Enteric Gram-Negative Rods (*Enterobacteiaceae*)

The *Enterobacteriaceae* are a large, heterogeneous group of gram-negative rods 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). Some enteric organisms, such as*Escherichia coli,* are part of the normal microbiota and incidentally cause disease, but others, the 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,but these bacteria may also be called coliforms**.**

CLASSIFICATION

The *Enterobacteriaceae* are the most common group of gram-negative rods cultured in clinical laboratories and along with staphylococci and streptococci are among the most common bacteria that cause disease. The taxonomy of the *Enterobacteriaceae* is complex and rapidly changing since the introduction of techniques that measure evolutionary distance, such as nucleic acid hybridization and nucleic acid sequencing. Members of the family *Enterobacteriaceae* have the following characteristics: They are gram-negative rods, either motile with peritrichous flagella or non motile; grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey agar; grow aerobically and anaerobically (are facultative anaerobes); ferment rather than oxidize glucose, of en with gas production; are catalase positive and oxidase negative (except for*Plesiomonas*) and reduce nitrate to nitrite; and have a 39–59% G + C DNA content . There are many others in addition to the ones listed. In the United States, commercially prepared kits or automated systems are used to a large extent for this purpose. The implementation of matrix-assisted laser desorption ionization time of fight mass spectroscopy (MALDI-TOF MS) for identification of culture isolates may soon replace the more traditional panels of biochemical currently in use in most clinical microbiology laboratories. The is new technology seems to work quite well for identification of most of the common *Enterobacteriaceae* encountered in clinical material except for *Shigella* species. The is technology is unable to differentiate *Shigella* from *E coli.*

The major groups of *Enterobacteriaceae* are described and discussed

briefly in the following paragraphs. Specific characteristics of salmonellae, shigellae, and the other medically important enteric gram-negative rods and the diseases they cause are discussed separately.

Morphology and Identification

A. Typical Organisms

The *Enterobacteriaceae* are short gram-negative rods (Figure-1 A). 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* species, less so in *Enterobacter* species, and uncommon in the other species.

B. 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.

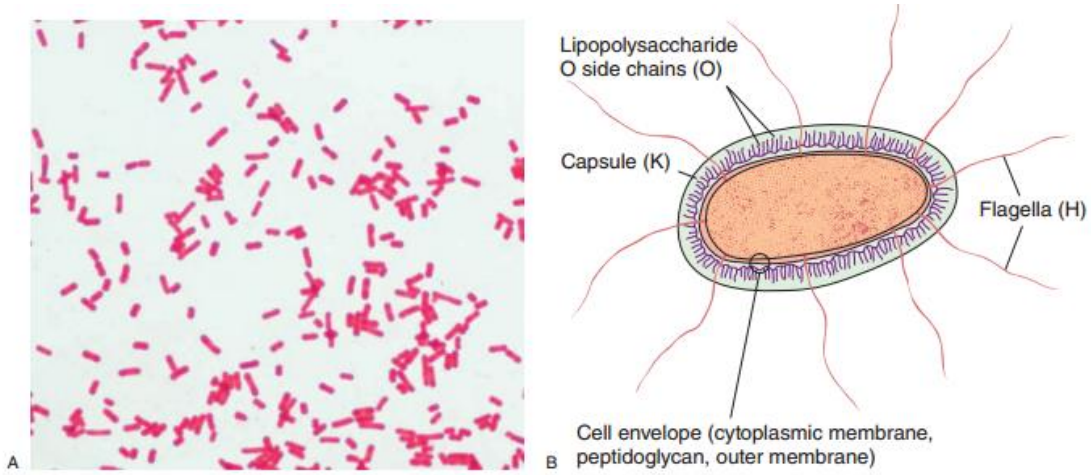

FIGURE 15-1 A: Gram stain of *Escherichia coli*. Original magnification ×1000. (Courtesy of H Reyes.)
B: Antigenic structure of *Enterobacteriaceae*.

C. Growth Characteristics

Carbohydrate fermentation patterns and the activity of amino acid decarboxylases and other enzymes are used in biochemical differentiation. Some tests, such as the production of in dole from tryptophan, are commonly used in rapid identification systems, but others, such as the Voges Proskauer reaction (production of acetyl methyl carbinol 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 non–lactose-fermenting colonies (non

pigmented) and may allow rapid presumptive identification of enteric bacteria (Table -1).

TABLE -1 Rapid, Presumptive Identification of Gram-Negative Enteric Bacteria

Lactose fermented rapidly
Escherichia coli: metallic sheen on differential media; motile; flat, nonviscous colonies
Enterobacter aerogenes: raised colonies, no metallic sheen; often motile; more viscous growth
Enterobacter cloacae: similar to *Enterobacter aerogenes*
Klebsiella pneumoniae: very viscous, mucoid growth; nonmotile
Lactose fermented slowly
Edwardsiella, Serratia, Citrobacter, Arizona, Providencia, Erwinia
Lactose not fermented
Shigella species: nonmotile; no gas from dextrose
Salmonella species: motile; acid and usually gas from dextrose
Proteus species: "swarming" on agar; urea rapidly hydrolyzed (smell of ammonia)
Pseudomonas species (see Chapter 16): soluble pigments, blue-green and fluorescing; sweetish smell

Many complex media have been devised to help in identification of the enteric bacteria. One such medium is triple sugar iron (TSI) agar, which is often used to help differentiate salmonellae and shigellae from other enteric gram-negative rods in stool cultures. The medium contains 0.1% glucose, 1% sucrose, 1% lactose, ferrous sulfate (for detection of H 2S production), tissue extracts (protein growth substrate), and a pH indicator (phenol red). It is poured into a test tube to produce a slant with a deep butt and is inoculated by stabbing bacterial growth into the butt. If only glucose is fermented, the slant and the butt initially turn yellow from the small amount of acid produced; as the fermentation products are subsequently oxidized to CO2 and H2O and released from the slant and as oxidative decarboxylation of proteins continues with formation of amines, the slant turns alkaline (red). If lactose or sucrose is fermented, so much acid is produced that the slant and butt remain yellow (acid). Salmonellae and shigellae typically yield an alkaline slant and an acid butt. Although *Proteus*, *Providencia*, and *Morganella* species produce an alkaline slant and acid butt, they can be identified by their rapid formation of red color in Christensen's urea medium. Organisms producing acid on the slant and acid and gas (bubbles) in the butt are other enteric bacteria.

1. *Escherichia*—*E. coli*** typically produces positive test results for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *E coli* by its hemolysis on blood agar, typical colonial morphology with an iridescent "sheen" on differential media such as EMB agar, and a positive spot indole test result. More than 90% of *E coli* isolates are positive for β-

glucuronidase using the substrate 4-methylumbelliferyl- β-glucuronide (MUG). Isolates fromanatomic sites other than urine, with characteristic properties (above plus negative oxidase test results) often can be confimed as *E coli* with a positive MUG test result.

2. *Klebsiella–Enterobacter–Serratia* group—*Klebsiella*** species exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive test results for lysine decarboxylase and citrate. Most *Enterobacter* species give positive test results for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Enterobacter aerogenes* has small capsules. *Serratia* species produces DNase, lipase, and gelatinase. *Klebsiella*, *Enterobacter*, and *Serratia* species usually give positive VogesProskauer reactions.

3. *Proteus–Morganella–Providencia* group—the members of this group deaminate phenylalanine, are motile, grow on potassium cyanide medium (KCN), and ferment xylose. *Proteus* species move very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals, such as phenylethyl alcohol or CLED (cystine-lactose-electrolytedefiient) medium. Whereas *Proteus* species and *Morganella morganii* are urease positive, *Providencia* species usually are urease negative. The *Proteus–Providencia* group ferments lactose very slowly or not at all. *Proteus mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group.

4. *Citrobacter*—these bacteria typically are citrate positive and differ from the salmonellae in that they do not decarboxylate lysine. They ferment lactose very slowly if at all.

5. *Shigella*—Shigellae are non-motile and usually do not ferment lactose but do ferment other carbohydrates, producing acid but not gas. Thy do not produce H2S. The four *Shigella* species are closely related to *E coli.* Many share common antigens with one another and with other enteric bacteria (eg, *Hafnia alvei* and *Plesiomonas shigelloides*).

6. *Salmonella*—Salmonellae are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce H 2S. They are often pathogenic for humans or animals when ingested. Organisms originally described in the genus *Arizona* are included as subspecies in the *Salmonella* group.

7. Other *Enterobacteriaceae—Yersinia species* .

Other genera occasionally found in human infections include *Cronobacter*, *Edwardsiella*, *Ewingella*, *Hafnia, Cedecea*, and *Kluyvera*.

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, and more than 50 H (flgellar) antigens (Figure-1B). In *Salmonella* serotype Typhi, the capsular antigens are called Vi antigens. 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. Although each genus of *Enterobacteriaceae* is associated with specific O groups, a single organism may carry several O antigens. Ths, most shigellae share one or more O antigens with *E coli. E coli* may cross-react with some *Providencia, Klebsiella,* and *Salmonella* species. Occasionally, O antigens may be associated with specifi human diseases (eg, specific O types of *E coli* are found in diarrhea and in urinary tract infections). 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 before 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. Human infections of the respiratory tract are caused particularly by capsular types 1 and 2 and those of the urinary tract by types 8, 9, 10, and 24.

H antigens are located on flagella and are denatured or removed by heat or alcohol. Thy are preserved by treating motile bacterial variants with formalin. Such H antigens agglutinate with anti-H antibodies, mainly IgG. The determinants in H antigens are a function of the amino acid sequence in flagella protein (flgellin). Within a single serotype, flagellar antigens may be present in either or both of two forms, called phase 1 (conventionally designated by lower-case letters) and phase 2 (conventionally designated by Arabic numerals), as shown in Table . The organism tends to change from one phase to the other; this is called phase variation. H antigens on the bacterial surface may interfere with agglutination by anti-O antibody.

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 inflenzae* or *Neisseria meningitidis.* Thus, The antigenic classification of

Enterobacteriaceae often indicates the presence of each specific antigen; eg, the antigenic formula of an *E coli* may be O55:K5:H21.

TABLE -2 Representative Antigenic Formulas of Salmonellae

O Group	Serotype	Antigenic Formula[a]
D	*Salmonella* Typhi	9, 12 (Vi):d:—
A	*Salmonella* Paratyphi A	1, 2, 12:a—
C_1	*Salmonella* Choleraesuis	6, 7:c:1,5
B	*Salmonella* Typhimurium	1, 4, 5, 12:i:1, 2
D	*Salmonella* Enteritidis	1, 9, 12:g, m:—

[a]O antigens: boldface numerals.
(Vi): Vi antigen if present.
Phase 1 H antigen: lower-case letter.
Phase 2 H antigen: numeral.

Colicins (Bacteriocins)

Many gram-negative organisms produce bacteriocins. These high-molecular-weight bactericidal proteins (pore-forming toxins) 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. Bacteriocin producing strains are resistant to their own bacteriocin; thus, bacteriocins can be used for "typing" of organisms.

Toxins and Enzymes

Most gram-negative bacteria possess complex lipopolysaccharides in their cell walls. These substances, cell envelope (cytoplasmic membrane, peptidoglycan, outer membrane endotoxins, have a variety of pathophysiologic effects. Many gram-negative enteric bacteria also produce exotoxins of clinical importance. Some
specific toxins are discussed in subsequent sections.

DISEASES CAUSED BY *ENTEROBACTERIACEAE* OTHER THAN *SALMONELLA* AND *SHIGELLA*

Causative Organisms

E coli is a member of the normal intestinal microbiota. Other enteric bacteria (*Proteus, Enterobacter, Klebsiella, Morganella, Providencia, Citrobacter,* and *Serratia* species) are also found as members of the normal intestinal microbiota but are considerably less common than *E coli.* The enteric bacteria are sometimes found in small numbers as part of the normal microbiota of the upper respiratory and genital tracts. The enteric bacteria generally do not cause disease, and in the intestine, they may even contribute

to normal function and nutrition. When clinically important infections occur, they are usually caused by *E coli,* but the other enteric bacteria are causes of hospital-acquired infections and occasionally cause community-acquired infections. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal microbiota sites. The most frequent sites of clinically important infection are the urinary tract, biliary tract, and other sites in the abdominal cavity, but any anatomic site (eg, bloodstream, prostate gland, lung, bone, meninges) can be the site of disease. Some of the enteric bacteria (eg, *Serratia marcescens, Enterobacter aerogenes*) are opportunistic pathogens. When normal host defenses are inadequate—particularly in infancy or old age, in the terminal stages of other diseases, aftr immunosuppression, or with indwelling venous or urethral catheters— localized clinically important infections can result, and the bacteria may reach the bloodstream and cause sepsis

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Pathogenesis and Clinical Findings

The clinical manifestations of infections with *E coli* and the other enteric bacteria depend on the site of the infection and cannot be differentiated by symptoms or signs from processes caused by other bacteria.

A. *E. coli*

1. *Urinary tract infection*—*E coli* is the most common cause of urinary tract infection and accounts for approximately 90% of fist urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection. None of these symptoms or signs is specific for *E coli* infection. Urinary tract infection can result in bacteremia with clinical signs of sepsis. Most of the urinary tract infections that involve the bladder or kidney in an otherwise healthy host are caused by a small number of O antigen types that have specifically elaborated virulence factors that facilitate colonization and subsequent clinical infections. These organisms are designated as uropathogenic *E coli*. Typically, these organisms produce hemolysin, which is cytotoxic and facilitates tissue invasion Strains that cause pyelonephritis express K antigen and elaborate a specific type of pilus, P fibriae, which binds to the P blood group antigen.

2. *E coli*–associated diarrheal diseases—*E. coli* that cause diarrhea are extremely common worldwide. These *E coli* are classified by the characteristics of their virulence properties (see later discussion), and each group causes disease by a different mechanism—at least six of which have been characterized. The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid or phage mediated. **Enteropathogenic *E coli* (EPEC)** are an important cause of diarrhea in infants, especially in developing countries. EPEC previously was associated with outbreaks of diarrhea in nurseries in

developed countries. EPEC adhere to the mucosal cells of the small bowel. Pathogenicity requires two important factors, the bundle forming pilus encoded by a plasmid EPEC adherence factor (EAF) and the chromosomal locus of enterocyte effacement (LEE) pathogenicity island that promote the tight adherence characteristic of EPEC(attachment and effacement). After attachment, there is loss of microvilli (effacement); formation of filamentous actin pedestals or cuplike structures; and, occasionally, entry of the EPEC into the mucosal cells. Characteristic lesions can be seen on electron micrographs of small bowel biopsy lesions. The result of EPEC infection in infants is severe, watery diarrhea; vomiting; and fever, which is usually self-limited but can be prolonged or chronic. EPEC diarrhea has been associated with multiple specific serotypes of *E coli;* strains are identified by O antigen and occasionally by H antigen typing A two-stage infection model using HEp-2 cells also can be performed. Tests to identify EPEC are performed in reference laboratories. The duration of the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

Enterotoxigenic *E coli* (ETEC) is a common cause of "traveler's diarrhea" and a very important cause of diarrhea in infants in developing countries. ETEC colonization factors (known as colonization factor antigens [CFAs]) specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a **heat-labile exotoxin** (LT) (molecular weight [MW], 80,000) that is under the genetic control of a plasmid. Its subunit B attaches to the GM1 ganglioside in the apical membrane of enterocytes and facilitates the entry of subunit A (MW, 26,000) into the cell, where the latter activates adenylyl cyclase. This markedly increases the local concentration of cyclic adenosine monophosphate (cAMP) after which ensues a complex cascade that involves the cystic fibrosis trans membrane conductance regulator. The end result is an intense and prolonged hypersecretion of water and chlorides and inhibition of the reabsorption of sodium. The gut lumen is distended with flied, and hypermotility and diarrhea ensue, lasting for several days. LT is antigenic and cross-reacts with the enterotoxin of *Vibrio cholerae*, which has an identical mechanism of action. LT stimulates the production of neutralizing antibodies in the serum (and perhaps on the gut surface) of persons previously infected with enterotoxigenic *E coli.* Persons residing in areas where such organisms are highly prevalent (eg, in some developing countries) are likely to possess antibodies and are less prone to develop diarrhea on reexposure to the LT-producing *E coli*. Assays for LT include

(1) flied accumulation in the intestines of laboratory animals

(2) typical cytologic changes in cultured Chinese hamster ovary cells or other cell lines

(3) stimulation of steroid production in cultured adrenal tumor cells

(4) binding and immunologic assays with standardized antisera to LT

(5) detection of the genes that encode the toxins. These assays are done only in reference laboratories.

Some strains of ETEC produce the **heat-stable enterotoxin** ST a (MW, 1500–4000), which is under the genetic control of a heterogeneous group of plasmids. STa activates guanylyl cyclase in enteric epithelial cells and stimulates flied secretion. Many STa-positive strains also produce LT. The strains with both toxins produce a more severe diarrhea. The plasmids carrying the genes for enterotoxins (LT, ST) also may carry genes for the CFAs that facilitate the attachment of *E coli* strains to intestinal epithelium. Recognized colonization factors occur with particular frequency in some serotypes. Certain serotypes of ETEC occur worldwide; others have a limited recognized distribution. It is possible that virtually any *E coli* may acquire a plasmid encoding for enterotoxins. There is no definite association of ETEC with the EPEC strains causing diarrhea in children. Likewise, there is no association between enter toxigenic strains and those able to invade intestinal epithelial cells. Care in the selection and consumption of foods potentially contaminated with ETEC is highly recommended to help prevent traveler's diarrhea. Antimicrobial prophylaxis can be effective but may result in increased antibiotic resistance in the bacteria and probably should not be uniformly recommended. When diarrhea develops, antibiotic treatment effectively shortens the duration of disease.

Shiga toxin-producing *E coli* (STEC) are named for the cytotoxic toxins they produce. There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and Shiga-like toxin 2. STEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. Shiga-like toxin 1 is identical to the Shiga toxin of *Shigella dysenteriae* type 1, and Shiga-like toxin 2 also has many properties that are similar to the Shiga toxin; however, the two toxins are antigenically and genetically distinct. Of the *E coli* serotypes that produce Shiga toxin, O157:H7 is the most common and is the one that can be identifid most readily in clinical specimens. STEC O157:H7 does not use sorbitol, unlike most other *E coli*, and is negative (clear colonies) on sorbitol MacConkey agar (sorbitol is used instead of lactose); O157:H7 strains also are negative on MUG tests. Many of the non-O157 serotypes may be sorbitol positive when grown in culture. Specific antisera are used to identify the O157:H7 strains. Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (EIAs) are done in many laboratories. Other sensitive test methods include cell culture cytotoxin testing using Vero cells and polymerase chain reaction for the direct detection of toxin genes directly from stool samples. Many cases of hemorrhagic colitis and its associated complications can be prevented by thoroughly cooking ground beef and avoiding unpasteurized products such as apple cider.

Enteroinvasive *E coli* (EIEC) produce a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. Similar to *Shigella*, EIEC strains are non

lactose or late lactose fermenters and are non motile. EIEC produce disease by invading intestinal mucosal epithelial cells.

Enteroaggregative *E coli* (EAEC) causes acute and chronic diarrhea (>14 days in duration) in persons in developing countries. These organisms also are the cause of foodborne illnesses in industrialized countries and have been associated with traveler's diarrhea and persistent diarrhea in patients with HIV. Thy are characterized by their specific patterns of adherence to human cells. This group of diarrheagenic *E coli* is quite heterogeneous, and the exact pathogenic mechanisms are still not completely elucidated. Some strains of EAEC produce ST-like toxin (see earlier discussion); others a plasmid-encoded enterotoxin that produces cellular damage; and still others, a hemolysis. Diagnosis can be suspected clinically but requires confirmation by tissue culture adhesion assays not readily available in most clinical laboratories.

3. *Sepsis*—When normal host defenses are inadequate, *E coli* may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to *E coli* sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infection.

4. *Meningitis*—*E coli* and group B streptococci are the leading causes of meningitis in infants. Approximately 75% of *E coli* from meningitis cases have the K1 antigen. This antigen cross-reacts with the group B capsular polysaccharide of *N meningitidis.* The mechanism of virulence associated with the K1 antigen is not understood.

B. *Klebsiella–Enterobacter–Serratia; Proteus– Morganella–Providencia;* and *Citrobacter*

The pathogenesis of disease caused by these groups of enteric gram-negative rods is similar to that of the nonspecific factors in disease caused by *E coli*.

1. *Klebsiella*—*K pneumoniae* is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (~1%) of bacterial pneumonias. *K pneumoniae* can produce extensive hemorrhagic necrotizing consolidation of the lung. It produces urinary tract infection and bacteremia with focal lesions in debilitated patients. Other enterics also may produce pneumonia. *Klebsiella* species rank among the top ten bacterial pathogens responsible for hospital-acquired infections. Two other Klebsielleae are associated with inflammatory conditions of the upper respiratory
tract: *Klebsiella pneumoniae* subspecies *ozaenae* has been isolated from the nasal mucosa in ozena, a fetid, progressive atrophy of mucous membranes; and *K pneumoniae* subspecies *rhinoscleromatis* form rhinoscleroma, a destructive granuloma of the nose and pharynx. *Klebsiella granulomatis* (formerly *Calymmatobacterium granulomatis*) causes a chronic genital ulcerative disease, **granuloma inguinale**, an uncommon sexually transmitted disease. The organism grows with difficulty on media containing egg yolk. Ampicillin or tetracycline is effective treatment.

2. *Enterobacter*—Thee species of*Enterobacter, Enterobacter cloacae, Enterobacter aerogenes,* and *Enterobacter sakazakii* (now in the genus *Cronobacter*), cause the majority of *Enterobacter* infections. These bacteria ferment lactose, may contain capsules that produce mucoid colonies, and are motile. These organisms cause a broad range of hospital-acquired infections such as pneumonia, urinary tract infections, and wound and device infections. Most strains possess a chromosomal β-lactamase called *ampC*, which renders them intrinsically resistant to ampicillin and fist- and second-generation cephalosporins. Mutants may hyperproduce β-lactamase, conferring resistance to thirdgeneration cephalosporins.

3. *Serratia*—*Serratia marcescens* is a common opportunistic pathogen in hospitalized patients. *Serratia* (usually nonpigmented) causes pneumonia, bacteremia, and endocarditis, especially in narcotics addicts and hospitalized patients. Only about 10% of isolates form the red pigment (prodigiosin) that has long characterized *S marcescens. S marcescens* is often multiply resistant to aminoglycosides and penicillins; infections can be treated with third generation cephalosporins.

4. *Proteus*—*Proteus* species produce infections in humans only when the bacteria leave the intestinal tract. Thy are found in urinary tract infections and produce bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving contaminated intravenous infusions. *P mirabilis* causes urinary tract infections and occasionally other infections. *Proteus vulgaris* and *M morganii* are important nosocomial pathogens.
Proteus species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia. This, in urinary tract infections with *Proteus* species, the urine becomes alkaline, promoting stone formation and making acidification virtually impossible. The rapid motility of *Proteus* may contribute to its invasion of the urinary tract. Strains of *Proteus* vary greatly in antibiotic susceptibility. *P mirabilis* is often inhibited by penicillins; the most active antibiotics for other members of the group are aminoglycosides and cephalosporins.
5. *Providencia*—*Providencia* species (*Providencia rettgeri, Providencia alcalifaciens,* and *Providencia stuartii*) are members of the normal intestinal flra. All cause urinary tract infections and occasionally other infections and are often resistant to antimicrobial therapy.

6. *Citrobacter*—*Citrobacter* species can cause urinary tract infections and sepsis.

Diagnostic Laboratory Tests
A. Specimens

Specimens include urine, blood, pus, spinal fluid, sputum, or other material, as indicated by the localization of the disease process.

B. Smears

The *Enterobacteriaceae* resemble each other morphologically. The presence of large capsules is suggestive of*Klebsiella* species.

C. Culture

Specimens are plated on both blood agar and differential media. With differential media, rapid preliminary identification of gram-negative enteric bacteria is often possible.

Immunity

Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, floroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential. Multiple drug resistance is common and is under the control of transmissible plasmids. Certain conditions predisposing to infection by these organisms require surgical correction, such as relief of urinary tract obstruction, closure of a perforation in an abdominal organ, or resection of a bronchiectasis portion of lung. Treatment of gram-negative bacteremia and impending septic shock requires rapid institution of antimicrobial therapy, restoration of fluid and electrolyte balance, and treatment of disseminated intravascular coagulation.

Various means have been proposed for the prevention of traveler's diarrhea, including daily ingestion of bismuth subsalicylate suspension (bismuth subsalicylate can inactivate *E coli* enterotoxin in vitro) and regular doses of tetracyclines or other antimicrobial drugs for limited periods. Because none of these methods are entirely successful or lacking in adverse effects, it is widely recommended that caution be observed in regard to food and drink in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim–sulfamethoxazole) be substituted for prophylaxis.

Epidemiology, Prevention, and Control

The enteric bacteria establish themselves in the normal intestinal tract within a few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora. *E coli* is the prototype. Enterics found in water or milk are accepted as proof of fecal contamination from sewage or other sources. Control measures are not feasible as far as the normal endogenous flora is concerned. Enteropathogenic *E coli* serotypes should be controlled like salmonellae .

Some of the enterics constitute a major problem in hospital infection. It is particularly important to recognize that many enteric bacteria are "opportunists" that cause illness when they are introduced into debilitated patients. Within hospitals or other institutions, these bacteria commonly are transmitted by personnel, instruments, or parenteral medications. Their control depends on hand washing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the urinary tract sterile (ie, closed drainage).

THE SHIGELLAE

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

Morphology and Identification

A. Typical Organisms

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

B. 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.

C. Growth Characteristics

All shigellae ferment glucose. With the exception of *Shigella sonnei*, they do not ferment lactose. The inability to ferment lactose distinguishes shigellae on differential media. Shigellae form acid from carbohydrates but rarely produce gas. They may also be divided into those that ferment mannitol and those that do not (Table -3).

TABLE -3 Pathogenic *Shigella Species*

Present Designation	Group and Type	Mannitol	Ornithine Decarboxylase
Shigella dysenteriae	A	–	–
Shigella flexneri	B	+	–
Shigella boydii	C	+	–
Shigella sonnei	D	+	+

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.
The pathogenic species are shown in Table -3.

Pathogenesis and 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 103 organisms (it usually is 105–108 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. Microabscesses 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

A. Endotoxin

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

B. *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 a protein that is antigenic (stimulating production of antitoxin) and lethal for experimental animals. Acting as an enterotoxin, it produces diarrhea as does the *E coli* Shiga-like toxin, perhaps by the same mechanism. In humans, the exotoxin also 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 flxneri* or *S sonnei* infections develop antitoxin that neutralizes *S dysenteriae* exotoxin in vitro. The toxic activity is distinct from the invasive property of shigellae in dysentery. The two may act in sequence, the toxin producing an early non bloody, voluminous diarrhea and the invasion of the large intestine, resulting in later dysentery with blood and pus in stools.

Clinical Findings

After a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an exotoxin acting in the small intestine. A day or so later, as the infection involves the ileum and colon, the number of stools

increases; they are less liquid but often contain mucus and blood. Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain. In more than half of adult cases, fever and diarrhea subside spontaneously in 2–5 days.
However, in children and elderly adults, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness caused by *S dysenteriae* may be particularly severe. On recovery, most persons shed dysentery bacilli for only a short period, but a few remain chronic intestinal carriers and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection.

Diagnostic Laboratory Tests

A. 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.

B. Culture

The materials are streaked on diffrential 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 TSI agar. Organisms that fail to produce H2S, that produce acid but not gas in the butt and an alkaline slant in TSI agar medium, and that are non motile should be subjected to slide agglutination by specific *Shigella* antisera.

C. Serology

Normal persons often have agglutinins against several *Shigella* species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is not used to diagnose *Shigella* infections.

Immunity

Infection is followed by a type-specific antibody response. Injection of killed shigellae stimulates production of antibodies in serum but fails to protect humans against infection. IgA antibodies in the gut may be important in limiting reinfection; these may be stimulated by live attenuated strains given orally as experimental vaccines. Serum antibodies to somatic *Shigella* antigens are IgM.

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. Thy 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. Opioids should be avoided in *Shigella* dysentery.

Epidemiology, Prevention, and Control

Shigellae are transmitted by "food, fingers, feces, and flies" from person to person. Most cases of *Shigella* infection occur in children younger than 10 years of age. Shigellosis, caused primarily by *S sonnei*, has become an important problem in daycare centers in the United States. *S dysenteriae* can spread widely. Mass chemoprophylaxis for limited periods of time (eg, in military personnel) has been tried, but resistant strains of shigellae tend to emerge rapidly. Because humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by

(1) sanitary control of water, food, and milk; sewage disposal and fly control

(2) isolation of patients and disinfection of excreta

(3) detection of subclinical cases and carriers, particularly food handlers

(4) antibiotic treatment of infected individuals

THE *SALMONELLA-ARIZONA* GROUP

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

Morphology and Identification

Salmonellae vary in length. Most isolates are motile with mperitrichous flagella. Salmonellae grow readily on simple media, but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce H2S. Thy survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria; such compounds are therefore useful for inclusion in media to isolate salmonellae from feces.

Classification

The classification of salmonellae is complex because the organisms are a continuum rather than a defied species. The members of the genus *Salmonella* were originally classified on the basis of epidemiology; host range; biochemical reactions; and structures of the O, H, and Vi (when present) antigens. The names (eg, *S typhi, Salmonella typhimurium*) were written as if they were genus and species; this form of the nomenclature remains in widespread but incorrect use. DNA–DNA hybridization studies have demonstrated that there are seven evolutionary groups. Currently, the genus *Salmonella* is divided into two species each with

multiple subspecies and serotypes. The two species are *Salmonella enterica* and *Salmonella bongori* (formerly subspecies V). *S enterica* contains fie subspecies, which are subspecies *enterica* (subspecies I), subspecies *salamae* (subspecies II), subspecies *arizonae* (subspecies IIIa), subspecies *diarizonae* (subspecies IIIb), subspecies *houtenae* (subspecies IV), and subspecies *indica* (subspecies VI). Most human illness is caused by the subspecies I strains, written as *S enterica* subspecies *enterica*. Rarely human infections may be caused by subspecies IIIa and IIIb or the other subspecies frequently found in cold-blooded animals. Frequently, these infections are associated with exotic pets such as reptiles. It seems probable that the widely accepted nomenclature for classification will be as follows: *S enterica* subspecies *enterica* serotype *Typhimurium*, which can be shortened to *S* Typhimurium with the genus name in italics and the serotype name in roman type. National and international reference laboratories may use the antigenic formulas following the subspecies name because they impart more precise information about the isolates (see Table -3).

There are more than 2500 serotypes of salmonellae, including more than 1400 in DNA hybridization group I that can infect humans. Four serotypes of salmonellae that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance. Thy are as follows: *Salmonella* Paratyphi A (serogroup A), *Salmonella* Paratyphi B (serogroup B), *Salmonella* Choleraesuis (serogroup C1), and *S* Typhi (serogroup D). *Salmonella* serotypes Enteritidis and Typhimurium are the two most common serotypes reported in the United States. The more than 1400 other salmonellae that are isolated in clinical laboratories are serogrouped by their O antigens as A, B, C1, C2, D, and E; some are non-type able with this set of antisera. The isolates are then sent to reference laboratories for definitive serologic identification. This allows public health offials to monitor and assess the epidemiology of *Salmonella* infections on statewide and nationwide basis.

Variation

Organisms may lose H antigens and become nonmotile. Loss of O antigen is associated with a change from smooth to rough colony form. Vi antigen may be lost partially or completely. Antigens may be acquired (or lost) in the process of transduction.

Pathogenesis and Clinical Findings

S Typhi, *S* 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; these include 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 *S* Typhi organisms).

Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbiota, and local intestinal immunity . Salmonellae produce three main types of disease in humans, but mixed forms are frequent (Table -4).

TABLE -4 Clinical Diseases Induced by Salmonellae

	Enteric Fevers	Septicemias	Enterocolitis
Incubation period	7–20 days	Variable	8–48 hours
Onset	Insidious	Abrupt	Abrupt
Fever	Gradual; then high plateau with "typhoidal" state	Rapid rise; then spiking "septic" temperature	Usually low
Duration of disease	Several weeks	Variable	2–5 days
Gastrointestinal symptoms	Often early constipation; later, bloody diarrhea	Often none	Nausea, vomiting, diarrhea at onset
Blood culture results	Positive in first to second weeks of disease	Positive during high fever	Negative
Stool culture results	Positive from second week on; negative earlier in disease	Infrequently positive	Positive soon after onset

A. The "Enteric Fevers" (Typhoid Fever)

This syndrome is produced by only a few of the salmonellae, of which *S* Typhi (typhoid fever) is the most important. The ingested salmonellae reach the small intestine, from which they enter the lymphatics and then the bloodstream. Thy 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. In the preantibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and 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 inflmmation of the gallbladder, periosteum, lungs, and other organs.

B. Bacteremia with Focal Lesions

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

C. Enterocolitis

This is the most common manifestation of salmonella infection. In the United States, *S* 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 immunodefiient persons. Blood culture results are usually negative, but stool culture results are positive for salmonellae and may remain positive for several weeks after clinical recovery.

Diagnostic Laboratory Tests

A. Specimens

Blood for culture must be taken repeatedly. In enteric fevers and septicemias, blood culture results are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine culture results 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, the stools yield positive results
during the fist week. A positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract in carriers.

B. Bacteriologic Methods for Isolation of Salmonellae

1. ***Diffrential medium cultures***—EMB, MacConkey, or deoxycholate medium permits rapid detection of lactose nonfermenters (not only salmonellae and shigellae but also ProteuSerratia, Pseudomonas, and so on). Gram-positive organisms are somewhat inhibited. Bismuth sulfide medium permits rapid detection of salmonellae, which form black colonies because of H 2S production. Many salmonellae produce H2S.

2. Selective medium cultures—The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, xylose-lysine decarboxylase (XLD) agar, or deoxycholatecitrate 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 identifiation—Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

C. Serologic Methods

Serologic techniques are used to identify unknown cultures with known sera (see later discussion) and may also be used to determine antibody titers in patients with unknown illness, although the latter is not very useful in diagnosis of Salmonella infections.

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 S 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 greater than 1:320 and against the H antigen of greater than 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 EIA 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.

Immunity

Infections with S Typhi or Salmonella Paratyphi usually confer a certain degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease. However, relapses may occur in 2–3 weeks aftr recovery despite antibodies. Secretory IgA antibodies may prevent attachment of salmonellae to intestinal epithelium. Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to Salmonella infections, particularly osteomyelitis. Persons with A/S

hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Treatment

Although 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. In most carriers, the organisms persist in the gallbladder (particularly if gallstones are present) and in the biliary tract. Some chronic carriers have been cured by ampicillin alone, but in most cases cholecystectomy must be combined with drug treatment.

Epidemiology

The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are "shedding" organisms. Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs. The high incidence of salmonellae in commercially prepared chickens has been widely publicized. The incidence of typhoid fever has decreased, but the incidence of other Salmonella infections has increased markedly in the United States. The problem probably is aggravated by the widespread use of animal feeds containing antimicrobial drugs that favor the proliferation of drug-resistant salmonellae and their potential transmission to humans.

A. Carriers

After manifest or subclinical infection, some individuals continue to harbor salmonellae in their tissues for variable lengths of time (ie, convalescent carriers or healthy permanent carriers). Thee percent of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder; biliary tract; or, rarely, the intestine or urinary tract.

B. 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; some outbreaks are traceable to the source of supply

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 humans
6. "Recreational" drugs—Marijuana and other drugs
7. Animal dyes—Dyes (eg, carmine) used in drugs, foods, and cosmetics
8. Household pets—Turtles, dogs, cats, exotic pets such as reptiles, and so on