

Enteric Gram-Negative Rods (Enterobacteriaceae)

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, enteric gram-negative rods (enteric bacteria), or (coliforms).

There are four major features:

- **All ferment glucose.**
- All reduce nitrates to nitrites.**
- **All are oxidase negative.**
- **All except *Klebsiella*, *Shigella* and *Yersinia* are motile.**

Morphology & Identification

The Enterobacteriaceae are short gram-negative rods (Figure1). Typical morphology is seen in growth on solid media in vitro, but morphology is highly variable in clinical specimens. Capsules are large and regular in *Klebsiella*, less so in *Enterobacter*, and uncommon in the other species.



CULTURE :

E coli and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. *Enterobacter* colonies are similar but somewhat more mucoid. *Klebsiella* colonies are large and very mucoid and tend to coalesce with prolonged incubation. The salmonellae and shigellae produce colonies similar to *E coli* but do not ferment lactose. Some strains of *E coli* produce hemolysis on blood agar.

GROWTH CHARACTERISTICS

- Carbohydrate fermentation patterns
- Activity of amino acid decarboxylases
- Enzymes production are used in biochemical differentiation.

The production of indole from tryptophan, are commonly used in rapid identification systems, while others, eg, the Voges-Proskauer reaction (production of acetylmethylcarbinol from dextrose), are used less often.

Culture on "differential" media that contain special dyes and carbohydrates (eg, eosin-methylene blue [EMB], MacConkey, or deoxycholate medium) distinguishes lactose-fermenting (colored) from nonlactose-fermenting colonies (nonpigmented) and may allow rapid presumptive identification of enteric bacteria.

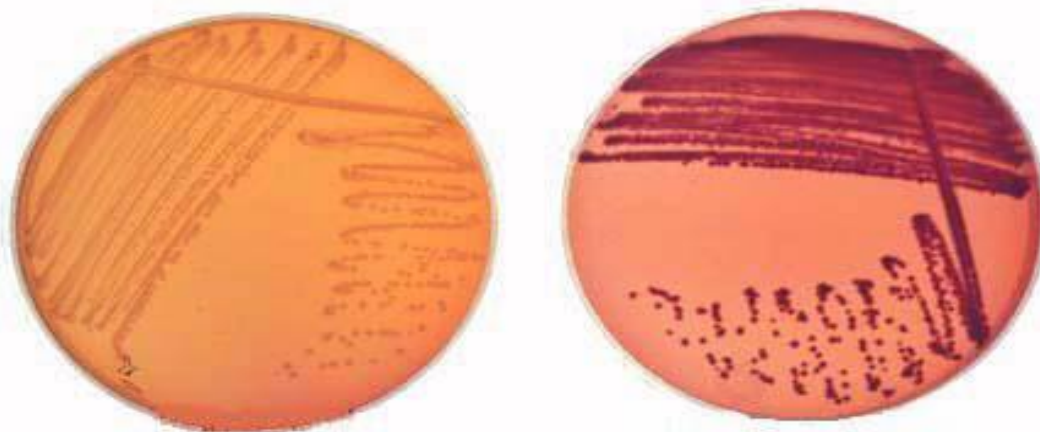


Figure :

Lactose fermentation and non-fermentation

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

Pathogenicity in *E. coli* strains is due to the presence of one or more virulence factors, including

-Invasiveness factors (invasins).

Heat-labile and heat-stable enterotoxins.

Verotoxins, and colonization factors or adhesins.

Pathogenic strains are usually identified by detection of a specific virulence factor or of a serotype associated with a virulence factor. The most recently identified *E. coli* disease is hemorrhagic colitis caused by strains of serotype O157:H7. The disease, characterized by painful abdominal cramping and bloody diarrhea, is caused by strains that produce verotoxins, and the same strains are associated with hemolytic uremic syndrome (HUS).

1. Escherichia :

Most *E. coli* strains are harmless, there are others that cause disease in humans and animals that have evolved to become important pathogens in their own right. Clinically, two distinct types of pathogenic *E. coli* are recognized.

- I- Extra-intestinal pathogenic *E. coli* (ExPEC) includes those *E. coli* associated with newborn meningitis (NBM) or sepsis and urinary tract infections (UTIs).
- II- Intestinal pathogenic *E. coli* (IPEC) includes *E. coli* responsible for a range of distinct classes of diarrhoeal disease.

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.

Detection and Isolation of *Escherichia coli*

When *E. coli* was recognized to be a useful marker for fecal pollution

The IMViC tests were developed in order to distinguish strains of *E. coli* from related species that also produced acid and gas from the fermentation of lactose. IMViC is an acronym in which the capital letters stand for Indole, Methyl red, Voges-Proskauer, and Citrate.)

Detection of *E. coli* in Clinical Specimens

If the number of *E. coli* is likely to be very low or the amount of specimen is limited, enrichment in a rich nutrient medium such as brain heart infusion broth may be used. A number of different commercially available kits are generally used to identify the isolates as *E. coli*.

Infections Caused by Pathogenic *E. coli*

E. coli is responsible primarily for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases.

The strains of this bacterium which associated with gastrointestinal disease are classified as follow:

- A- 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.

B- 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-Enterotoxigenic *E. coli* (EAEC) :

Cause diarrhea by adhering to the mucosal surface of the intestine; watery diarrhea; symptoms may persist for over two weeks, EAegEC (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 EAegEC 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 <i>E. coli</i>	ETEC	Secretion of: heat-labile (LT)/ heat-stable (ST)/	Traveler’s diarrhoea Watery, mild abdominal cramp ,(small intestine) dehydration, vomiting
Enterotoxigenic <i>E. coli</i>	EaggEC	Adhere to epith. cells	Watery diarrhoea, vomit, dehydration, abdominal pain
Enteropathogenic <i>E. coli</i>	EPEC	Adhere to epithelial cells (pili)/effacing lesions	Infants (18-24month); low fever, malaise, vomiting, diarrhoea → (duodenum)
Enteroinvasive <i>E. coli</i>	EIEC	Invade colonic mucosa ;Causing dysenteric-like diarrhoea	Dysentery; fever, colitis, diarrhoea with blood, mucus, Leukocytes
Enterohaemorrhagic <i>E. coli</i>	EHEC	Production of cytotoxin serotype 0157;H7	Bloody diarrhoea, WBCs, →Haemorrhagic colitis &Haemolytic uraemic syndrome (HUS)/Acute renal failure

Table : Pathogenicity of *E.coli*

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 infection , it is member of Normal flora that may cause severe systemic infection under certain condition immunocompromised, debilitation.

Klebsiella pneumoniae

It is responsible for the most infection which may cause 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-antibiotic resistance due to species 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 unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystine-lactose-electrolyte-deficient) medium. 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.

Pathogenicity: 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, producing acid but not gas. They do not produce H₂S. The four *Shigella* species are closely related to *E coli*.

5. *Salmonella*—Salmonellae are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce H₂S. They are often pathogenic for humans or animals when ingested.

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.

- More than 50 H (flagellar) antigens . In *Salmonella typhi*, the capsular antigens are called Vi antigens.

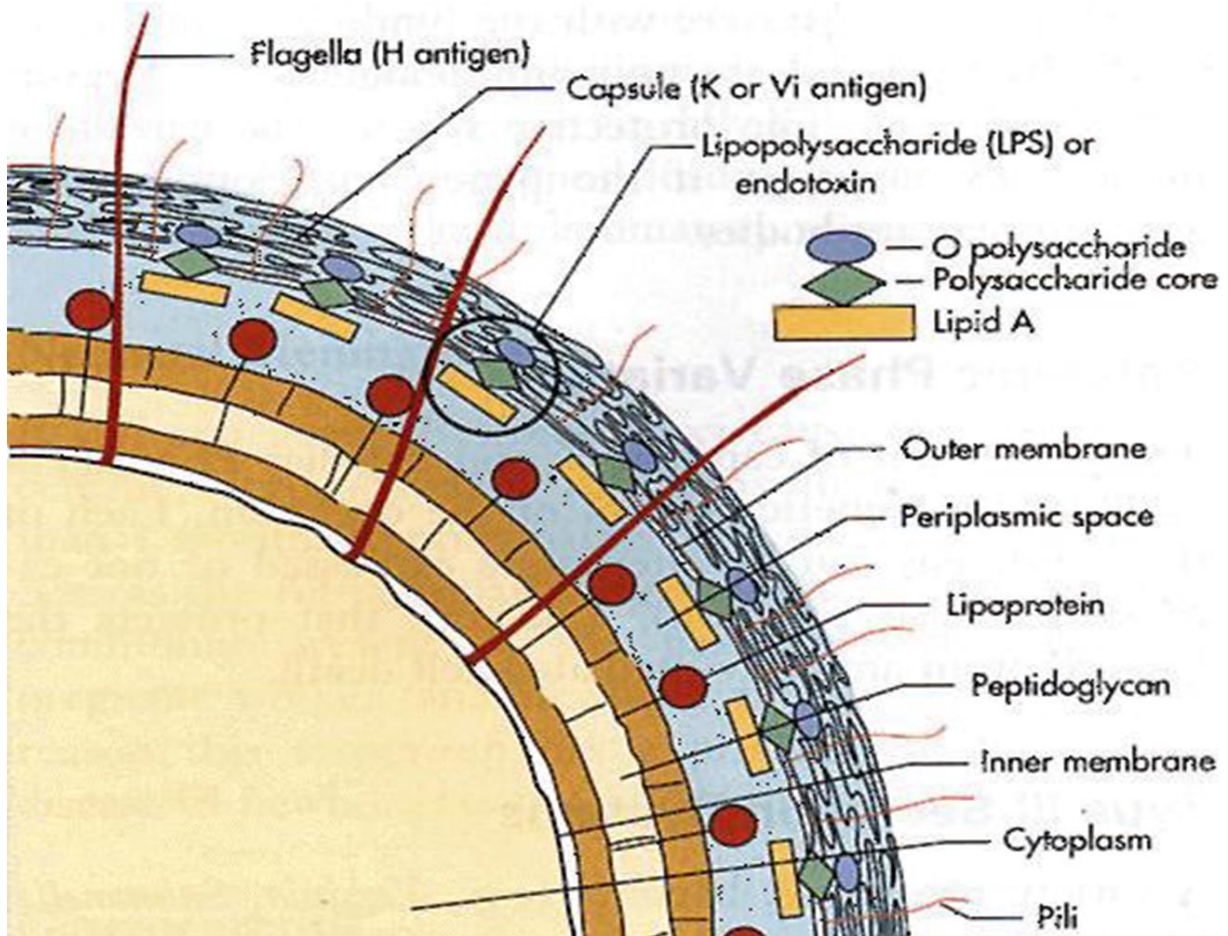


Figure : Antigenic structure of Enterobacteriaceae.

O antigens : are the most external part of the cell wall lipopolysaccharide and consist of repeating units of polysaccharide. Some O-specific polysaccharides contain unique sugars. O antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM.

K antigens : are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides, including the K antigens of *E coli*; others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence (eg, *E coli* strains producing K1 antigen are prominent in neonatal meningitis, and K antigens of *E coli* cause attachment of the bacteria to epithelial cells prior to gastrointestinal or urinary tract invasion).

Klebsiellae form large capsules consisting of polysaccharides (K antigens) covering the somatic (O or H) antigens and can be identified by capsular swelling tests with specific antisera.

H antigens : are located on flagella and are denatured or removed by heat or alcohol. They are preserved by treating motile bacterial variants with formalin. Such H antigens agglutinate with anti-H antibodies, mainly IgG.

There are many examples of overlapping antigenic structures between Enterobacteriaceae and other bacteria. Most Enterobacteriaceae share the O14 antigen of *E coli*. The type 2 capsular polysaccharide of *Klebsiella* is very similar to the polysaccharide of type 2 pneumococci. Some K antigens cross-react with capsular polysaccharides of *Haemophilus influenzae* or *Neisseria meningitidis*.

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.

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

Morphology & Identification

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

CULTURE :

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

GROWTH CHARACTERISTICS :

All shigellae ferment glucose. They do not ferment lactose. Shigellae form acid from carbohydrates but rarely produce gas. They may also be divided into those that ferment mannitol and those that do not .

Antigenic Structure :

Shigellae have a complex antigenic pattern. There is great overlapping in the serologic behavior of different species, and most of them share O antigens with other enteric bacilli. The somatic O antigens of shigellae are lipopolysaccharides. Their serologic specificity depends on the polysaccharide. There are more than 40 serotypes. The classification of shigellae relies on biochemical and antigenic characteristics.

Pathogenesis & Pathology

Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. Shigellae are highly communicable; the infective dose is on the order of 10^3 organisms (whereas it usually is 10^5 – 10^8 for salmonellae and vibrios).

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 epithelial cell cytoplasm, and passage to adjacent cells.

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. As the process subsides, granulation tissue fills the ulcers and scar tissue forms.

Toxins

ENDOTOXIN :

Upon autolysis, 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:

- protein that is antigenic (stimulating production of antitoxin)
- lethal for experimental animals.
- Acting as an enterotoxin, it produces diarrhea as does the *E coli* Shiga-like 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 and to the central nervous system reactions observed in them (ie, meningismus, coma).

Patients with *Shigella flexneri* or *Shigella sonnei* infections develop antitoxin that neutralizes *S dysenteriae* exotoxin in vitro.

Diagnostic Laboratory Tests

SPECIMENS :

Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically. Serum specimens, if desired, must be taken 10 days apart to demonstrate a rise in titer of agglutinating antibodies.

CULTURE :

The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or *Salmonella-Shigella* agar), which suppress other Enterobacteriaceae and gram-positive organisms. Colorless (lactose-negative) colonies are inoculated into triple sugar iron agar. Organisms that fail to produce H₂S, that produce acid but not gas in the butt and an alkaline slant 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. They may fail to eradicate the organisms from the intestinal tract. Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread. Many cases are self-limited. dysentery.

Salmonella :

Salmonella are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause enteritis, systemic infection, and enteric fever.

Morphology & Identification :

Salmonellae vary in length. Most isolates are motile with peritrichous flagella. Salmonellae grow readily on simple media, but they almost never ferment lactose or sucrose. They usually produce H₂S. 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*, and perhaps *Salmonella paratyphi* A and *Salmonella paratyphi* B are primarily infective for humans, and infection with these organisms implies acquisition from a human source. 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 mean infective dose to produce clinical or subclinical infection in humans is 10⁵–10⁸ salmonellae (but perhaps as few as 10³ *Salmonella* Typhi organisms). Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbial flora, and local intestinal immunity .

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 fever rises to a high plateau, and 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%.

The principal lesions are hyperplasia and necrosis of lymphoid tissue (eg, Peyer's patches), hepatitis, focal necrosis of the liver, and inflammation of the gallbladder, periosteum, lungs, and other organs.

2- BACTEREMIA WITH FOCAL LESIONS

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

3- ENTEROCOLITIS

This is the most common manifestation of salmonella infection. In the United States, *Salmonella typhimurium* and *Salmonella enteritidis* are prominent, but enterocolitis can be caused by any of the more than 1400 group I serotypes of salmonellae. 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 septicemias, 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.

A positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract in carriers.

BACTERIOLOGIC METHODS FOR ISOLATION OF SALMONELLAE

1. Differential medium cultures—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 H₂S production. Many salmonellae produce H₂S.

2. Selective medium cultures—The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, or deoxycholate-citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae.

3. Enrichment cultures—The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is 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 preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C₁, C₂, D, and E.

2. Tube dilution agglutination test (Widal test)—Serum agglutinins rise sharply during the second and third weeks of *Salmonella* Typhi infection. 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 titer. Serial dilutions of unknown sera are tested against antigens from representative salmonellae. False-positive and false-negative results occur. The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of >1:320 and against the H antigen of >1:640 is considered positive. High titer of antibody to the Vi antigen occurs in some carriers. Alternatives to the Widal test include rapid colorimetric and enzyme immunoassay methods. There are conflicting reports in the literature regarding superiority of these methods to the Widal test. Results of serologic tests for *Salmonella* infection cannot be relied upon to establish a definitive diagnosis of typhoid fever and are most often used in resource poor areas of the world where blood cultures are not readily available.

Treatment :

While enteric fevers and bacteremias 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 enterocolitis, clinical symptoms and excretion of the salmonellae may be prolonged by antimicrobial therapy. 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 adjunct to selecting a proper antibiotic.

CARRIERS :

After manifest or subclinical infection, some individuals continue to harbor salmonellae in their tissues for variable lengths of time (convalescent carriers or healthy permanent carriers). Three percent of survivors of typhoid become permanent carriers, harboring 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 salmonellae. 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

Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae. Infected poultry, meats, and eggs must be thoroughly cooked. Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.

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

Vaccination is recommended for travelers to endemic regions especially if the traveler visits rural areas or small villages where food choices are limited. Both vaccines have an efficacy of 50–80%. The time required for primary vaccination and age limits for each vaccine varies and individuals should consult the CDC Web site or obtain advice from a travel clinic regarding the latest vaccine information.

References:

- 1- Jawetz, Melnick, & Adelberg's Medical Microbiology, 27e**
(Chapter 15: Enteric Gram-Negative Rods (Enterobacteriaceae))
- 2-Essential microbiology for dentistry 4th edition 2012.**