Anbar University

Science College

Biotechnology Department

Enterobacteriaceae

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Textbook of Diagnostic Microbiology (Mahon, Textbook of Diagnostic Microbiology), Connie R. Mahon MS, Donald C. Lehman EdD MLS(ASCP)cm SM(NRCM), George Manuselis Jr. MA MT(ASCP)

Jawetz Melnick & Adelbergs Medical Microbiology, Stefan Riedel (Author), Stephen Morse (Author), Timothy Mietzner (Author), Steve Miller.

Mims' Medical Microbiology and Immunology, International Edition, Goering.

Enterobacteriaceae

Including more than15 different genera

Escherichia, Shigella, Edwardsiella, Salmonella, Citrobacter, Klebsiella, Enterobacter, Hafnia, Serratia, Proteus, Providencia, Morganella, Yersinia, Erwinia, Pectinobacterium.

Morphology and General Characteristics

Gram-negative, non-spore forming, rod shaped bacteria. Many are normal inhabitants of the intestinal tract of human and other animals. Some are enteric pathogens and others are urinary or respiratory tract pathogens. Differentiation is based on biochemical reactions and differences in antigenic structure. Most grow well on a variety of lab media including a lot of selective and differential media originally developed for the selective isolation of enteric pathogens.

Most of these media is selective by incorporation of dyes and bile salts that inhibit G+ organisms and may suppress the growth of nonpathogenic species of *Enterobacteriaceae.*

Three major classes of antigens are found:

1-Somatic O antigens – these are the heat stable polysaccharide part of the LPS.

2-Flagellar H antigens – are heat labile

3-Envelope or capsule K antigens – overlay the surface O antigen and may block agglutination by O specific antisera.

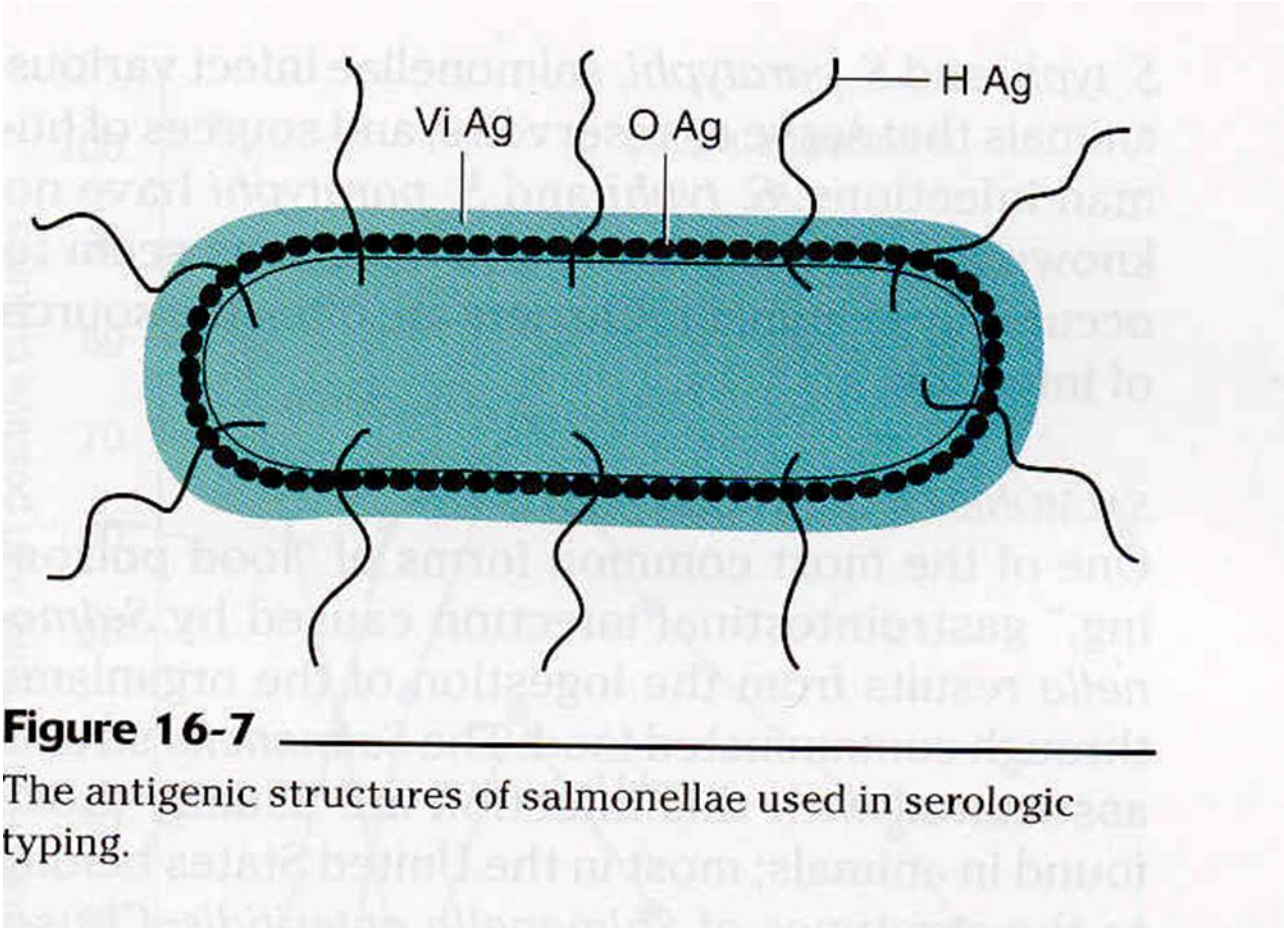

Figure 16-7

The antigenic structures of salmonellae used in serologic typing.

Escherichia coli

Normal inhabitant of the G.I. tract, some strains cause various forms of gastroenteritis. It is a major cause of urinary tract infection and neonatal meningitis and septicemia. It may have a capsule. Most are motile.

Antigenic structure - has O, H, and K antigens. K1 has a strong association with virulence, particularly meningitis in neonates.

Virulence factors

Toxins

Enterotoxins

They are produced by enterotoxigenic strains of *E. coli* (ETEC), causes a movement of water and ions from the tissues to the bowel resulting in watery diarrhea. There are two types of enterotoxin:

LT – is heat labile and binds to specific gangliosides on the epithelial cells of the small intestine where it ADP-ribosylates which stimulates adenylate cyclase to increase production of cAMP.

Increased cAMP alters the activity of sodium and chloride transporters producing an ion imbalance that results in fluid transport into the bowel.

ST – is heat stable and binds to specific receptors to stimulate the production of cGMP with the same results as with LT.

Both enterotoxins are composed of five beta subunits (for binding) and 1 alpha subunit which have the toxic enzymatic activity.

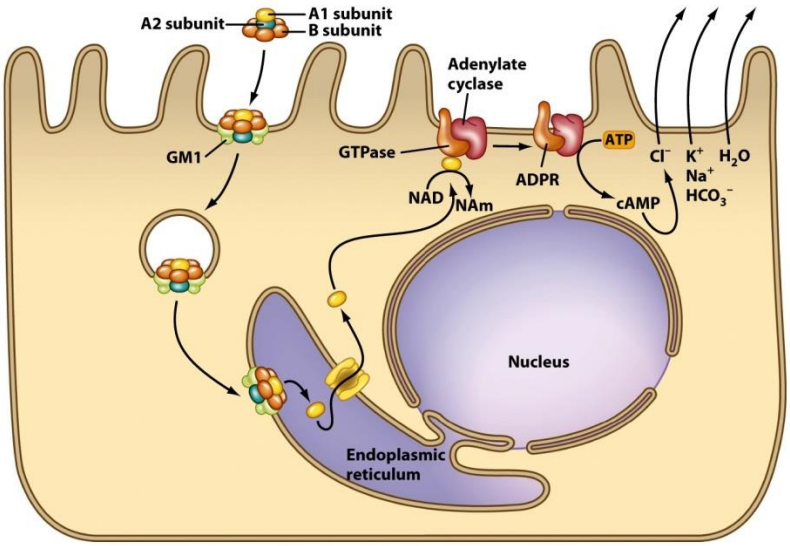

Figure 25.19a Microbiology: An Evolving Science
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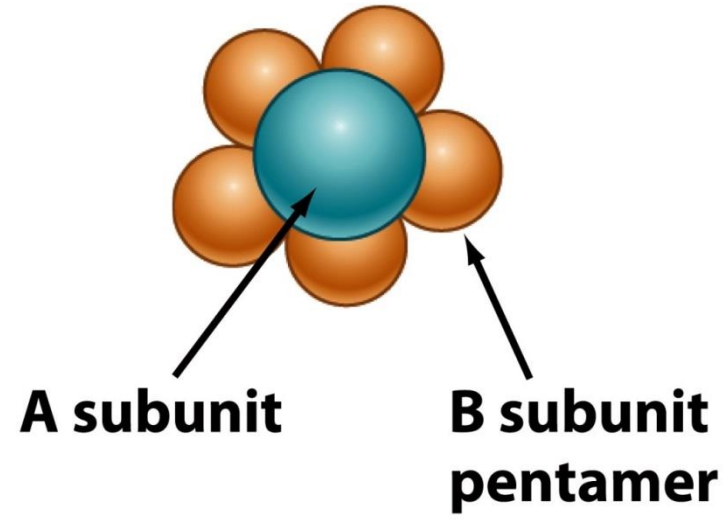

Figure 25.16a Microbiology: An Evolving Science
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Shiga-type toxin – also called the verotoxin -produced by enterohemorrhagic strains of *E. coli* (EHEC) – is cytotoxic, enterotoxic, neurotoxic, and may cause diarrhea and ulceration of the G.I. tract.

There are two types of shiga toxin, shiga-like toxin 1 and shiga-like toxin 2.Inhibit protein synthesis by cleaving a 28S rRNA.

-Enteroaggregative ST-like toxin – produced by enteroaggregative strains of *E. coli* (EAEC) – causes watery diarrhea.

-Hemolysins – two different types may be found: cell bound and secreted. They lyse RBCs and leukocytes and may help to inhibit phagocytosis when cell bound.

-Endotoxin

-Adhesions – are also called colonization factors and include both pili or fimbriae and non-fimbrial factors involved in attachment.

-**Capsule**

-Iron capturing ability

Outer membrane proteins - are involved in helping the organism to invade by helping in attachment (acting as adhesion) and in initiating endocytosis.

Neonatal meningitis – is the leading cause of neonatal meningitis and septicemia with a high mortality rate. Usually it is caused by strains with the K1 capsular antigen.

Gastroenteritis – there are several distinct types of *E. coli* that are involved in different types of gastroenteritis:

enterotoxigenic *E. coli* (ETEC)
enteroinvasive *E. coli* (EIEC)
enteropathogenic *E. coli* (EPEC)
enteroaggregative *E. coli* (EAEC)
enterohemorrhagic *E. coli* (EHEC)

ETEC – is a common cause of traveler's diarrhea and diarrhea in children in developing countries. The organism attaches to the intestinal mucosa via colonization factors and then liberates enterotoxin. The disease is characterized by a watery diarrhea, nausea, abdominal cramps and low-grade fever for 1-5 days. Transmission is via contaminated food or water.

EPEC – Bundle forming pili are involved in attachment to the intestinal mucosa. Diarrhea with large amounts of mucous without blood or pus occurs along with vomiting, malaise and low grade fever. This is a problem mainly in hospitalized infants and in day care centers.

EIEC – The organism attaches to the intestinal mucosa via pili and outer membrane proteins are involved in direct penetration, invasion of the intestinal cells, and destruction of the intestinal mucosa. Symptoms include fever, severe abdominal cramps, malaise, and watery diarrhea followed by scanty stools containing blood, mucous, and pus.

EAEC – Mucous associated autoagglutinins cause aggregation of the bacteria at the cell surface and result in the formation of a mucous biofilm. The organisms attach via pili and liberate a cytotoxin.

Symptoms include watery diarrhea, vomiting, dehydration and occasional abdominal pain.

EHEC – The organism attaches via pili to the intestinal mucosa and liberates the shiga-like toxin. The symptoms start with a watery diarrhea that progress to bloody diarrhea without pus and crampy abdominal pain with no fever or a low-grade fever.

Shigella

It contains four species that differ antigenically and, to a lesser extent, biochemically.

S. dysenteriae (Group A)

S. flexneri (Group B)

S. boydii (Group C)

S. sonnei (Group D) may show delayed lactose fermentation

Antigenic structure

Differentiation into groups (A, B, C, and D) is based on O antigen serotyping; K antigens may interfere with serotyping, but are heat labile.

Virulence factors

Shiga toxin – is produced by *S. dysenteriae* and in smaller amounts by *S. flexneri* and *S. sonnei.* It Acts to inhibit protein synthesis by inactivating the 60S ribosomal subunit by cleaving a glycoside bond in the 28S rRNA constituents. This plays a role in the ulceration of the intestinal mucosa.

Pathogenesis

Shigella destroy the vacuoles to escape into the cytoplasm. From there they spread laterally (Polymerization of actin filaments propels them through the cytoplasm.) to epithelial cells where they multiply but do not usually disseminate beyond the epithelium.

Clinical significance

It causes shigellosis or bacillary dysentery. Transmission is via the fecal-oral route. There is an incubation of 1-7 days followed by fever, cramping, abdominal pain, and watery diarrhea (due to the toxin) for 1-3 days. This may be followed by frequent, scant stools with blood, mucous, and pus (due to invasion of intestinal mucosa). It is rare for the organism to disseminate.

The severity of the disease depends upon the species one is infected with. *S. dysenteria* is the most pathogenic followed by *S. flexneri*, *S. sonnei* and *S. boydii*.

Salmonella

Classification has been changing in the last few years. There is now 1 species: *S. enteritica*, and 7 subspecies: 1, 2 ,3a ,3b ,4 ,5, and 6.

Virulence factors

Endotoxin – may play a role in intracellular survival capsule (for *S. typhi* and some strains of S*. paratyphi*)

Adhesions – both fimbrial and non-fimbrial

Type III secretion systems and effector molecules – 2 different systems may be found:

One type is involved in promoting entry into intestinal epithelial cells. The other type is involved in the ability of *Salmonella* to survive inside macrophages.

Outer membrane proteins - involved in the ability of *Salmonella* to survive inside macrophages

Flagella – help bacteria to move through intestinal mucous

Enterotoxin - may be involved in gastroenteritis

Iron capturing ability

Clinical Significance

It causes two different kinds of disease: enteric fevers and gastroenteritis. Both types of disease begin in the same way, but with the gastroenteritis the bacteria remains restricted to the intestine and with the enteric fevers, the organism spreads. Transmission is via a

fecal-oral route, i.e., via ingestion of contaminated food or water.The organism moves through the intestinal mucosa and adheres to intestinal epithelium.

Salmonella invasion of epithelial cells

The endosome moves to the basal side of the cell and *Salmonella* are released and may be phagocytosed by macrophages. For gastroenteritis the *Salmonella* multiply and their presence induces a strong inflammatory response which causes most of the symptoms seen in gastroenteritis (mild to moderate fever with diarrhea and abdominal cramps).

The inflammatory response prevents the spread beyond the GI tract and eventually kills the bacteria. In enteric fevers (typhoid and paratyphoid) the Salmonella disseminate before they multiply to high enough levels to stimulate a strong inflammatory response so the initial symptoms are only a low-grade fever and constipation.

Pathogenesis

The organism moves through the intestinal mucosa and adheres to intestinal epithelium. The bacteria move via the lymphatics and bloodstream to the liver and spleen where phagocytosis and multiplication occurs. The bacteria re-enter the bloodstream to disseminate throughout the body to all organs causing fever, headaches, myalgia, and GI problems.

Rose spots (erythematous, muculopapular lesions) are seen on the abdomen. Osteomyelitis, cystitis, and gall bladder infections may occur. Symptoms of paratyphoid fevers (due to *S. paratyphi* A,

B, or C) are similar to but less severe than those that occur with typhoid fever (due to *S. typhi*).

Antimicrobial therapy

Enteric fevers – use chloramphenicol usually. Resistant strains have emerged making antimicrobial susceptibility testing essential. **Gastroenteritis** – usually doesn't require antimicrobial therapy. Replace lost fluids and electrolytes.

Citrobacter

Are opportunistic pathogens causing urinary tract or respiratory tract infections and occasionally wound infections, osteomyelitis, endocarditis, and meningitis.

Klebsiella

Klebsiella is a genus of gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. *Klebsiella* species are found everywhere in nature.

Virulence factors

Capsule

Adhesions

Iron capturing ability

Clinical significance

It causes pneumonia, mostly in immunocompromised patients. Permanent lung damage is a frequent occurrence (rare in other types of bacterial pneumonia). A major cause of nosocomial infections such as septicemia and meningitis

Proteus

It is part of the NF of the GI tract, motile, with *Proteus* swarming

Virulence factors

Urease – the ammonia produced may damage the epithelial cells of the UT

Clinical Significance

UT infections, as well as pneumonia, septicemia, and wound infections. These bacteria create alkaline conditions in the urine because of the ability to produce urease enzyme. These bacteria may provoke the formation of calculi (stones) in the urinary tract**.**

Yersinia

Three species are important pathogens in human

Yersinia pestis – causes plague

Yersinis enterocolitica – enteropathogenic

Yersinia pseudotuberculosis – enteropathogenic

Identification

Y. pestis can be separated from *Y. enterocolitica* and *Y. pseudotuberculosis* by the fact that it is non-motile. *Y. enterocolitica* and *Y. pseudotuberculosis* are both non-motile at 37^0 C, and motile at 22^0 C.

Virulence characteristics

Endotoxin – is responsible for many of the symptoms

Fraction 1 – a protein component of the antiphagocytic protein capsule.

V antigen – a secreted protein that controls expression of many of the virulence genes plus it appears to have another unknown function that is essential for virulence

Pla – a protease that activates plasminogen activator (acts as a fibrinolysin) and degrades C3b (prevents formation of complement membrane attack complex) and C5a (prevents attraction of phagocytes)

-**Psa** – a pilus adhesion for attachment

-Iron acquisition and sequestering system

-**YopB** and **YopD** – disrupt actin cytoskeleton in phagocytic cells to evade phagocytosis

***Y. pestis* – clinical significance**

In human plague occurs in two forms; bubonic and pneumonic

Bubonic plague – transmitted by fleas from an infected rodent, the bacteria travel in the blood to the nearest lymph node where they are engulfed by fixed macrophages. A high fever develops and the lymph

nodes in the groin and armpit become enlarged (buboes) as the bacteria proliferate and stimulate an inflammatory response.

The bacteria growing in the lymph node leak into the bloodstream. Lysis of the bacteria releases LPS, causing septic shock. Subcutaneous hemorrhages, probably due to LPS causing disseminated intravascular coagulation gave the disease the name, the Black Death, in the Middle Ages. The untreated mortality rate is quite high.

Eventually bacteria reach the lungs where they are ingested by lung macrophages to cause pneumonic plague.

Pneumonic plague – this can be transmitted directly to others via aerosol. Direct inhalation of aerosols containing the organism produces a form of the disease that progresses much more rapidly and the mortality rate is close to 100%.

Treatment for plague

Streptomycin and tetracycline are effective