagellar) antigens .



- **Colicins (Bacteriocins)**

Many gram-negative organisms produce bacteriocins. These high-molecular-weight bactericidal proteins are produced by certain strains of bacteria active against some other strains of the same or closely related species. Their production is controlled by plasmids. Colicins are produced by *E coli*. Bacteriocin-producing strains are resistant to their own bacteriocin; thus, bacteriocins can be used for "typing" of organisms.

- **Toxins & Enzymes**

Most gram-negative bacteria possess complex lipopolysaccharides in their cell walls. These substances, cell envelope (cytoplasmic membrane, peptidoglycan, outer membrane) endotoxins. Many gram-negative enteric bacteria also produce exotoxins of clinical importance.

- **Human pathogen :**

Enterobacteriaceae as a group were originally divided into pathogens and non-pathogens based on their ability to cause diarrheal disease of humans.

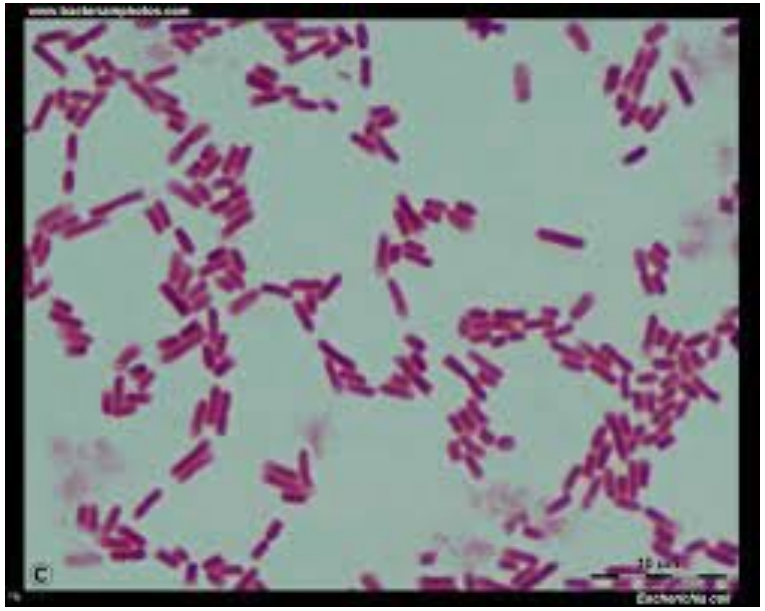
The most important pathogenic genera are *Salmonella* and *Shigella.* However, it is now known that *E. coli* causes at least five types of gastrointestinal disease in humans.

1.Escherichia coli :

- Most *E. coli* strains are harmless however there are strains that cause disease in humans and animals that making them important pathogens in their own right.

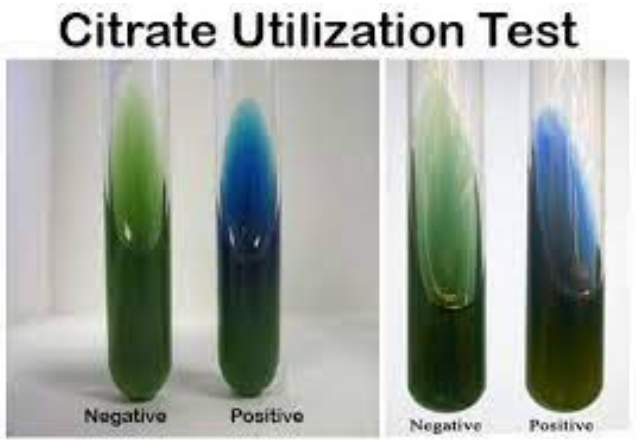
Clinically, two distinct types of pathogenic *E. coli* are recognized.

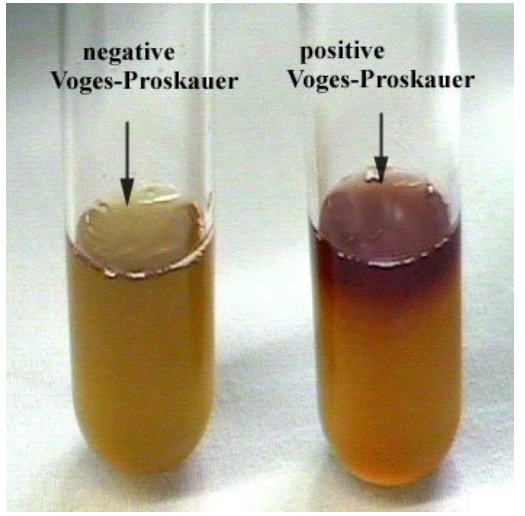
- Extra-intestinal pathogenic *E. coli* (ExPEC) includes those *E. coli* associated with newborn meningitis (NBM) or sepsis and urinary tract infections (UTIs).

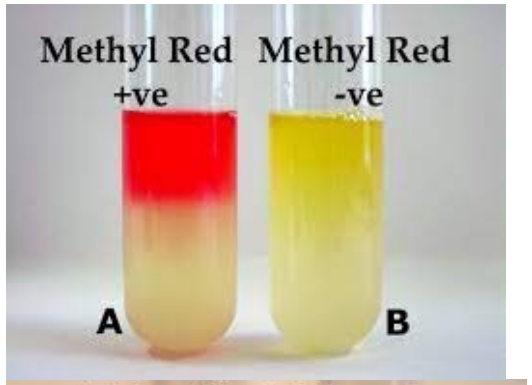


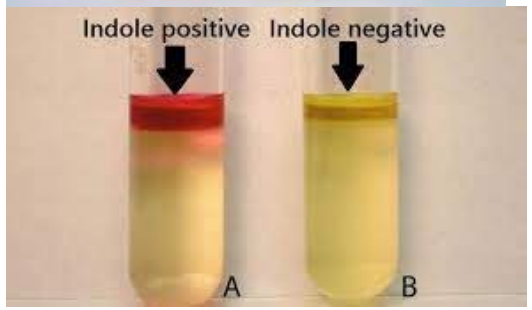
- Intestinal pathogenic *E. coli* (IPEC) includes *E. coli* responsible for a range of distinct classes of diarrhoeal disease.

- This bacteria produce pink (lactose positive) colony with surrounding pink area on MacConkey*, Ferments glucose,* lactose, trehalose, & xylose, Positive indole and methyl red tests, Does NOT produce H2S or phenylalanine deaminase, Simmons citrate negative, Usually motile, Voges-Proskauer test negative.









The strains that associated with gastrointestinal disease are classified as follow:

- **Enteroinvasive E. coli (EIEC) :**

Strains belonging to this group are biochemically, genetically and pathogenically closely related to *Shigella* spp. The most common symptom is watery diarrhoea which may precede dysenteric stools containing mucus and blood. In severe cases, the bacteria may attack the colonic mucosa, invading epithelial cells, multiplying and causing ulceration of the bowel.

- **Enterotoxigenic *E. coli* (ETEC) :**

Strains belonging to the ETEC pathotype are characterized by the production of at least one of two types of enterotoxin: LT (heat-labile enterotoxin) and ST (heat-stable enterotoxin). Cause “traveler’s diarrhea”; watery diarrhea without blood; self-limiting; usually not identified**.**

- **C-Enteroaggregative *E. coli* (EAEC*) :***

Cause diarrhea by adhering to the mucosal surface of the intestine; watery diarrhea; symptoms may persist for over two weeks, EAggEC (or EAEC) are a major cause of chronic infantile diarrhoea and they have also emerged as a cause of diarrhoeal disease in adults and children in developed countries. Toxins that have also been associated with strains of EAggEC include an *E. coli* heat-stable-like enterotoxin termed enteroaggregative heat-stable toxin-1 (EAST-1) and a heat-labile toxin.

- **D-Enteropathogenic *E. coli* (EPEC) :**

Primarily in infants and children; outbreaks in hospital nurseries and day care centers; stool has mucous but not blood; identified by serotyping.

- **E-Enterohaemorrhagic *E. coli* (EHEC) :**

(EHEC serotype 0157:H7) – associated with hemorrhagic diarrhea and hemolytic-uremic syndrome (HUS), which includes low platelet count, hemolytic anemia, and kidney failure; potentially fatal, especially in young children; undercooked hamburger, unpasteurized milk and apple cider have spread the infection; does NOT ferment sucrose; identified by serotyping. EHECs have emerged as one of the most important threats to human health .

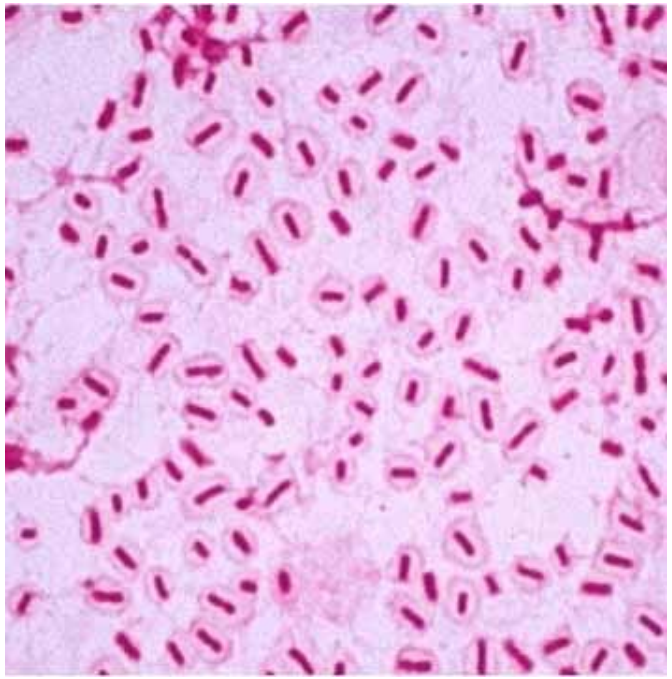
Term	Abbreviation	Pathogenic Phenotype	Signs& Symptoms
Enterotoxigenic *E.coli*	ETEC	Secretion of: heat-Labile (LT)/ heat-stable (ST)/	Traveler's diarrhoea Watery, mild abdominal cramp ,(small intestine) dehydration,vomiting
Enteroaggregative E.*coli*	EaggEC	Adhere to epith.cells	Watery diarrhoea, vomit, dehydration, abdominal pain
Enteropathogenic *E.coli*	EPEC	Adhere to epithelial cells (pilli)/effacing lesions	Infants (18-24month); low fever,malaise,vomiting, diarrhoea→ (duodenum)
Enteroinvasive *E.coli*	EIEC	Invade colonic mucosa ;Causing dysenteric-like diarrhoea	Dysentery;fever, colitis,diarrhoea with blood, mucus, Leukocytes
Enterohaemorrhagic *E.coli*	EHEC	Production of cytotoxin serotype 0157;H7	Bloody diarrhoea,WBCs, →Haemorrhagic.colitis &Haemolytic uraemic syndrome (HUS)/Acute renal failure

- ***2- Klebsiella*:**

It is gram negative , non-motile, capsulate, thick& bacilli producing mucoid pink colonies on MacConky medium, it is found in mucosa of upper respiratory tract, intestinal & urinary tract , it is member of Normal flora that may cause sever systemic infection under certain condition such as immunocompromise debilitation.

- ***Klebsiella pneumoniae***

Is the causative agent of pneumonia &lung abscesses also may cause urinary tract infections .



- **Virulence factor for *Klebsiella pneumoniae***

1-capsular mucoid polysaccharide which can resist to action of phagocytes.

2-some strain carry plasmid coding for production heat – stable enterotoxine

3-they contain resistance plasmids (R-plasmids) which confer resistance to antibiotic.

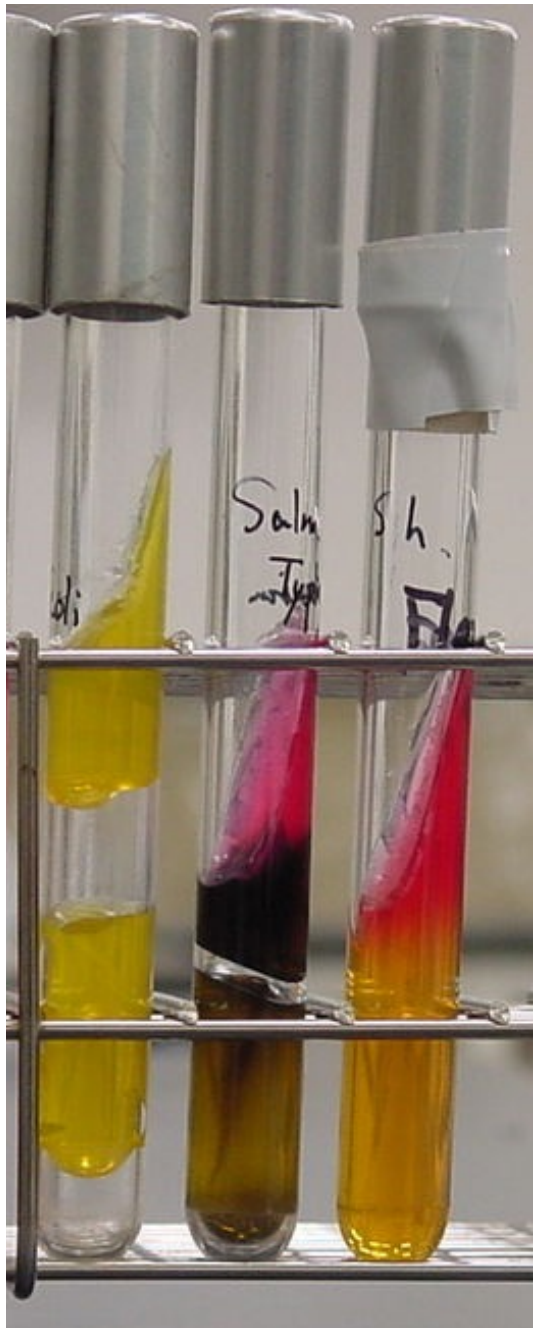
- ***3- Proteus*:**

Proteus species move very actively by means of peritrichous flagella, resulting in "swarming" on solid media.

Strains of Proteus vary greatly in antibiotic sensitivity. *P mirabilis* is often inhibited by penicillins; the most active antibiotics for other members of the group are aminoglycosides and cephalosporins. *Proteus* species are urease-positive, ferments lactose very slowly or not at all.

- **Pathogenecity:** it is opportunistic pathogen cause urinary tract infection ,may produce Pyogenic lesion like abscess infection of wound ,ear or respiratory tract.

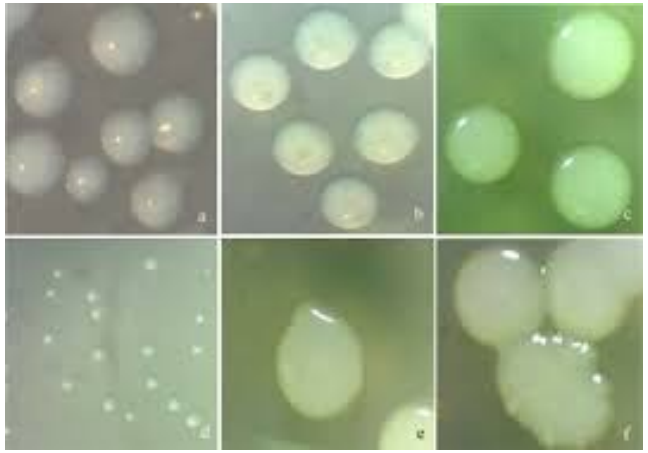
- **4. *Shigella*—**Shigellae are nonmotile and usually do not ferment lactose but do ferment other carbohydrates like glucose, producing acid but not gas. They do not produce H_2S (in triple sugar iron test). The four *Shigella* species are closely related to *E coli.*



The natural habitat of shigella is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.

- **Morphology & Identification**

Shigella are slender gram-negative rods; coccobacillary forms occur in young cultures.



- **Culture :**

Shigella are facultative anaerobes but grow best aerobically. Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours.

Pathogenesis & Pathology

- *Shigella* infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare.
- The essential pathologic process is :-

-Invasion of the mucosal epithelial cells (eg, M cells) by induced phagocytosis

-Escape from the phagocytic vacuole.

-Multiplication and spread within the cell cytoplasm, and passage to adjacent cells.

- This bacteria cause micro abscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a "pseudomembrane" on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane, and bacteria.

Toxins

- **Endotoxin :**

all shigellae release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall.

- ***Shigella dysenteriae* exotoxin :**

S. dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut and the central nervous system. The exotoxin is:

- Acting as an enterotoxin, it produces diarrhea as does the *E coli* toxin

- inhibits sugar and amino acid absorption in the small intestine.

-Acting as a "neurotoxin," this material may contribute to the extreme severity and fatal nature of *S dysenteriae* infections.

Diagnostic Laboratory Tests

- **Specimens :**

Specimens include fresh stool, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

- **Culture :**

The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or *Salmonella-Shigella* agar).

Colorless (lactose-negative) colonies are inoculated into triple sugar iron agar.

Organisms that fail to produce H_2S, that do not produce gas in triple sugar iron agar medium, and that are nonmotile should be subjected to slide agglutination by specific *Shigella* antisera.

- **Treatment :**

Ciprofloxacin, ampicillin, doxycycline, and trimethoprim-sulfamethoxazole are most commonly inhibitory for *Shigella* isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms.

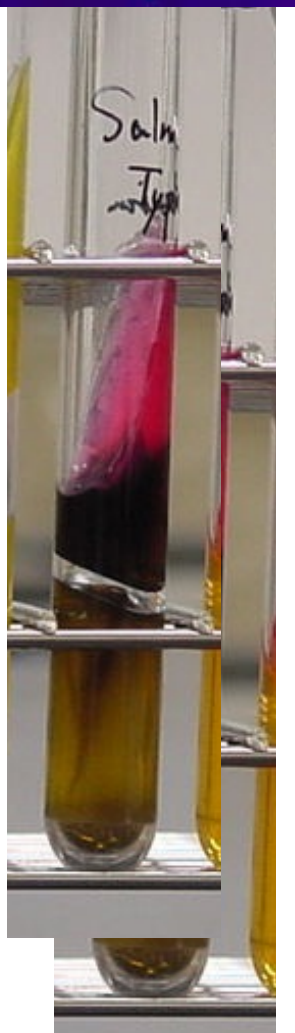
5. *Salmonella*—They are pathogenic for humans or animals when ingested.

Salmonella are transmitted from animals and animal products to human, where they cause **enteritis, systemic infection, and enteric fever.**

- **Morphology & Identification :**

Salmonella vary in length. Most isolates are motile with peritrichous flagella.

Salmonellae grow on simple media, it ferments glucose and mannose without producing gas but they almost never ferment lactose or sucrose. They usually produce H_2S. They survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) .



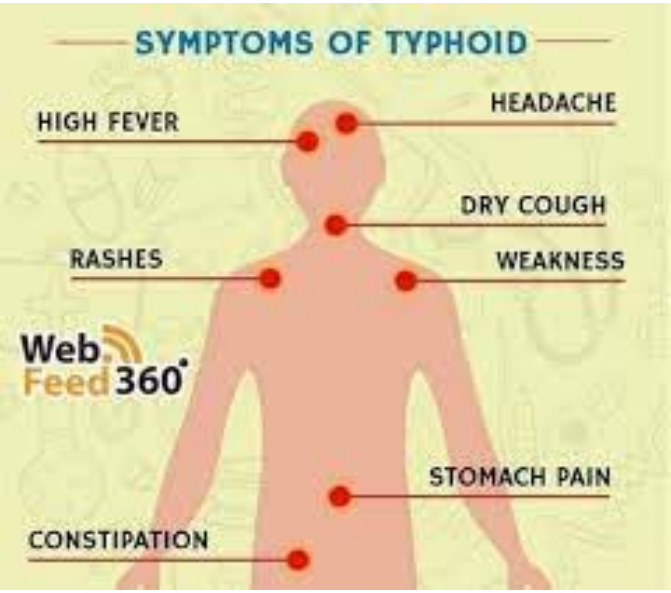
Pathogenesis & Clinical Findings :

- *Salmonella typhi*, *Salmonella choleraesuis*, *Salmonella paratyphi* A and *Salmonella paratyphi* B are primarily infective for humans. The vast majority of salmonellae, however, are chiefly pathogenic in animals that constitute the reservoir for human infection: poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others.

- The organisms almost always enter via the oral route, usually with contaminated food or drink. The infective dose to produce clinical or subclinical infection in humans is 10^5–10^8 salmonellae (but perhaps as few as 10^3 *Salmonella* Typhi organisms).

Salmonellae produce three main types of disease in humans, but mixed forms are frequent

1-THE "ENTERIC FEVERS" (TYPHOID FEVER)



This syndrome is produced by only a few of the salmonellae, of which *Salmonella* Typhi (typhoid fever) is the most important. The ingested salmonellae reach the small intestine, from which they enter the lymphatics and then the bloodstream. They are carried by the blood to many organs, including the intestine. The organisms multiply in intestinal lymphoid tissue and are excreted in stools.

After an incubation period of 10–14 days, fever, malaise, headache, constipation, bradycardia, and myalgia occur. The spleen and liver become enlarged. Rose spots, usually on the skin of the abdomen or chest, are seen briefly in rare cases. The white blood cell count is normal or low. the mortality rate was 10–15%. Treatment with antibiotics has reduced the mortality rate to less than 1%.

2- BACTEREMIA WITH FOCAL LESIONS

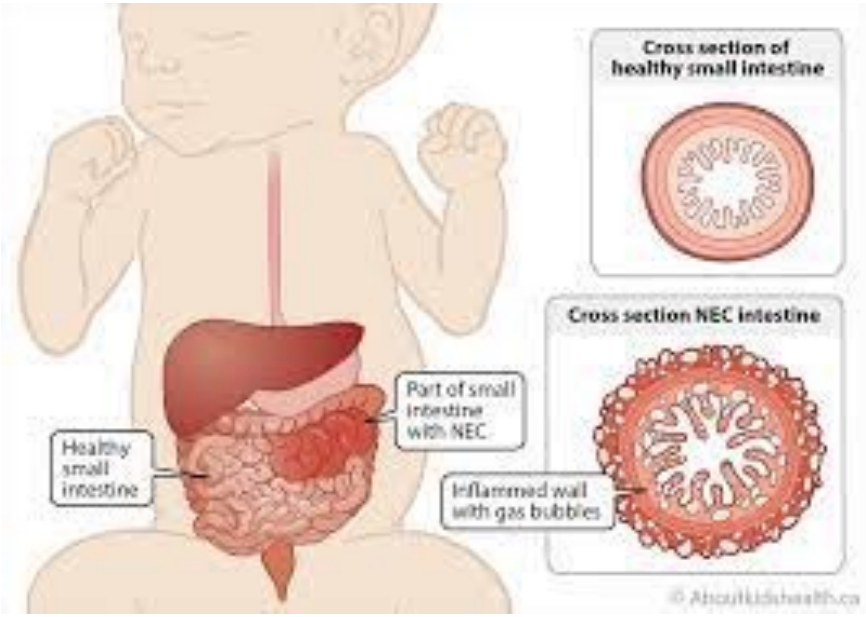
This is associated commonly with *S.choleraesuis* but may be caused by any salmonella serotype. Following oral transmission, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, etc) intestinal manifestations are often absent. Blood cultures are positive.

3- ENTEROCOLITIS

This is the most common manifestation of salmonella infection. Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days. Inflammatory lesions of the small and large intestine are present.

Bacteremia is rare (2–4%) except in immunodeficient persons.

Blood cultures are usually negative, but stool cultures are positive for salmonellae and may remain positive for several weeks after clinical recovery.



Diagnostic Laboratory Tests

- **Specimens**

Blood for culture must be taken repeatedly. In **enteric fevers and septicaemias**, blood cultures are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine cultures may be positive after the second week.

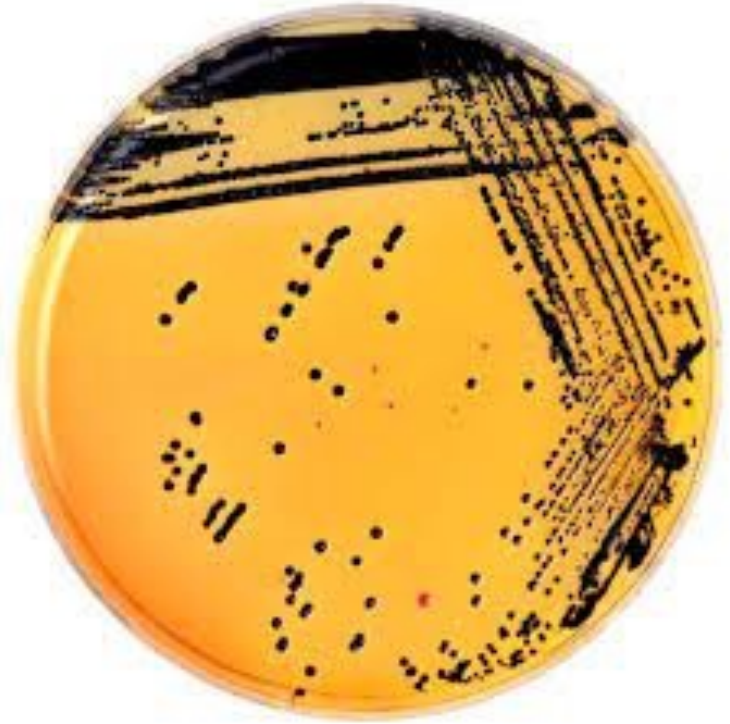
Stool specimens also must be taken repeatedly. In enteric fevers, the stools yield positive results from the second or third week on; in enterocolitis, during the first week.

Bacteriologic methods for isolation of salmonellae

- **1. Differential medium cultures— (**Differential media are used to differentiate closely related organisms or groups of organisms).

EMB, MacConkey, or deoxycholate medium permits rapid detection of lactose non-fermenters (not only salmonellae and shigellae but also *Proteus*, *Serratia*, etc).

Bismuth sulfite medium permits rapid detection of salmonellae which form black colonies because of H_2S production. Many salmonellae produce H_2S.

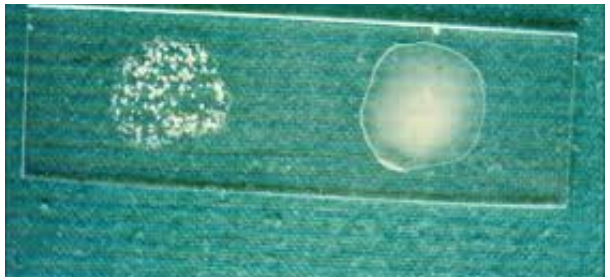


- **2. Selective medium cultures—** (Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms). For salmonella, The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar which favor growth of salmonellae and shigellae over other Enterobacteriaceae.

- **3. Enrichment cultures—**The specimen (usually stool) also is put into tetrathionate broth, which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, the resulted colonies have to be plated on differential and selective media.
- **4. Final identification—**Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

SEROLOGIC METHODS

1. **Agglutination test—**In this test, known sera and unknown culture are mixed on a slide.

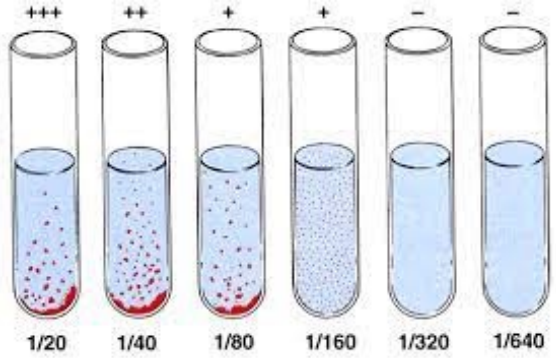


Clumping, when it occurs, can be observed within a few minutes.

This test is particularly useful for rapid identification of cultures.

There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C_1, C_2, D, and E.

2. Tube dilution agglutination test (Widal test)—The Widal test to detect these antibodies against the O and H antigens has been in use for decades.



At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titre.

Serial dilutions of unknown sera are tested against antigens from representative salmonellae. False-positive and false-negative results occur.

Serum agglutinins rise sharply during the second and third weeks of *Salmonella* Typhi infection.

Treatment :

- While enteric fevers and bacteraemia with focal lesions **require antimicrobial treatment**, the vast majority of cases of enterocolitis do not.
- Antimicrobial treatment of *Salmonella* enteritis in neonates is important.
- In severe diarrhea, replacement of fluids and electrolytes is essential.
- Antimicrobial therapy of invasive *Salmonella* infections is with ampicillin, trimethoprim-sulfamethoxazole, or a third-generation cephalosporin.
- Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in *Salmonella* infections.
- Susceptibility testing is an important in order to select a proper antibiotic.

- **Carriers :**

After manifest or subclinical infection, some individuals continue to harbour salmonellae in their tissues for variable lengths of time (healthy permanent carriers). harbouring the organisms in the gallbladder, biliary tract, or, rarely, the intestine or urinary tract.

Sources of infection :

The sources of infection are food and drink that have been contaminated with salmonella. The following sources are important:

1. Water—Contamination with feces often results in explosive epidemics.

2. Milk and other dairy products (ice cream, cheese, custard)— Contamination with feces and inadequate pasteurization or improper handling.

3. Shellfish—From contaminated water.

4. Dried or frozen eggs—From infected fowl or contaminated during processing.

5. Meats and meat products—From infected animals (poultry) or contamination with feces by rodents or human

- **Prevention & Control** salmonella infections

1-prevent contamination of food and water by rodents or other animals that excrete salmonellae.

2-Infected poultry, meats, and eggs must be thoroughly cooked.

3-Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.

4-Two typhoid vaccines are currently available: an oral live, attenuated vaccine and a Vi capsular polysaccharide vaccine for intramuscular use.

5-Vaccination is recommended for travellers to endemic regions.