The Family Enterobacteriaceae

1972: 12 genera and 26 species in the family Enterobacteriaceae
1994: 27 genera and 102 species!

Most important genera:
*Escherichia coli, Shigella, Salmonella, Yersinia*

Others include: *Klebsiella, Enterobacter, Serratia, Proteus, Providencia, Edwardsiella, Citrobacter*

- Gram-negative rods
- nonspore-forming
- facultatively anaerobic
- ferment glucose
- simple growth requirements
- most are motile with peritrichous flagella
- many produce fimbriae (pili), capsules, or both.

Coliform - denotes all the enteric bacilli, inhabitants of the gastrointestinal tract; normal flora or pathogens.
The bulk of the gut flora (~1011 organisms/gram feces) = obligate anaerobes
Predominant facultative anaerobe (108 bacteria/g feces) = *Escherichia coli*

Habitat - worldwide in soil, water, vegetation, decaying matter, and the large intestines of most animals and humans.

The *Enterobacteriaceae* cause different types of infections in different populations:

**Underdeveloped countries**: acute gastroenteritis is widespread among malnourished small children; principal cause of death in this age group.

**Developed countries**: infections with members of the *Enterobacteriaceae* (other than the frank pathogens like *Salmonella, Shigella, Yersinia*) - usually opportunistic and occur outside of the intestines.

Increase in the number of nosocomial diseases caused by enteric bacilli due to:
- suppression of other organisms by the overuse of antibiotics
- immunosuppressive and cytotoxic agents
- survival of patients with impaired immune responses

Various kinds of extra-intestinal disease are increasingly associated with ordinarily harmless enteric organisms.

Enteric organisms are the most common agents of urinary tract infections (UTI). They are also the predominant etiologic agents in cases of endogenous systemic infections and nosocomial infections.

They can be isolated from feces, urine, blood, wounds, pulmonary aspirates, and cerebrospinal fluid.

Treatment - difficult because of drug resistance and also because of the presence of underlying serious diseases or impaired host defenses.
I. *Escherichia coli*

Most common isolate of the hospital microbiology lab. *E. coli* is normally the most common facultative anaerobe in the large bowel. Most *E. coli* strains adhere to the mucus overlying the surface of the large bowel and the distal small bowel. The doubling time of *E. coli* within the intestine has been estimated to be ~40 hours (contrast with 20 min in the lab). *E. coli* of the normal flora provide protection against colonization by harmful microbes.

Only a small number of *E. coli* strains actually cause disease. Virulent strains differ from avirulent strain in possessing genetic elements (Tn's, plasmids, pathogenicity islands) for virulence factors. *E. coli* can cause 3 kinds of disease. Different strains are associated with different diseases.

1. urinary tract infections (UTI)
2. neonatal meningitis
3. intestinal (diarrheal) diseases

**Virulence factors include:**

a. adherence to specific host receptors
b. elaboration of exotoxins
c. invasion of host cells
d. capsules that are antiphagocytic and inhibit the opsonizing and lytic activities of complement
e. synthesis of iron-chelating siderophores (aerobactin and enterobactin). In addition, iron can be released from host cells by production of hemolysins.

**Clinical:**

1. **UTI**: *E. coli* is responsible for >80% of UTI's. Most UTIs originate from a pathogenic strain of *E. coli* that is resident in the gut. In females, these *E. coli* uropathogenic strains subsequently colonize the vaginal and periurethral region (endogenous infection). This colonization facilitates the ascent of bacteria from the urethra into the bladder. Females suffer more UTI's than males, probably because of a shorter urethra.

In the bladder, *E. coli* colonizes the uroepithelium leading to large numbers of bacteria in the urine (>10⁵/ml) - *bacteriuria*. Symptoms arise when invasion of the mucosa, cell death, and inflammation (cystitis) occur. If the invading bacteria pass up the ureters to the kidney, *pyelonephritis* results. One of the main host defenses of the urinary tract is the washing action of urine. Bacteria that do not adhere will be washed out of the bladder faster than they can multiply. **Key feature**: adherence to bladder mucosa.

a. **type 1 common pili** are called "mannose sensitive" because the bind to a mannose-containing receptor in the host. Their binding to host receptors is inhibited by mannose. These pili are probably responsible for anchoring *E. coli* to mucus in the large intestine and contributing to colonization of the vaginal tract. Type 1 pili are found in virulent and avirulent *E. coli* strains.

**b. P pili** - expressed by almost all pyelonephritis *E. coli* isolates, ~50% of cystitis isolates, and <10% of fecal isolates. Named for their ability to agglutinate human RBCs carrying the P blood group antigen.

c. Uropathogenic strains of *E. coli* may also have adhesins that are not pili - **afimbrial adhesins**.
How does colonization of the bladder lead to the strong inflammatory response that appears to be responsible for the symptoms of an acute UTI?

- LPS
- cytolytic hemolysin that creates pores in eukaryotic cell membranes.

Other factors that contribute to uropathogenicity include:
- resistance to the inhibitory properties of normal human serum
- production of aerobactin and enterochelin - iron-scavenging molecules
- capsular polysaccharide (K antigen)

2. Neonatal meningitis - *E. coli* is a common cause of neonatal bacterial meningitis. Neonates acquire the strains from their mothers and become colonized in the nasopharynx or the intestine. The organisms then invade the bloodstream and are carried to the meninges. The mortality rate: 40 to 75%.

   a. **K1 capsular polysaccharide** - approximately 80% of the isolates synthesize the K1 antigen. K1 is also common in strains associated with septicemia and UTI. *E. coli* K1 strains are found in the colonic flora of 20 to 40% of individuals. The K1 capsule = homopolymer of sialic acid. It is antiphagocytic and provides some resistance against the usual sensitivity of *E. coli* to complement-mediated lysis. The host does not produce antibodies to the K1 capsule because of its similarity to host sialic acid polymers. The meningococcal Group B capsule and *E. coli* K1 polysaccharides are chemically and immunologically identical.

   b. **siderophores**

   c. **S fimbriae** - bind to vascular endothelium and epithelial lining of brain tissues.

3. Intestinal diseases - *E. coli* causes ~4 classes of diarrheal diseases with distinct features in their pathogenesis, clinical syndrome, and epidemiology.

   The biochemical activities of the pathogenic species of *E. coli* are identical to those of the nonpathogenic species of the gut. Thus, special tests are needed to detect specific groups of diarrheagenic *E. coli*. These tests may include enterotoxin testing and serotyping. DNA probes are now being developed to identify toxigenic strains of *E. coli* in the clinical lab.

   A. **Enterotoxigenic *E. coli*** (ETEC) are an important cause of traveler's diarrhea and diarrhea in infants in less developed countries. The disease is rare in infants in industrialized countries. Adults in endemic areas are evidently immune. Disease caused by ETEC is rarely observed in the US.

   - Acquired by the ingestion of contaminated food and water; incubation period is 1-3 days. Large numbers of organisms (108) must be consumed to cause disease in a susceptible individual.

   - The disease is characterized by 1-7 days of watery diarrhea with minor discomfort to severe cholera-like symptoms; vomiting; little to no fever.

   Worldwide incidence: 650 million cases

   800,000 deaths (mostly <5 yrs of age)

   Prerequisites for disease:
   - susceptible individual (no abs to enterotoxins)
   - adherence (plasmid-encoded adhesins)
   - enterotoxin production (plasmid encoded toxin genes)
a. **Adhesins** - ETEC possess specialized pili called **colonization factor antigens** (CFAs) which act as ligands to bind the bacterial cells to specific complex carbohydrate receptors on the epithelial cell surfaces of the **small intestine**. CFAs are found only on *E. coli* strains that cause diarrheal disease. Genes for the production of CFAs reside on the ETEC virulence **plasmids**, usually on the same plasmids that carry the enterotoxin genes.

b. **Enterotoxins** - Most ETEC synthesize one or both of two **plasmid-mediated** enterotoxins; both cause net secretion of fluid and electrolytes into the bowel.

  o **heat-labile toxin** (LT). LT is an 86,000 dalton protein with 80% aa identity to cholera toxin. A subunit + 5 identical binding B subunits. The B subunits bind the toxin to the target cells via a specific receptor that has been identified as GM1 ganglioside. The A subunit is then activated by cleavage of a peptide bond and internalized.

    In the crypt cells of the intestine, the A subunit **ADP-ribosylates the adenyl cyclase regulatory subunit Gs**. This results in elevated levels of intracellular cAMP that leads to hypersecretion of water and electrolytes into the bowel lumen: a profuse watery, non-inflammatory diarrhea.

  o **heat-stable toxin** (ST; stable at 100°C for 30 min) - small polypeptides, ranging in size from 18 to 50 amino acids long. All the STs are structurally related; they have a large number of disulfide bonds that contribute to their heat stability.

    ST acts as a hormone analog and **binds to guanylate cyclase** that is located in the apical membrane of the host cell which stimulates this host enzyme resulting in an increase in intracellular cGMP levels. Intracellular accumulation of cGMP inhibits intestinal fluid uptake, resulting in net fluid secretion.

    *E. coli* strains that produce both LT and ST cause the most severe disease. Most ETEC make CFA, LT, and ST. Stool from colonized patients would yield almost a pure culture of ETEC. However, diagnosis is usually made clinically; suitable assays for CFA, LT, and ST are not available in the clinical lab.

**Therapy** - simple restoration of fluid balance by IV or oral glucose and electrolytes and the use of pharmacologic agents to reduce diarrhea. Antimicrobial therapy in travelers has not been effective. Vaccines are not available (Expt'l vaccine: LT-B fragment cloned into potatoes or bananas - protects against cholera and ETEC.). The disease is self-limiting.

**Host defenses** against ETEC diarrhea: gastric acidity, small-intestinal motility, a large population of normal flora in the large intestine, breast-feeding of infants, and intestinal secretory IgA directed against the CFAs and the toxins.

**Transmission** is from person to person (via water, food; fecal/oral) with no known important animal vectors. The incidence of *E. coli* diarrhea is clearly related to hygiene and care in food processing.

B. **Enteropathogenic *E. coli* (EPEC)** are a major cause of pediatric diarrhea and death in developing countries. Nonimmune adults can also acquire EPEC diarrhea. EPEC strains do **not** produce LT or ST.

Stage one: bacteria **associate** with the host cell through non-intimate binding mediated by a **bundle-forming pilus** (Bfp). Bfp may mediate bacterium-bacterium interaction and/or adherence between bacterium and host cell. Genes for Bfp are plasmid-encoded.

Stage two: bacteria **attach** to the host cell triggering a signal transduction event that is associated with activation of host cell tyrosine kinases and results in increased host cell intracellular Ca²⁺ levels.
Stage three: bacteria adhere tightly to the surface of cells in the colon, indenting the cell membrane and causing localized destruction of the microvilli. Extensive rearrangement of host cell actin occurs in the vicinity of the adherent bacteria resulting in the formation of a cup-like pedestal structure under the bacteria. The pedestal-like structure is composed of a dense mat of actin fibers. This intimate contact with the host cell causes the so-called attaching and effacing lesions leading to loss of microvilli which then causes malabsorption and osmotic damage. Diarrhea is apparently caused by the loss of the absorptive capacity of mucosal cells. The diarrhea is chronic and accompanied by fever.

**Therapy** - Oral nonabsorbable antibiotics like gentamicin are used, together with the maintenance of fluid and electrolyte balances. No vaccine is available. Diagnosis is not made in the clinical lab.

C. **Enteroinvasive E. coli (EIEC)** closely resemble Shigella's pathogenic mechanisms and the clinical illness that they produce. Most strains of *E. coli* are motile and ferment lactose, but these strains are characteristically nonmotile and lactose-negative (like *Shigella*). They cross-react with certain *Shigella* O antigens and possess a large plasmid that confers the capacity to invade human epithelial cells (similar to a *Shigella* plasmid).

EIEC actively invade colonic cells and spread laterally to adjacent cells. Invasion and cell-to-cell spread are virtually identical to steps in invasion and cell-to-cell spread of *Shigella* spp (actin polymerization). The clinical syndrome is identical to *Shigella* dysentery, as is its treatment and prevention. However, EIEC do not produce Shiga toxin.

Infectious inoculum is low (<100 bacteria); little fluid in stool; much blood and mucus.

D. **Enterohemorrhagic (EHEC) E. coli** - most frequent diarrheagenic type of *E. coli* isolated in North America today. The first documented EHEC outbreak of hemorrhagic colitis in the US was reported in 1982 (origin was undercooked hamburger from a fast-food chain). Outbreaks of *E. coli* O157:H7 infections have been reported >60 times since that first cluster. In January 1993, a hamburger-borne outbreak of hemorrhagic colitis (bloody diarrhea) associated with ingestion of undercooked fast-food hamburgers occurred in the Seattle-Tacoma, Washington area. By Feb 93, the CDC reported 732 cases of culture-confirmed *E. coli* O157:H7 infection. Approximately 7.2% of the infected individuals (children) also developed the hemolytic uremic syndrome (toxicity for the kidney); four infected children died.

Oct 96: Washington apple cider (unpasteurized) - 45 cases.

1996: Japan - 9,578 affected; 11 deaths; 20-30 critically ill; affected 62 schools in Sakai City. Traced to contaminated radish sprouts.

1997: 15 cases - led to recall of frozen beef patties throughout U.S.

1997: 100 cases in Michigan and Virginia: alfalfa sprouts - same *E. coli* strain.

1998: 45 outbreaks of O157:H7 in U.S.

1999: 38 outbreaks, 1897 cases; 2% (37) were hemolytic uremic syndrome (HUS); 4 deaths (0.2%). *E. coli* serotype O157:H7 causes this diarrheal syndrome worldwide. The disease is distinct from the bacillary dysentery caused by *Shigella* and EIEC because there is no fever, and the stool is bloody and copious.
**Transmission:** The most likely source is contaminated meat; spread by contact with infected individuals or by consumption of contaminated unpasteurized milk, water, or apple cider. Infectious inoculum is low (2 to 2000 CFU).

Incubation period: 2-5 days

The organism has been isolated from dairy cattle, calves, chickens, swine, and sheep. Infected animals are not sick. Meat is contaminated when the intestinal contents from infected cattle contact the carcasses during slaughter. Retail meats (1 to 2.5%) - harbor *E. coli* O157:H7.

Ground meat appears to be particularly susceptible to contamination because grinding inoculates any organisms present on the surface of a cut of meat into its interior, where the increased surface area encourages growth. This distributive mixing of the bacteria throughout the product helps to explain why the entire portion needs to be thoroughly cooked.

Capable of surviving cold storage, *E. coli* O157:H7 remains infectious in contaminated meat unless destroyed by thorough cooking at 155-160°F (70°C).

The USDA launched an E.coli 0157:H7 testing program for meat plants and retail stores in 1994. Since the program began, USDA scientists have tested more than 20,000 samples and found 13 contaminated with the virulent bacteria that can cause bloody diarrhea and kidney failure.

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**Virulence:**

a. **attachment:** EHEC strains bind tightly to cultured mammalian cells and produce the same attachment-effacement phenomenon seen with EPEC strains. They are not invasive. No animal model of infection has been developed.

b. **toxins:** No LT or ST is made. These strains produce relatively large amounts of one or more types of Shiga-like toxins (also called verotoxin because of its cytopathic effect in the Vero cell line of tissue culture cells) that is probably responsible for its pathogenicity. Encoded by a bacteriophage; growth-limiting iron conditions enhance production. SLTs are cytotoxins with an A-B subunit structure; AB(5)
The B subunits bind specifically to glycolipid receptors. The A subunit enzymatically disrupts the structural integrity of the 60S ribosomal subunit causing protein synthesis to cease.

The EHEC O157H7 clone apparently emerged with an EPEC progenitor was lysogenized by a bacteriophage containing Shiga-like toxin genes.

*E. coli* O157:H7 - can be detected in the clinical laboratory because it does not ferment sorbitol; 95% of "regular" *E. coli* isolates are sorbitol positive (SMAC plates). Confirm by serologic testing for the O157 and H7 antigens.

**Treatment:** In most cases of gastrointestinal infections, antibiotics are not recommended. Successful treatment depends on the replacement of water and electrolytes.

**Control/prevention:** sanitation, handwashing, proper food preparation, adequate cooking, chlorination of water, sewage treatment and disposal

For gastroenteritis in the U.S.: Campylobacter > Salmonella > EHEC

4. **Opportunistic infections**

The *Enterobacteriaceae* are responsible for about half of nosocomial infection in the US. About 1/4 of these are caused by *E. coli*. *E. coli* is an important cause of bacteremia, surgical infections and respiratory tract disease, mostly in patients whose normal defense mechanisms have been breached.
The hospital environment selects for strains that are drug-resistant (via R plasmids), so they are often difficult to eradicate. 

*E. coli* recovered from extraintestinal infections often produce capsule, soluble and cell bound hemolysins, siderophores, and adherence pili.

II. *Shigella*

*Shigella* spp. are the principal agents of bacillary dysentery, characterized by a small volume of feces containing blood, mucus, and inflammatory cells. This contrasts with the profuse, watery diarrhea of other enteric pathogens. *Shigella* show a high degree of relatedness to *E. coli*. EIEC caused an illness indistinguishable from that of *Shigella*. There are four species of *Shigella: S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. The most severe infections are caused by *Shigella dysenteriae*.

**Transmission:** by ingestion; usually spread from person to person by the fecal-oral route - contaminated food, water, or fomites (flies, fingers, forks). Highly infectious: 10 to 100 organisms can lead to infection. *Shigella* organisms survive gastric acidity (probably why the infective dose is so low). The patient experiences abdominal pain, cramps, and fever w/in 24 - 48 h of ingestion. Duration ~7 days. Mostly affects children in developing countries.

**Habitat:** Only humans and apes serve as the natural host and reservoir in nature.

**Steps in pathogenesis:**

a. colonization of the colon

b. invasion of the colon epithelium by the pathogen. The current hypothesis about how shigellae invade the colonic mucosa through normally phagocytic M cells in the gut.

c. the organisms are internalized within the cell in an intracellular vacuole

d. organisms digest the vacuole membrane and are found free in the cytoplasm

e. bacteria multiply and infect adjacent epithelial cells causing cell death. *Shigella* spreads from cell to cell by actin polymerization (like *Listeria*).

When Shigellae reach the submucosal space, they invade mucosal cells through the basal surface where the integrins are located.

Integrins - a family of receptors that promote attachment of eukaryotic cells to extracellular matrix proteins like fibronectin and collagen. Integrins function in cell-to-cell interactions and signal transduction.

Growing within the mucosal epithelial layer, the shigellae are protected from phagocytes and other host defenses.

The epithelial layer is destroyed and microabscesses form, leading eventually to mucosal ulcerations. When the invading bacteria reach the underlying lamina propria, they evoke an intense inflammatory reaction that efficiently destroys the microbes. *Shigella* rarely penetrate beyond the epithelial cells past the lamina propria to other parts of the body. The ulceration process leads to high concentrations of neutrophils in the stools.

**Virulence factors:**

1. *Shigella virulence plasmid:* 180-240 kb - present in all virulent (invasive) *Shigella* strains as well as enteroinvasive *E. coli* strains. It encodes OMPs that mediate attachment to the epithelial cell. Plasmid-
encoded proteins initiate parasite-induced phagocytosis and break down the membrane of the phagocytic vacuole, allowing bacteria to multiply within the cytoplasm.

2. **Shiga toxin** is a potent exotoxin that is:
   a. *neurotoxic* - causes paralysis when injected into small animals;
   b. *enterotoxic* in the rabbit ileal loop assay;
   c. *cytotoxic* to tissue culture cells.

   It is encoded by a **chromosomal gene** and is composed of an active A subunit (32 kd) and 5 identical receptor-binding B subunits (7 kDa). The B subunits recognize a host cell surface glycolipid called Gb3.

   The toxin irreversibly inactivates the mammalian 60S ribosomal subunit causing **cessation of protein synthesis** and death of the cell. The enterotoxic effects of Shiga toxin is due to **blocked absorption** of electrolytes, glucose, and amino acids from the intestinal lumen.

   *Shigella* species other than *S. dysenteriae* produce a serologically cross-reactive toxin referred to as **Shiga-like toxin** - it is not produced in sufficient quantities to cause disease so severe as that caused by *S. dysenteriae*.

3. **Actin polymerization** is involved in cell to cell spread. *Shigella* lack flagella associated with motility. Now it is recognized that (like *Listeria*) intracellular *Shigella* cause a **localized polymerization of actin microfilaments** within the cytoplasm. This apparently provides a motive force for bacterial movement and intracellular spread. A virulence **plasmid-encoded** outer membrane protein is implicated in actin polymerization.

   Shigellae form tails of polymerized actin and penetrate into adjacent cells at the head of long projections of polymerized actin filaments. Interestingly, no genetic similarity has been detected to date between the machinery for intracellular movement of *Shigella* and *Listeria*.

**Treatment:** As in all diarrheal disease, the most important consideration is to maintain fluid and electrolyte balance. Because the disease is self-limiting, most experts consider antibiotic treatment only for the young and for those infected with *S. dysenteriae*. Antibiotics reduce the average duration of the illness from 5 days to 3 days and reduce stool excretion of viable organisms.

*Shigella* frequently acquire R plasmids and become resistant to many common antibiotics. Treatments might include norfloxacin, ampicillin, tetracycline, or trimethoprim-sulfamethoxazole.

**Prevention:** **good sanitation**, particularly regarding the fecal-oral route. Frequent hand washing has a significant impact in minimizing transmission from patients to family members or other members of the community.

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### III. Salmonella

The classification and naming of Salmonella has been controversial. The current classification calls for only a single species of *Salmonella*: *S. enterica*. This species is divided into ~1800 serovars. Common nomenclature:

- *S. typhi* - enteric fever - only in man
- *S. cholerasuis* - bacteremia
- *S. enteritidis* - gastroenteritis
- *S. typhimurium* - enteric fever in the mouse; gastroenteritis in humans
A. Habitat: *Salmonella* are ubiquitous pathogens capable of causing disease in both animals and humans. *Salmonella* can be found in humans and their livestock, wild mammals, reptiles, birds, and even insects. However, certain strains like *S. typhi* (the typhoid bacillus), are highly adapted to humans and have no known reservoir outside of humans. Other strains rarely cause disease in humans but are usually restricted to specific hosts other than humans. Most *Salmonella*, however, cause both human and non-human infection.

Salmonellae are hardy and can survive in moist environments or frozen for several months. *Salmonella* disease is the result of ingestion of the bacilli from contaminated water, food, or fomites. Minimum inoculum ~$10^6$ CFU.

B. Host defenses: Normal gastric acidity (pH <3.5) is lethal to *Salmonella*. In healthy individuals, the number of ingested *Salmonella* is reduced in the stomach so that fewer organisms enter the intestine. (Other host protective factors: normal intestinal motility, normal flora, lactoferrin, IgA).

C. Clinical: *Salmonella* is the etiologic agent of a number of clinical syndromes. The factors that determine the severity of infection by salmonellae are size of the inoculum and the state of health of the host. In addition, particular serotypes show a strong propensity to produce a particular syndrome.

1. **Gastroenteritis** usually follows the ingestion of food (meat, poultry, eggs) or water contaminated by feces (human or animal). This accounts for almost 15% of foodborne infection in the US. The episode usually begins 18 to 24 hours after the ingestion with nausea and vomiting, followed by, or concomitant with, abdominal cramps and diarrhea. Diarrhea persists for 3 to 4 days and is usually gone within a week. Infection with non-typhoidal strains of *Salmonella* is relatively mild and self-limiting ("stomach flu"). However, the syndrome varies markedly in its severity, with the most severe cases occurring in infants and in adults over the age of 50. ~2 million cases/yr in US.

**Pathogenesis:** bacteria pass through the stomach, adsorb to and invade epithelial cells lining the terminal small intestine and colon. Bacteria migrate to the lamina propria, multiply, and stimulate an inflammatory response. Inflammation is accomplished by release of prostaglandins, production of cAMP, and active fluid secretion (diarrhea).

2. **Enteric fever (typhoid-like disease)** is caused by *Salmonella typhi*. The source is human, but may be from food or water contaminated by the carrier. The early pathogenesis of this disease is similar to that of other *Salmonella* infections, but the ingested bacilli that cause enteric fever are more invasive than those that cause only gastroenteritis.

**Pathogenesis:** Where *Salmonella* contacts the epithelial surface, there is localized degeneration of the brush border of the epithelial cell. The microbe is internalized by the cytoplasmic membrane so that it resides in a vacuole within the cytoplasm. Salmonellae are transported through the epithelial cells within the vacuoles. The salmonellae eventually enter the lamina propria; the organisms proliferate and destroy the underlying lymphoid and adjacent epithelial cells. During the first 7 to 10 days of infection, individuals are mostly asymptomatic, and the salmonellae disappear from the stool. *Salmonella* can multiply within macrophages and enter the circulation, from there they can infect any tissue of the body. As the bacteria spread from the infected cells of the reticuloendothelial system (RES) into the blood stream, fever and other symptoms of endotoxemia appear. The liver and biliary tree become infected, resulting in reinvasion of the intestinal tract (diarrhea).
If no complications occur, the disease begins to terminate during the 3rd wk. Typhoid fever is a severe disease. It is quite common in developing countries, with high rates in South American and Asia. 20-30 million cases of typhoid fever occur annually in the world, causing about 600,000 deaths. Seldom seen in the US. When untreated, there is an average of 30 days of fever, during which about 20% of patients die. Those who recover, whether or not on chemotherapy, may continue to excrete the organism for long periods of time.

Transmission involves person-to-person spread because S. typhi lacks a significant animal reservoir. Chronic carriers may important reservoirs of infection (e.g. "typhoid Mary").

3. **Asymptomatic carriage:** 1 month after the symptoms - 1/2 of infected persons excrete salmonellae. 5 months later - 1 in 20 persons excrete the organisms.

Source of the organism

D. Virulence factors:

1. **Mechanisms for the invasion** of the gastrointestinal mucosa are still somewhat unclear. Bacilli in the GI tract penetrate the epithelial mucosal cells of the ileum. The invading bacteria replicate intracellularly but cause little mucosal damage or inflammation. They rapidly pass through the epithelial barrier and eventually proliferate in the lamina propria, penetrating the subepithelial tissues. An inflammatory response with infiltration by PMNs is typical.

2. **Intracellular survival and multiplication.** Salmonella survive intracellularly within phagolysosomes (contrast with Shigella and EIEC which both escape the phagosome). Intracellular survival, particularly within macrophages, makes Salmonella a facultative intracellular parasite.

3. **Vi capsular antigen (S. typhi only).** Vi = virulence. Decreases the susceptibility of the bacterium to phagocytosis and diminishes its ability to be killed by C'-mediated bacteriolysis. Target of protective antibodies.

4. **toxins - ill defined.** Some strains of Salmonella produce an LT or ST similar to those of E. coli. However, the toxins are cell-associated and not secreted by the bacterium. Diarrhea associated with salmonellosis is thought to be associated primarily with the inflammatory response, which stimulates local synthesis of prostaglandins and proinflammatory cytokines -- > fever, chills, abdominal pain, diarrhea. Adenyl cyclase is activated which increases the secretion of fluid and electrolytes into the bowel lumen.

During Salmonella-induced gastroenteritis, there may also be cytopathic changes; probably attributable to a cytotoxin associated with the outer membrane fraction of the bacteria. The cytotoxin is distinct from the Shiga-like toxins, but it also inhibits protein synthesis. Its precise structure and role in disease are not understood.

5. **LPS - lipid A can activate macrophages,** resulting in pyrogenicity, leukocytosis, and shock. Many of the symptoms of systemic Salmonella infection are attributable to the toxic moiety of LPS.

6. **Adherence - Salmonella** adhere to the intestinal epithelium in the terminal ileum. Salmonellae synthesize both mannose-sensitive type 1 fimbriae, and some strains produce a mannose-resistant adhesin.

7. **Flagella - nonmotile mutants are unable to invade epithelial cells.** In addition, the nonflagellated mutants have a decreased capacity for survival and growth in macrophages. The H antigen (flagella) also provides a useful epidemiologic tool with which to investigate outbreaks of salmonellosis.
E. Epidemiology. In the developing world, typhoid fever is still a major cause of disease. In the US only about 500 isolates are reported annually. The incidence of non-typhoidal salmonellosis however, has increased markedly in the U.S. This reflects changes in animal husbandry, the mechanization of food processing (e.g. eggs), and the mass distribution of food.

F. Treatment: Patients with bacteremia, meningitis, enteric fever, or other extraintestinal infections require antimicrobial treatment. Drugs of choice: chloramphenicol or ampicillin. Alternatives include amoxicillin and trimethoprim-sulfamethoxazole. For gastroenteritis, antibiotics are contraindicated unless chronic bacteremia is also present. As in any diarrheal disorder, fluid and electrolyte balances should be maintained. Antimicrobial therapy does not reduce the duration and severity of symptoms and may prolong convalescence and intestinal carriage of Salmonella.

G. Prevention and control - Salmonella infections can be controlled or at least reduced by:
1. Proper sanitation especially maintenance of unpolluted water supplies
2. Thorough cooking and subsequent refrigeration of food. The most common animal reservoirs are chickens, turkeys, pigs, and cows. Proper handling and cooking of eggs can minimize the risk of salmonellosis. Thorough cooking kills Salmonella.

H. Vaccines - 3 available in the US (only for typhoid fever - not gastroenteritis):
1. Killed bacteria; subq - effective, but many side effects.
2. Ty21 - attenuated live bacteria; oral - limited replication in vivo.
3. Vi antigen; IM - capsular polysaccharide; >2 yrs; free of side effects.
None used in U.S., but they are employed for military personnel and those traveling to areas where typhoid fever is endemic (Africa, Asia, Central and South America, south East Europe).

IV. Other members of the Enterobacteriaceae - Klebsiella, Enterobacter, Serratia
Habitat: water, soil, food, GI tract. When water sources are examined for fecal coliforms, they are looking for E. coli, not Enterobacter that may be present on vegetation. E. coli is indicative of fecal contamination.
Clinical: Members of this group (commensals of the GI tract) are second to E. coli as causes of gram-negative bacteremia. They have simple growth requirements, and can readily proliferate in contaminated IV solutions containing glucose. These organisms rarely cause disease unless host defenses are compromised. Destruction of the normal intestinal flora by antibiotic therapy may allow resistant nosocomial strains to colonize or overgrow. The skin and mucosa may be breached by disease, trauma, intubation, catheterization, etc. Immunosuppressive therapy also increases the risk of infection.