V. BACILLUS

The medically important, aerobic and facultatively anaerobic, gram-positive bacilli that form spores are classified in the genus *Bacillus.* Only *Bacillus anthracis* is always considered a pathogen. *Bacillus cereus* can cause disease or be isolated as an insignificant contaminant. Other *Bacillus* species, such as *Bacillus subtilis*, are oportunistic pathogens, causing disease in the presence of a foreign body (e.g., catheter, shunt, prosthesis) when intoduced by surgery or trauma into normally sterile tissues, or in intravenous drug abusers.

V.1 Bacillus anthracis

Physiology and Structure

Bacillus anthracis is a large (1 X 3 to 5 μm) organism that is arranged as single or paired bacilli in clinical specimens and in long serpentine chains and clumps in culture. Although spores are readily observed in 2- or 3-day-old cultures, they are not seen in clinical specimens. A prominent polypeptide capsule (consisting of glutamic acid) is observed in clinical specimens, but it is not produced in vitro unless special growth conditions are used. In addition to the capsular andgen, a polysaccharide cell wall somadc antigen and a toxin are associated with *B. anthracis*. **Anthrax toxin** consists of three antigenically distinct, heat-labile components: protective antigen (PA – mediates binding to epithelium and entry of LF and EF into cells), lethal factor (LF), and edema factor (EF – an adenylate cyclase, cuses increasing levels of cAMP). Although none of the components is active alone, the combination of protective andgen with either ofthe other two components has toxic properties.

Pathogenesis

The two major factors responsible for *B. anthracis* virulence are presence of the **capsule and toxin production**. The capsule is antiphagocytic, and antibodies directed against the capsule are not protective. Only one capsular type has been identified, presumably because the capsule is composed of only glutamic acid. **The anthrax toxin protective antigen produces edema in experimental animals when combined with the edema factor and death when combined with the lethal factor.** This toxin can be detected in edematous fluid collected from patients with anthrax.

Epidemiology

B. anthracis is an organism found in soil and on vegetation. The ability to form spores allows the organism to survive under adverse conditions for years without the need to replicate. Anthrax is a **disease primarily of herbivores**; humans may be accidentally infected by exposure to contaminated animals or animal products. Anthrax is rarely seen in developed counties, and most infections are reported in Iran, Turkey, Pakistan, and Sudan.

Human disease is acquired by one of three routes: **inoculation, inhalation, or ingestion**. Approximately 95% of anthrax infections are due to the inoculation of *Bacillus* spores through exposed skin surfaces, either from contaminated soil or infected animal products such as hides, goat hair, or wool. Inhalation anthrax, also called Woolsorter's disease, results from inhalation of *B. anthracis* spores during the processing of goat hair. Ingestion anthrax is very rare in humans but is a common route of infection in herbivores. Person-to-person transmission does not occur.

Clinical Syndromes

1. Cutaneous anthrax is characterized by the development of a painless papule at the site of inoculation that rapidly progresses to an ulcer surrounded by vesicles and then to a necrotic eschar. Massive edema due to the anthrax toxin and systemic signs can develop. Mortality in patients with untreated cutaneous anthrax is 20%.

2.Inhalation anthrax may initially mimic a viral respiratory illness and then rapidly progress to diffuse pulmonary involvement leading to respiratory failure. Mortality is high even in appropriately treated patients because the disease is usually not suspected until the course is irreversible.

3.Gastrointestinal anthrax is a very rare human disease with varied clinical presentations. Mesenteric adenopathy, hemorrhage, and ascites production are all reported and associated with high mortality.

Lab tests

Gram positive, aerobic, extracellular, nonmotile, "boxcar-shaped" rods. On blood agar growth forming grey-white colonies without hemolysis. Catalase is positive. Polychrome methylene blue is used to stain the peptide capsule.

Treatment, Prevention, and Control

Penicillin is the antibiotic of choice for treating anthrax, with **tetracycline or chloramphenicol used as alternative agents** for penicillin – allergic persons. Control of human disease requires control of animal anthrax. This involves **vaccination of animal herds in endemic regions** and **burning or burial of animals** who died of anthrax. Vaccination is an effective control measure in animal herds. It has also been used to protect humans in areas with endemic disease or humans who work with animal hides, furs, bone meal, wool, and animal hair imported from countries with endemic anthrax. Complete eradication of anthrax is unlikely because the spores of the organism can exist for many years in soil.

V.2 *Bacillus cereus* and other *Bacillus* species

Pathogenesis

Gastroenteritis caused by *Bacillus cereus* is mediated by one of two enterotoxins. The **heat-stable enterotoxin** is responsible for disease characterized by vomiting (emetic form), and the heat-labile enterotoxin causes the diarrheal form of *B. cereus* disease. The **heat-labile entero-toxin** is similar to the enterotoxin produced by *Escherichia coli* and *Vibrio cholera*; each stimulates the adenylate cyclase – cyclic AMP system in intestinal epithelial cells and can be assayed by measuring fluid accumulation in rabbit ileal loops inoculated with the toxin. The mechanism of action of the heat-stable enterotoxin is unknown.

The pathogenesis of *B. cereus* **panophthalmitis** is also incompletely defined. At least three toxins have been implicated: necrotic toxin (a heat-labile enterotoxin), cereolysin (a potent hcmolysin named after the species), and phospholipase C (a potent lecithinase). It is likely that the rapid destruction of the eye that is characteristic of *B. cereus* infections is the result of the interaction of these toxins and other unidentified factors.

Epidemiology

B. cereus, B. subtilis, and other *Bacillus* species are ubiquitous organisms, present in virtually all environmental sites. The isolation of the bacteria from clinical specimens, in the absence of characteristic disease, usually represents insignificant contamination.

Clinical Syndromes

As mentioned previously, *B. cereus* is responsible for **two forms of food poisoning: vomiting disease (emetic form) and diarrheal disease (diarrheal form).** The emetic form is associated with consumption of **contaminated rice**. During the initial cooking of the rice, the vegetative bacilli are killed but the heat-resistant spores survive. If the rice is not refrigerated, the spores germinate and the bacilli can multiply rapidly. The heat-stable enterotoxin that is released is not destroyed when the rice is reheated. After ingestion of the enterotoxin and a 1- to 6-hour incubation period, the patient develops a disease of short duration (less than 24 hrs), characterized by vomiting, nausea, and abdominal cramps. Fever and diarrhea are generally absent. In contrast, the diarrheal form of *B. cereus* food poisoning is associated with **consumption of contaminated meat or vegetables**. There is a longer incubation period during which the organism multiplies in situ and produces a heat-labile toxin, and then the diarrhea, nausea, and abdominal cramps develop. This form of disease generally lasts 1 day or longer.

B. cereus is the most common cause of **traumatic eye infections**. The source of the organisms can be either soil contamination of the object penetrating the eye or direct inoculation of organisms colonizing the eye surface. Bacillus panophthalmitis is a rapidly progressive disease that almost universally ends in complete loss of light perception within 48 hours of the injury. Massive destruction of the vitreal and retinal tissue is observed.

Other infections seen with *B. cereus*, *B. subtilis*, and other *Bacillus* species include **intravenous catheter and central nervous system shunt infections, endocarditis** (most commonly in drug abusers), as well as **pneumonitis, bacteremia, and meningitis** in severely immuno-suppressed patients. Most isolates of *Bacillus* in blood cultures represent insignificant conta-minants from the patient's skin surface or in the blood culturing system.

Lab tests - Not usually done.

Treatment, Prevention, and Control

Because of the short and uncomplicated course of *B. cereus* gastroenteritis, symptomatic treatment is adequate. Treatment of other *Bacillus* infections is complicated by the rapid, progressive course of the infections and the high incidence of multidrug resistance that is observed with these organisms (most isolates are resistant to penicillins and cephalosporins, as well as other antibiotics). **Clindamycin, vancomycin, and the aminoglycosides** can be used, although susceptibility must be confirmed by in vitro testing. Food poisoning can be prevented by proper refrigeration of food products after cooking and before serving.

VI. MISCELLANEOUS GRAM-POSITIVE BACILLI

The aerobic, non-spore-forming, gram-positive bacilli are a heterogeneous group of organisms, many of which are poorly characterized. The most common isolates are collectively called coryneform bacteria or diphtheroids (named after the most prominent member, ***Corynebacterium diphtheriae***). However, despite the fact that most of these organisms are morphologically similar (i.e., small, pleomorphic gram-positive bacilli that stain irregularly), many isolates that were originally classified in the genus *Corynebacterium* are now known to belong to other genera. Three other genera of aerobic gram-positive bacilli are also discussed in this chapter: *Listeria, Erysipelothrix,* and *Gardnerella*. The isolation of *Listeria* and *Erysipelothrix* in clinical specimens is virtually always significant. *Gardnerella* strains, which are commonly isolated in genital specimens, play a poorly defined role in the pathogenesis of bacterial vaginosis (vaginitis).

VI.1 *Corynebacterium*

Physiology and Structure

The corynebacteria are **small, usually pleomorphic, gram-positive bacilli that appear in short chains (V or Y configurations) or in clumps resembling "Chinese letters."** Metachromatic granules within the cells may be seen with special stains. Corynebacteria are **aerobic or facultatively anaerobic and generally grow slowly on enriched media**. The organisms are **nonmotile, catalase-positive**, and ferment carbohydrates, producing lactic acid. Corynebacteria are ubiquitous in plants and animals, and they normally colonize the skin, upper respiratory tract, gastrointestinal tract, and urogenital tract of humans.

Pathogenesis and Immunity

Although most of these organisms are opportunistic pathogens, specific virulence factors have been identified in the more pathogenic species.

Diphtheria Exotoxin

The mechanism of action of diphtheria **exotoxin** is well known. The "tox" gene that codes for the exotoxin is introduced into strains of *Corynebacterium diphtheriae*, *Corynebacterium pseudo-tuberculosis*, and *Corynebacterum ulcerans* by a lysogenic bacteriophage (B-cory-nephage). Diphtheria exotoxin is a 63,000 d protein that consists of two fragments (**A-B toxin**). The B fragment mediates binding to the cell surface, permitting the enzymatically active A fragment to enter the cell. In the presence of limiting amounts of iron, the **A fragment blocks protein synthesis** in a manner similar to *Pseudomonas aeruginosa* exotoxin A. The concentration of diphtheria exotoxin found in strains of *C. ulcerans* and *C. pseudotubercu-losis* that have the "tox" B-corynephage is generally lower than in *C. diphtheriae*.

Phospholipase D

Phospholipase D (also called dermonecrotic toxin) is reduced by strains of *C. pseudo-tuberculosis* and *C. ulcerans*. This toxin promotes the spread of the organisms by increasing vascular permeability through hydrolysis of sphingomyelin in endothelial cell membranes.

Urease Production

The concentration of urease produced by *C. urealyticum* is sufficient to cause alkalinization of urine with subsequent formation of struvite calculi or stones. The association between high

urease production and renal one formation is also seen with some other urinary tract pathogens, such as *Staphylococcus saprophyticus, Klebsiella pneumoniae,* and *Proteus mirabilis*.

Antibiotic Resistance

Although a number of different species of corynebacteria colonize the skin and mucosal surfaces, ***C. jeikeium* is a common cause of hospital-acquired infections in immunocompromised patients.** This is largely due to the selection of these antibiotic-resistant coryneforms during antibiotic therapy. Likewise, *C. urealyticum* is resistant to most antibiotics commonly used to manage urinary tract infections.

Epidemiology

Diphtheria is a disease with worldwide distribution among poor urban areas where crowding exists and a protective level of vaccine-induced immunity is low. *Corynebocterium diphtheriae* is maintained in the population by **asymptomatic carriage in the oropharynx** or on the **skin of immune individuals** (after either exposure to *C. diphtheriae* or immunization). It is **transmitted person to person by respiratory droplets or skin contact**.

Human infection with some strains of corynebacteria (e.g., *C. ulcerans* and *C. pseudotuberculosis*) requires exposure to an animal reservoir (e.g., cattle, sheep, horses, goats, deer). Other corynebacteria are part of the indigenous human oropharyngeal or skin flora (e.g., *C. jeikeium, C. urealyticum, C. pseuedodiphtheriticum, C. xerosis*). With only a few exceptions the incidence of disease caused by these organisms is relatively rare. *C. jeikeium* is a well-recognized opportunistic pathogen in immunocompromised patients, particularly those with hematologic disorders, and patients with intravascular catheters. **Carriage of this organism is uncommon in healthy persons,** but as many as 40% of hospitalized patients can be colonized. Predisposing conditions for disease include prolonged hospitalization, granulocytopenia, prior or concurrent antimicrobial therapy or chemotherapy, and a mucocutaneous portal of entry. The highest incidence of disease is seen in elderly men.

Risk factors associated with *C. urealyticum* infections include immunosuppression, underlying genitourinary disorders, an antecedent urologic procedure, and prior antibiotic therapy. Since as many as one fourth to one third of all hospitalized patients are colonized with *C. urealyticum*, prior antibiotic therapy may select for the organism. A urologic procedure may introduce organisms on the skin surface into previously damaged tissue.

Clinical Syndromes

Diphtheria

The clinical presentation of diphtheria is determined by the site of infection, immune status of the patient, and virulence of the organism. Exposure to *Corynebacterium diphtheriae* can result in asymptomatic colonization in fully immune individuals, **mild respiratory disease in partially immune patients, or a fulminant, sometimes fatal disease in nonimmune patients**. The Centers for Disease Control has defined respiratory diphtheria as an upper respiratory tract illness characterized by sore throat, low-grade fever, and an adherent membrane of the tonsil, pharynx, and/or nose without other apparent cause. Patients with diphtheria involving the respiratory tract will develop symptoms after a 2- to 6-day incubation period. Organisms will multiply locally on epithelial cells in the pharynx or adjacent surfaces and initially produce localized damage by exotoxin activity. The onset is sudden, with malaise, sore throat, exudative pharyngitis, and a low-grade temperature. The exudate evolves into a **thick pseudo-membrane**, composed of bacteria, lymphocytes, plasma cells, fibrin, and dead cells, that can cover the tonsils, uvula, and palate and extend up into the nasopharynx or down into the larynx. The pseudomembrane firmly adheres to the respiratory tissue and is difficult to dislodge without causing bleeding of the underlying tissue.

As the patient recovers after the approximately 1-week course of the disease, the membrane dislodges and is expectorated. Complications in patients with severe disease include breathing obstruction and myocarditis.

Cutaneous diphtheria is acquired by skin contact with other infected persons. The organism colonizes the skin surface and gains entry into the subcutaneous tissue through breaks in the skin (e.g., following an insect bite). A papule will develop and evolve into a chronic non-healing ulcer, sometimes covered with a grayish membrane. Systemic signs of disease due to the exotoxin can be seen.

Other Clinical Syndromes

A variety of diseases have been associated with other species of *Corynebacterium*. The most important include **mild to severe pharyngitis or a diphtheria-like illness** produced by toxigenic *C. ulcerans*, opportunistic infections by *C. jeikeium*, and urinary tract infections by *C. urealyticum*. The development of **catheter-related infections with bacteremia**, as well as **pneumonitis, wound infections, and other diseases by *C. jeikeium***, is particularly troublesome because successful treatment is frequently difficult with these antibiotic-resistant bacteria.

C. jeikeium is a well-recognized opportunistic pathogen in immunocompromised patients, particularly those with hematologic disorders, and patients with intravascular catheters.

Carriage of this organism is uncommon in healthy persons, but as many as 40% of hospitalized patients can be colonized. Predisposing conditions for disease include prolonged hospitalization, granulocytopenia, prior or concurrent antimicrobial therapy or chemotherapy, and a mucocutaneous portal of entry. The highest incidence of disease is seen in elderly men .

Other corynebacteria are part of the indigenous human oropharyngeal or skin flora (e.g., C. jeikeium, C. urealyticum, C. pseuedodiphtheriticum, C. xerosis). The incidence of disease caused by these organisms is relatively rare.

Lab tests

Gram positive rods with metachromatic staining of storage granules. Growth on Loeffler medium, Tellurite medium (dark gray or black colonies).

Treatment, Prevention, and Control

The most important factor for treatment of diphtheria is early use of **diphtheria antitoxin** for the specific neutralization of exotoxin. Antibiotic therapy with **penicillin or erythromycin** has proved effective in eliminating *C. diphtheriae* from patients who have the disease and also from those who are asymptomatic carriers. Bed rest, isolation to prevent secondary spread, and maintenance of an open airway in patients with respiratory diphtheria are all appropriate.

Symptomatic diphtheria can be prevented by **active immunization with diphtheria toxoid during childhood and booster immunizations every 10 years throughout life.** The nontoxic, immunogenic toxoid is prepared by formalin treatment of toxin. Immunization with this preparation, in conjunction with pertussis and tetanus (DPT vaccine), is initially performed in three monthly injections followed by regular booster injections (normally combined with tetanus only). At 2, 6 months: DPT+HBSAg, first Revaccination: 12 months: DPT, second revaccination: 30-35 months: DPT, third revaccination: 7 years : DT, R4: 14 years: DT. Than DT at 10 years interval

Immunity to diphtheria can be determined by measuring for the presence of neutralizing antibodies in an individual's circulatory system (Schick test). Is done by injecting diphtheria toxin intradermally. No skin reaction will be observed if neutralizing antibodies are present, but localized edema with necrosis indicates neutralizing antibodies are absent and the individual is susceptible to diphtheria.

Close contacts of patients with documented diphtheria are at risk for developing disease. Nasopharyngeal cultures should be collected from all close contacts and antimicrobial prophylaxis with either penicillin or erythromycin should be started immediately. Contacts who have not completed the series of diphtheria immunizations or who have not received a booster dose within the previous 5 years should receive a booster dose of toxoid. Exposure to cutaneous diphtheria should be managed in the same manner as respiratory diphtheria. If the respiratory or cutaneous infection is known to be caused by a nontoxigenic strain, then prophylaxis of contacts is unnecessary.

VII. ENTEROBACTERIACEAE

The family *Enterobacteriaceae* is the largest, most heterogeneous collection of medically important bacilli. At present, at least 27 genera and 102 species, as well as eight enteric groups (isolates with undefined genus affiliation), have been described. These genera have been classified on the basis of DNA homology, biochemical properties, serologic reactions, susceptibility to genus – specific and species – specific bacteriophages, and antibiotics susceptibility patterns. Despite the complexity of this family, more than 95% of medically important isolates belong to fewer than 25 species.

Enterobacteriaceae are **ubiquitous organisms** that are found worldwide in soil, water, vegetation and are part of the normal intestinal flora of most animals, including humans. Some members of the family (e.g., *Shigella, Salmonella, Yersinia pestis*) are always associated with disease when isolated from humans, whereas others (e.g., *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*) are members of the normal commensal flora that can cause opportunistic infections. Infections can originate from an animal reservoir (e.g., most *Salmonella* infections), from a human carrier (e.g., *Shigella* and *Salmonella typhi*), or by endogenous spread of organisms in a susceptible patient (e.g., *Escherichia*), involving virtually all body sites. The Enterobacteriaceae are responsible for 30% to 35% of all septicemias, more than 70% of urinary tract infections, and many intestinal infections.

Physiology and Structure

Members of this family are moderate – size (0.3 – 1.0 x 1.0 – 6.0 μm) **gram – negative bacilli, usually motile with peritrichous flagella or nonmotile,** and **do not form spores**. All members grow aerobically and anaerobically (**facultative anaerobes**), with growth observed generally after 18 to 24 hours of incubation on a variety of nonselective media (e.g., blood agar) and selective media (e.g., MacConkey agar). The *Enterobacteriaceae* have **simple nutritional requirements, ferment glucose, reduce nitrate, and are catalase – positive and oxidase – negative.** The absence of cytochrome oxidase activity is an important characteristic because it can be rapidly measured and used to distinguish the Enterobacteriaceae from many other fermentative and nonfermentative gram – negative bacilli.

Morphological characteristics on differential selective media have been used to identify members of the *Enterobacteriaceae.*family. For example, the ability to ferment lactose has been exploited as a differential characteristic for separating **lactose – fermenting strains** (e.g., *Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia*) from **strains do not ferment lactose** (e.g., *Salmonella, Shigella, Yersinia*). The red – colored colonies of lactose – fermenting organisms are readily differentiated on MacConkey agar from the colorless non – lactose fermenting colonies. **Resistance to bile salts** present in some selective media has also been used to separate the enteric pathogens *Shigella* and *Salmonella* from commensal *Enterobacteriaceae* and other organisms present in the gastrointestinal tract. Some members of the family have prominent capsule (e.g., *Klebsiella*), whereas other strains are surrounded by a loose – fitting, diffusible slime layer.

The serologic classification of *Enterobacteriaceae* is based on three major groups of antigens: **somatic O lipopolysaccharides, capsular K antigens, and the flagellar H proteins**. **The heat stable lipopolysaccharide (LPS)** is the major cell wall antigen and consists of three components: the antigenically variable O polysaccharide, a core polysaccharide common to all *Enterobacteraiceae* (common antigen), and lipid A. Endotoxin activity is associated with the lipid A component of LPS. **Specific O antigens** are present in each genus, although cross – reactions between closely related genera are common (e.g., *Salmonella* with *Citrobacter, Escherichia* with

Shigella). The antigens are detected by agglutination with specific antisera. The capsular K antigens are either protein or polysaccharides. The capsular antigen of *Salmonella typhi* is referred to as the Vi antigen. K antigens are shared by different genera both within and outside the *Enterobacteriaceae* family (e.g., *Escherichia coli* K1 cross – reacts with *Neisseria meningitidis* and *Haemophilus influenzae*; *Klebsiella pneumoniae* cross – reacts with *Streptococcus pneumoniae*). The H antigens are heat – labile, flagellar proteins. These can be absent from a cell or undergo antigenic variation and be present in two phases.

The *Enterobacteriaceae* is a large family of gram-negative rods found primarily in the colon of humans and other animals, many as part of the normal flora. These organisms are the major facultative anaerobes in the large intestine but are present in relatively small numbers compared with anaerobes such as *Bacteroides*. Although the members of the *Enterobacteriaceae* are classified together taxonomically, they cause a variety of diseases with different pathogenetic mechanisms.

Members of this heterogeneous family are united both by their anatomic location and by the following four metabolic processes: **(1) they are all facultative anaerobes; (2) they all ferment glucose (fermentation of other sugars varies); (3) none have cytochrome oxidase (ie, they are oxidase-negative); and (4) they reduce nitrates to nitrites as part of their energy-generating processes.**

These four reactions can be used to distinguish the *Enterobacteriaceae* from another medically significant group of organisms, the nonfermenting gram-negative rods, the most important of which is *Pseudomonas aeruginosa*. *P. aeruginosa*, a significant cause of urinary tract infections and sepsis in hospitalized patients, does not ferment glucose or reduce nitrates, and is oxidase-positive. In contrast to the *Enterobacteriaceae*, it is a strict aerobe and derives its energy from oxidation, not fermentation.

PATHOGENESIS AND IMMUNITY

Consistent with the large and diverse composition of the *Enterobacteriaceae* family is the observation of many virulence factors in the pathogenic strains: endotoxin, capsule, antigenic phase variation, exotoxin production, expression of adhesion factors, intracellular survival and multiplication, sequestration of growth factors, resistance to serum killing, antimicrobial resistance.

Antigens The antigens of several members of the Enterobacteriaceae, especially *Salmonella* and *Shigella*, are important; they are used for identification purposes both in the clinical laboratory and in epidemiologic investigations. The three surface antigens are as follows.

(1) The cell wall antigen (also known as the somatic or O antigen) is the outer polysaccharide portion of the lipopolysaccharide (LPS). The O antigen, which is composed of repeating oligosaccharides consisting of three or four sugars repeated 15 or 20 times, is the basis for the serologic typing of many enteric rods. The number of different O antigens is very large; eg, there are approximately 1500 types of *Salmonella* and 150 types of *E. coli*.

(2) The H antigen is on the flagellar protein. Only flagellated organisms, such as *Escherichia* and *Salmonella*, have H antigens, whereas the nonmotile ones, such as *Klebsiella* and *Shigella*, do not. The H antigens of certain *Salmonella* species are unusual because the organisms can reversibly alternate between two types of H antigens called phase 1 and phase 2. The organisms may use this change in antigenicity to evade the immune response.

(3) The capsular or K polysaccharide antigen is particularly prominent in heavily encapsulated organisms such as *Klebsiella*. The K antigen is identified by the quellung (capsular swelling) reaction in the presence of specific antisera and is used to serotype *E. coli* and *Salmonella typhi*

for epidemiologic purposes. In *S. typhi*, the cause of typhoid fever, it is called the Vi (or virulence) antigen.

Endotoxin is a virulence factor shared among all aerobic and some anaerobic gram-negative bacteria. This toxicity resides in the lipid A component of LPS, which is released upon cell death and lysis. Many of the systemic manifestations of gram-negative infections are initiated by endotoxin: fever, leukopenia followed by leukocytosis, activation of complement, thrombocytopenia, disseminated intravascular coagulation, decreased peripheral circulation and perfusion to major organs, shock, and death.

Capsule Encapsulated Enterobacteriaceae are protected from phagocytosis because the hydrophilic capsular antigens repel the hydrophobic phagocytic cell surface. These antigens also obscure cell wall antigens and thus interfere with antibody binding to the bacteria. The capsular antigens are also poor immunogens or activators of complement. However, when specific anticapsular antibodies develop, the protected role of the capsule is diminished.

Antigenic Phase Variation Expression of capsular (K) and flagellar (H) antigens is under genetic control of the organism. Each of these antigens can be alternately expressed or not (phase variation), which can serve to protect bacteria from antibody mediated cell death.

Exotoxin Production A number of important toxins have been identified in the *Enterobacteriaceae*, including **heat-stable and heat-labile enterotoxins**, Shiga and Shiga-like toxins, and hemolysins. The heat-labile enterotoxins, as well as Shiga and Shiga-like toxins, are A-B type toxins (i.e., they consist of an A subunit and one or more B subunits). The A subunit is responsible for the enzymatic, intracellular activity of the toxin, while the B subunit(s) mediates cell binding to facilitate intracellular transfer of the A subunit.

Heat-labile enterotoxin is virtually identical to cholera toxin. This toxin, produced primarily by *Escherichia coli* and occasional isolates of *Klebsiella* and *Salmonella*, catalyzes the ADP ribosylation of the adenylate-cyclase regulatory protein, G_5. This leads to elevated levels of cyclic AMP and subsequent altered electrolyte transport with a resultant secretory diarrhea. The **heat-stable toxin**, present in *Escherichia coli* and occasional *Yersinia enterocolitica* and *Citrobacter freundii*, is a small molecular weight protein extensively cross-linked with disulfide bonds that impart heat stability. This toxin also stimulates a secretory diarrhea by increasing cyclic activation of guanylate cyclase. *Shigella dysenteriae* produces Shiga toxin, which in animal models has been demonstrated to be neurotoxic, enterotoxic, and cytotoxic. The role this toxin plays in human disease, however, is ill defined. The toxin inhibits protein synthesis by the enzymatic inactivation of 60S ribosomes. Related toxins, called Shiga-like toxins or verotoxins, are present in other *Shigella* species and *Escherichia coli*. These toxins have been demonstrated to produce a pronounced cytopathic effect in tissue culture, mouse death, and gastrointestinal toxicity. Hemolysins are also present in many species and can cause cell destruction (e.g., lysis of erythrocytes and leukocytes) and increase the extracellular pool of iron.

Intracellular Survival and Multiplication : Intracellular survival has the obvious benefit of protecting the bacteria from many antibiotics and the patient's immune reaction. *Shigella*, *Salmonella*, enteroinvasive *Escherichia coli*, and *Yersinia* are facultative intracellular parasites (i.e., these organisms can invade and multiply inside cells but do not require intracellular host factors for survival). *Shigella* enters the colonic epithelium through bacterial-directed endocytosis. After internalization, the bacteria rapidly escape from the phagocytic vacuole and initiate replication, leading to the eventual lysis of the host cell and spread to adjoining cells. Polymerization of actin filaments at the late stages of this replicative cycle coordinates the migration of bacteria from one cell to another.

Antimicrobial Resistance As rapidly as new antibiotics are introduced, organisms are able to develop resistance. This process can be dramatically illustrated by examining the speed with which resistant strains of bacteria can develop following exposure to an antibiotic. The spread of this resistance is also a significant problem because resistance can be encoded on transferable plasmids and exchanged among species, genera, and even families of bacteria.

Laboratory Diagnosis

Specimens suspected of containing members of the *Enterobacteriaceae* and related organisms are usually inoculated onto two media, a blood agar plate and a selective differential medium such as MacConkey's agar or eosin-methylene blue (EMB) agar. The differential ability of these latter media is based on lactose fermentation, which is the most important metabolic criterion used in the identification of these organisms. On these media, the non-lactose fermenters, eg. *Salmonella* and *Shigella*, form colorless colonies, whereas the lactose fermenters form colored colonies. The selective effect of the media in suppressing unwanted gram-positive organisms is exerted by bile salts or bacteriostatic dyes in the agar.

An additional set of screening tests, consisting of triple sugar iron (TSI) agar and urea agar, is done prior to the definitive identification procedures. The results of the screening process are frequently sufficient to identify the genus of an organism; however, an array of 20 or more biochemical tests is required to identify the species.

Coliforms & Public Health

Contamination of the public water supply system by sewage is detected by the presence of coliforms in the water. In a general sense, the term "coliform" includes not only *E. coli* but also other inhabitants of the colon such as *Enterobacter* and *Klebsiella*. However, because only *E. coli* is exclusively a large-intestine organism, whereas the others are found in the environment also, it is used as the indicator of fecal contamination. In water quality testing, An *E. coli* colony count above 4/dL in municipal drinking water is indicative of unacceptable fecal contamination. Because *E. coli* and the enteric pathogens are killed by chlorination of the drinking water, there is rarely a problem with meeting this standard. Disinfection of the public water supply is one of the most important advances of public health in this century.

Antibiotic Therapy

The appropriate treatment for infections caused by members of the *Enterobacteriaceae* and related organisms must be individually tailored to the antibiotic sensitivity of the organism. Generally speaking, a wide range of antimicrobial agents are potentially effective, eg, some **penicillins and cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, quinolones, and sulfonamides.** The specific choice usually depends upon the results of antibiotic sensitivity tests.

Note that many isolates of these enteric gram-negative rods are highly antibiotic resistant because of the production of β-lactamases and other drug-modifying enzymes. These organisms undergo conjugation frequently, at which time they acquire plasmids (R factors) that mediate multiple drug resistance.

VII.1 *Salmonella*

Salmonellae are gram-negative rods that **do not ferment lactose but do produce H_2S** – features that are used in their laboratory identification. Their antigens—**cell wall O, flagellar H, and capsular Vi (virulence)—**are important for taxonomic and epidemiologic purposes. The O

antigens, which are the outer polysaccharides of the cell wall, are used to subdivide the salmonellae into **groups A-I.** There are two forms of the **H antigens, phases 1 and 2.**

Kaufman and White assign different species names to each serotype; there are roughly 1500 different species, usually named for the city in which they were isolated. *Salmonella dublin* according to Kaufman and White would be *S. enteritidis* serotype *dublin* according to Ewing. Both forms are used in the literature; the Centers for Disease Control and Prevention use the Ewing system.

Epidemiology

Salmonella are found in virtually all animals, including poultry, reptiles, livestock, rodents, domestic animals, birds, and humans. An animal reservoir is maintained by animal-to-animal spread and the use of *Salmonella* – contaminated animal feeds. Serotypes such as *Salmonella typhi* and *Salmonella paratyphi* are highly adapted to man and do not cause disease in nonhuman hosts. Other *Salmonella* strains are adapted to animals and, when they infect humans, can cause severe human disease (e.g.. *Salmonella choleraesuis*). Finally, many strains have no host specificity and cause disease in both human and nonhuman hosts.

The source of most infections is ingestion of contaminated water or food products or direct fecal-oral spread in children. The peak incidence of disease is in young children infected during the warm months of the year when consumption of contaminated food such as egg salad can occur at outdoor social gatherings. The **most common sources of human infections are poultry, eggs, and dairy products**. Interestingly, the outside surface of eggs, as well as the yolk, can be contaminated with the bacteria. Thus consumption of foods with undercooked or raw eggs substantially increases the risk of infection. Approximately 50,000 cases of *Salmonella* infections are reported annually, although this probably represents only 10% of all human infections. The most common incidence of salmonellosis is in children, particularly those younger than 1 year of age, and infections are most severe in the very young and the elderly.

Salmonella typhi is spread by ingestion of food or water contaminated by **infected food handlers.** Although exposure to *Salmonella* is frequent, a large inoculum (10^{6-8} bacteria) is required for the development of symptomatic disease. Disease occurs when the organism has an opportunity to multiply to a high density, such as in improperly refrigerated contaminated food products. The infectious dose is reduced for individuals at increased risk for disease because of age, immunosuppression or underlying disease (leukemia, lymphoma, sickle cell disease), or reduced gastric acidity.

Pathogenesis

The three types of Salmonella infections (enterocolitis, enteric fevers, and septicemia) have different pathogenetic features.

(1) Enterocolitis is characterized by an invasion of the epithelial and subepithelial tissue of the small and large intestines. Strains that do not invade do not cause disease. The organisms penetrate both through and between the mucosal cells into the lamina propria, with resulting inflammation and diarrhea. A polymorphonuclear leukocyte response limits the infection to the gut and the adjacent mesenteric lymph nodes; bacteremia is infrequent in enterocolitis. In contrast to *Shigella enterocolitis*, in which the infectious dose is very small (on the order of 100 organisms), **the dose of Salmonella required is much higher, at least 100,000 organisms**. Gastric acid is an important host defense; gastrectomy or use of antacids lowers the infectious dose significantly.

(2) In typhoid and other enteric fevers, infection begins in the small intestine but few gastrointestinal symptoms occur. The organisms enter, **multiply in the mononuclear phagocytes of Peyer's patches, and then spread to the phagocytes of the liver, gallbladder, and spleen.**

This leads to **bacteremia**, which is associated with the onset of fever and other symptoms, probably due to endotoxin. Survival and growth of the organism in phagocytic cells are a striking feature of this disease, as is the predilection for invasion of the gallbladder, which can result in establishment of the carrier state and excretion of the bacteria in the feces for long periods.

Asymptomatic Carriage – The *Salmonella* responsible for typhoid and paratyphoid fevers are maintained by human carriage. **Chronic carriage** for more than 1 year after symptomatic disease will develop in **1% to 5% of patients**, with the gall bladder the reservoir in most patients. Chronic carriage with other *Salmonella* occurs in less than 1% of patients and does not represent a significant source of human infection.

(3) Septicemia accounts for only about 5-10% of Salmonella infections and occurs in one of two settings: a patient with an underlying chronic disease such as sickle cell anemia or cancer or a child with enterocolitis. The septic course is more indolent than that with many other gram-negative rods. Bacteremia results in the seeding of many organs, **with osteomyelitis, pneumonia, and meningitis as the most common sequelae**. Osteomyelitis in a child with sickle cell anemia is an important example of this type of salmonella infection. Previously damaged tissues, such as infarcts and aneurysms, especially aortic aneurysms, are the most frequent sites of metastatic abscesses.

Clinical Findings

After an incubation period of 6 – 48 hours, **enterocolitis** begins with nausea and vomiting and then progresses to abdominal pain and diarrhea, which can vary from mild to severe, with or without blood; Usually the disease lasts a few days, is self-limited, causes nonbloody diarrhea, and does not require medical care except in the very young and very old. The most common cause of enterocolitis is *Salmonella typhimurium*, but virtually every species has been implicated.

In typhoid fever, caused by *S. typhi*, and in enteric fever, caused by organisms such as *Salmonella paratyphi* A, B, and C (*S. paratyphi* B and C are also known as *Salmonella schottmuelleri* and *Salmonella hirschfeldii*, respectively), the onset of illness is slow, with fever and constipation rather than vomiting and diarrhea predominating. After the first week, as the bacteremia becomes sustained, **high fever, delirium, tender abdomen, and enlarged spleen occur. "Rose spots,"** ie, rose-colored macules on the abdomen, are associated with typhoid fever but occur only rarely. The disease begins to resolve by the third week, but severe **complications such as intestinal hemorrhage or perforation can occur**. About 3% of typhoid fever patients become chronic carriers. The carrier rate is higher among women, especially those with previous gallbladder disease and gallstones.

Septicemia is most often caused by *S. choleraesuis*. The symptoms begin with fever but little or no enterocolitis and then proceed to focal symptoms associated with the affected organ, frequently bone, lung, or meninges.

Laboratory Diagnosis

In enterocolitis, the organism is most easily isolated from a **stool sample**. However, in the enteric fevers, a blood culture is the procedure most likely to reveal the organism during the first 2 weeks of illness.

Salmonellae form non-lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. On TSI agar, an alkaline slant and an acid butt, frequently with both gas and H_2S (black color in the butt), are produced. *S. typhi* is the major exception; it does not form gas and produces only a small amount of H_2S. If the organism is urease-negative (*Proteus* organisms, which can produce a similar reaction on TSI agar, are urease-positive), the Salmonella isolate can be identified and grouped by the slide agglutination test. Definitive serotyping of the O, H, and Vi antigens is done

by special public health laboratories for epidemiologic purposes. Salmonellosis is a notifiable disease, and an investigation to determine its source should be undertaken. In certain cases of enteric fever and sepsis, when the organism is difficult to recover, the diagnosis can be made serologically by detecting a rise in antibody titer in the patient's serum (Widal test).

Treatment

Enterocolitis caused by *Salmonella* is usually a self-limited disease that resolves without treatment. **Fluid and electrolyte replacement** may be required. Antibiotic treatment does not shorten the illness or reduce the symptoms; in fact, it may prolong excretion of the organisms, increase the frequency of the carrier state, and select mutants resistant to the antibiotic. **Antimicrobial agents are indicated only for neonates or persons with chronic diseases** who are at risk of septicemia and disseminated abscesses. Plasmid-mediated antibiotic resistance is common, and antibiotic sensitivity tests should be done. Drugs that retard intestinal motility (ie, that reduce diarrhea) appear to prolong the duration of symptoms and the fecal excretion of the organisms.

The treatment of choice for enteric fevers such as **typhoid fever**, and septicemia with metastatic infection is either **ceftriaxone or ciprofloxacin**. Ampicillin or ciprofloxacin should be used in patients who are chronic carriers of *S. typhi*. Cholecystectomy may be necessary to abolish the chronic carrier state. Focal abscesses should be drained surgically whenever feasible.

Prevention

Salmonella infections are prevented mainly by public **health and personal hygiene** measures. Proper sewage treatment, a chlorinated water supply that is monitored for contamination by coliform bacteria, cultures of stool samples from food handlers to detect carriers, hand washing prior to -food handling, pasteurization of milk, and proper cooking of poultry, eggs, and meat are all important.

Two vaccines are available, but they confer limited (50-80%) protection against *S. typhi*. One consists of acetone-killed *S. typhi* organisms and is administered intramuscularly. The other vaccine consists of live, attenuated *S. typhi* and is taken orally.

VII.2 *Shigella*

Shigellae are **non-lactose-fermenting, gram-negative rods** that can be distinguished from salmonellae by three criteria: they produce no gas from the fermentation of glucose, they do not produce H_2S, and they are nonmotile. All shigellae have O antigens (polysaccharide) in their cell walls, and these antigens are used to divide the genus into four groups: A, B, C, and D.

Epidemiology

Unlike the genus *Salmonella*, the taxonomic classification of *Shigella*. is quite simple. **Four groups,** consisting of approximately 38 O-antigen-based serotypes, have been described: ***Shigella dysenteriae* (group A), *Shigella flexneri* (group B), *Shigella boydii* (group C), and *Shigella sonnei* (group D).** *Shigella sonnei* is the most common cause of shigellosis in the industrial world, and *Shigella flexneri* is the most common in underdeveloped countries. **Shigellosis is primarily a pediatric disease,** with most infections in children from 6 months to 10 years of age. Endemic disease in adults is frequently due to contact with infected children. Infections in male homosexuals are also observed. Epidemic outbreaks of disease are associated with day-care centers, nurseries, and custodial institutions. Shigellosis is transmitted by the fecal-oral route, primarily by **contaminated hands** and less commonly **in water or food.** Bacilli can

remain viable in contaminated water for as long as 6 months. In contrast to *Salmonella* infections, food-borne disease is uncommon. Because as few as 200 bacilli can establish disease, shigellosis spreads rapidly in communities where sanitary standards and the level of personal hygiene are low.

Pathogenesis

Shigellosis is **only a human disease**. The organism is **transmitted from person to person**, **usually by asymptomatic carriers**. **The four F's—fingers, flies, food, and feces—are the principal factors in transmission**. Food-borne outbreaks outnumber water-borne outbreaks by 2 to 1. Outbreaks occur in **day-care nurseries and in mental hospitals**, where fecal-oral transmission is likely to occur. Children under 10 years of age account for approximately half of *Shigella*-positive stool cultures.

Shigellae, which cause disease **almost exclusively in the gastrointestinal tract**, produce **bloody diarrhea (dysentery)** by invading the mucosa of the distal ileum and colon. **Local inflammation accompanied by ulceration occurs**, but the organisms rarely penetrate the wall or enter the bloodstream, unlike salmonellae. Although some strains produce an enterotoxin, invasion is the critical factor in pathogenesis. The evidence for this is that mutants that fail to produce enterotoxin but are invasive can still cause disease, whereas noninvasive mutants are nonpathogenic.

Clinical Findings

After an incubation **period of 1-4 days, symptoms begin with fever and abdominal cramps**, followed by diarrhea, which may be watery at first but later contains **blood and mucus**. The disease varies from mild to severe depending on two major factors: the species of *Shigella* and the age of the patient, with young children and elderly people being the most severely affected. *Shigella dysenteriae*, which causes the most severe disease, is usually seen in developed countries only in travelers returning from abroad. *Shigella sonnei*, which causes mild disease, is isolated from approximately 75% of all individuals with shigellosis in the United States. The diarrhea frequently resolves in 2 or 3 days; in severe cases, antibiotics can shorten the course. Serum agglutinins appear after recovery but are not protective, because the organism does not enter the blood. The role of intestinal IgA in protection is uncertain.

Laboratory Diagnosis

Shigellae form non-lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. On TSI agar, they cause an alkaline slant and an acid butt, with no gas and no H_2S. Confirmation of the organism as *Shigella* and determination of its group are done by slide agglutination.

One important adjunct to laboratory diagnosis is a methylene blue stain of a fecal sample to determine whether PMNs are present. If they are found, an invasive organism such as *Shigella, Salmonella*, or *Campylobacter* is involved rather than a toxin-producing organism such as *V. cholerae, E. coli,* or *Clostridium perfringens*. (Certain viruses and the parasite *Entamoeba histolytica* can also cause diarrhea without PMNs in the stool.)

Treatment

The main treatment for shigellosis is **fluid and electrolyte replacement**. In mild cases, no antibiotics are indicated. In severe cases, a fluoroquinolone, eg, ciprofloxacin, is the drug of choice, but the incidence of plasmids conveying multiple drug resistance is high enough that antibiotic sensitivity tests must be performed. **Trimethoprim-sulfamethoxazole** is an alternative choice. Antiperistaltic drugs are contraindicated in shigellosis, because they prolong the fever, diarrhea, and excretion of the organism.

Prevention

Prevention of shigellosis is dependent on **interruption of fecal-oral transmission** by proper sewage disposal, chlorination of water, and personal hygiene (hand washing by food handlers). There is no vaccine, and prophylactic antibiotics are not recommended.

VII.3 *Yersinia*

The genus *Yersinia* consists of seven species, of which ***Yersinia pestis, Yersinia pseudotuberculosis,* and *Yersinia enterocolitica*** are the best-known human pathogens. The other species can occasionally cause opportunistic human disease. Because the clinical presentation of *Y. pestis* is distinct, it will be considered separately.

Yersinia pestis

Virulence of *Y. pestis* is multifactorial and includes adaptation to **intracellular survival**, presence of a **protein-polysaccharide capsule** that is antiphagocytic (called fraction 1 antigen), production of an exotoxin (adrenergic antagonist) and **endotoxin** (as with other gram-negative bacteria), ability to absorb organic iron (by a siderophore-independent mechanism), and the presence of coagulase and fibrinolysin. The **ability of the bacteria to cause disseminated infections** is encoded on a 10-kd plasmid, which is believed to be important for the organism's intracellular survival; a 100-kd plasmid encodes for the fraction 1 antigen and exotoxin.

Y. pestis is a small gram – negative rod that exhibits **bipolar staining**; ie, it resembles a safety pin, with a central clear area. Freshly isolated organisms possess a capsule, which can be lost with passage in the laboratory; loss of the capsule is accompanied by loss of virulence. It is one of the most virulent bacteria known.

Epidemiology

One of the most devastating diseases in history was caused by *Yersinia pestis*. During a 5-year period in the middle of the fourteenth century, **epidemic plague (the "Black Death")** claimed 25 million people – almost one fourth of the European population. Epidemics continued through the beginning of the twentieth century, and sporadic infections are still reported primarily from Asia and Africa.

Y. pestis infections are maintained in two epidemiologic forms: **urban plague**, the disease that was so devastating in the Middle Ages, and **sylvatic plague**, the disease that persists today in many countries. Urban plague is maintained in **rat populations** and spread between rats or from rats to humans by infected fleas. **Fleas become infected during a blood meal** from a bacteremic rat. Following replication of the bacteria in the flea gut, the organisms can be transferred to another rodent or accidentally to humans. With effective control of rats and better hygiene, urban plague has been eliminated from most communities. In contrast, sylvatic plague will be difficult or impossible to eliminate because the mammalian reservoirs (prairie dogs, mice, rabbits, rats) and flea vectors are widespread. *Y. pestis* produces a fatal infection in the animal reservoir. Thus cyclic patterns of human disease are observed as the opportunity for contact with the reservoir population increases or decreases. Infections can also be acquired by ingestion of contaminated animals (by rodents, domestic cats or dogs, etc.) or handling contaminated animal tissues. Although the organism is highly infectious, human-to-human spread is uncommon unless the patient has pulmonary involvement.

Clinical Syndromes

Two forms of *Y. pestis* infections have been observed: **bubonic plague and pneumonic plague. *Bubonic plague* is characterized by an incubation period of 7 days or less after a bite from an infected flea.** Patients will have a high fever and a painful bubo (inflammatory swelling of lymph node) in the groin or axilla. In the absence of treatment patients will rapidly progress to bacteremia and as many as 75% will die. This was the form of plague that was so common during the pandemic of the Middle Ages. Patients with the second form of *Y. pestis* infection, ***pneumonic plague*, experience a shorter incubation period** (2 to 3 days), initially have fever and malaise, and then develop pulmonary signs within 1 day. The fatality rate with pneumonic plague is greater than 90% in untreated patients.

Yersinia enterocolitica

Epidemiology

Yersinia enterocolitica is a common **cause of enterocolitis in Scandinavian and other European countries,** as well as in the **colder areas of North America**. Although most studies indicate that infections are more common during the **cold months** of the year, not all investigators have documented this observation. The speculation that *Y. enterocolitica* is clinically more active in cold climates is attractive because this parallels the increased metabolic activity of the organisms at 22° C to 25° C. Virulence with these organisms has also been associated with **specific serotypes: O3 and O9 in Europe**, Africa, Japan, and Canada, and O8 in the United States. *Y. enterocolitica* has been isolated in a variety of sources, including water, milk, and wild and domestic animals. Although an animal reservoir is generally considered to be important, the source of sporadic infections is rarely identified. Epidemic outbreaks have been associated with contaminated meat or milk.

Clinical Syndromes

Approximately two thirds of all *Y. enterocolitica* infections are **enterocolitis**, as the name would imply. The gastroenteritis is characterized by diarrhea, fever, and abdominal pain lasting for as long as 1 to 2 weeks, although a chronic form of the disease can develop and persist for months to more than 1 year. Disease involves the terminal ileum and, with enlargement of the mesenteric lymph nodes, can mimic acute appendicitis. *Yersinia* infections are **most common in children**, **with pseudoappendicitis particularly troublesome in this age-group**. ***Y. pseudotuberculosis* can also produce a disease with this presentation.** Other manifestations reported in adults include **septicemia, arthritis, intraabdominal abscess, hepatitis, and osteomyelitis.**

Laboratory diagnosis

Smear and culture of blood or pus from the bubo is the best diagnostic procedure. Great care must be taken by the physician during aspiration of the pus and by laboratory workers doing the culture not to create an aerosol that might transmit the infection. Giemsa's or Wayson's stain reveals the typical safety-pin appearance better than does Gram's stain. Fluorescent-antibody staining can be used to identify the organisms in tissues. A rise in antibody titer to the envelope antigen can be useful retrospectively.

Treatment

The treatment of choice is a combination of streptomycin and tetracycline, although streptomycin alon can be used. There is no significant antibiotic resistance.

Prevention

Prevention of plague involves **controlling the spread of rats in urban areas**, preventing rats from entering the country by ship or airplane, and avoiding both flea bites and contact with dead wild rodents. A patient with plague must be placed in strict isolation (quarantine) for 72 hours

after antibiotic therapy is started. Only close contacts need receive prophylactic tetracycline, but all contacts should be observed for fever. Reporting a case of plague to the public health authorities is mandatory.

A vaccine consisting of formalin-killed organisms provides a partial protection against bubonic but not pneumonic plague.

VII.4 *Escherichia coli*

E. coli is the most common cause of **urinary tract infections** and gram-negative rod **sepsis.** It is one of the two important causes of **neonatal meningitis** and the agent most frequently associated with **"traveler's diarrhea,"** a watery diarrhea. Some strains of *E. coli* are enterohemorrhagic and cause **bloody diarrhea**.

E. coli is the most abundant **facultative anaerobe in the colon and feces.** It is, however, greatly outnumbered by the obligate anaerobes such as *Bacteroides*.

E. coli **ferments lactose**, a property that distinguishes it from the two major intestinal pathogens, *Shigella* and *Salmonella*. It has three antigens that are used to identify the organism in epidemiologic investigations: the O or cell wall antigen, the H or flagellar antigen, and the K or capsular antigen. Because there are **more than 150 O, 50 H, and 90 K antigens**, the various combinations result in more than 1000 antigenic types of *E. coli*. Specific serotypes are associated with certain diseases; eg, O55 and O111 cause outbreaks of neonatal diarrhea.

Epidemiology

The genus *Escherichia* consists of five species, with *Escherichia coli* the most frequently isolated. Large numbers of *Escherichia coli* are present in the gastrointestinal tract and are the *Enterobacteriaceae* most frequently associated with bacterial sepsis, neonatal meningitis, infections of the urinary tract, and gastroenteritis in travelers to countries with poor hygiene. **Most infections** (with the exception of neonatal meningitis and gastroenteritis) **are endogenous** (i.e., the individual's normal microbial flora is able to establish infection under conditions in which the host defenses are compromised).

The antigenic composition of *Escherichia coli* is complex, with a very large number of O, H, and K antigens described. The serologic classification of *Escherichia coli* isolates is useful for epidemiologic purposes, and specific serotypes are associated with increased virulence.

Pathogenesis

E. coli has several clearly identified components that contribute to its ability to cause disease: pili, a capsule, endotoxin, and two exotoxins (enterotoxins).

A. Intestinal Tract Infection: The first step is the adherence of the organism to the cells of the jejunum and ileum by means of pili that protrude from the bacterial surface. Once attached, the bacteria synthesize enterotoxins (exotoxins that act in the enteric tract), which act on the cells of the jejunum and ileum to cause diarrhea. The toxins are strikingly cell-specific; the cells of the colon are not susceptible, probably because they lack receptors for the toxin. Enterotoxigenic strains of *E. coli* can produce either or both of two enterotoxins.

(1) The high-molecular-weight, heat-labile toxin (LT) acts by stimulating adenylate cyclase. Both LT and cholera toxin act by catalyzing the addition of ADP-ribose to the G protein that stimulates the cyclase. The resultant increase in intracellular cyclic AMP (cAMP) concentration stimulates cAMP-dependent protein kinase, causing an outpouring of fluid, potassium, and chloride from the enterocytes.

(2) The other enterotoxin is a low-molecular-weight, heat-stable toxin (ST), which stimulates guanylate cyclase.

The enterotoxin-producing strains do not cause inflammation, do not invade the intestinal mucosa, and cause a watery, non-bloody diarrhea. However, **certain strains of *E. coli* are enteropathic (entero-invasive)** and cause disease not by enterotoxin formation but by invasion of the epithelium of the large intestine, causing bloody diarrhea (dysentery) accompanied by inflammatory cells (neutrophils) in the stool. **Certain enterohemorrhagic strains of *E. coli*, ie, those with the O157:H7 serotype, also cause bloody diarrhea** but do not cause inflammation; therefore no neutrophils are found in the stool. These O157:H7 strains produce **verotoxin**, so called because it is toxic to Vero (monkey) cells in culture and presumably to the cells lining the colon. These strains are **associated with outbreaks of diarrhea following ingestion of undercooked hamburger at fast-food restaurants**. Some patients with bloody diarrhea caused by O157:H7 strains also have a life-threatening complication called **hemolytic-uremic syndrome**. This syndrome consists of a nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure.

B. Systemic Infection: The other two structural components, the **capsule and the endotoxin**, play a more prominent role in the pathogenesis of systemic, rather than intestinal tract, disease. The capsular polysaccharide interferes with phagocytosis, thereby enhancing the organism's ability to cause infections in various organs. The endotoxin of *E. coli* is the cell wall lipopoly-saccharide, which causes several features of gram-negative sepsis such as **fever, hypotension, and disseminated intravascular coagulation.** Certain O serotypes of *E. coli* preferentially cause **urinary tract infections. These uropathic strains are characterized by pili** with adhesin proteins that bind to specific receptors on the urinary tract epithelium. The binding site on these receptors consists of dimers of galactose (Gal-Gal dimers).

Clinical Syndromes

Septicemia

Escherichia coli is the **most common gram-negative bacillus isolated from septic patients.** The focus of infection from which the organisms spread into the bloodstream is commonly either the urinary tract or the gastrointestinal tract. The mortality associated with *Escherichia coli* septicemia is influenced by the source of infection and the underlying disease of the patient, with a significantly higher incidence of death in immunocompromised patients or with infections originating from intestinal perforation.

Urinary Tract Infections

Escherichia coli is **responsible for more than 80% of all community-acquired urinary tract infections** and **the majority of hospital-acquired infections**. Infecting strains originate from the gastrointestinal tract, with disease associated with specific serotypes, primarily O4, O6, and O75. The ability of these bacteria to resist killing in serum, produce hemolysins, and bind to uroepithelial cells is associated with increased virulence.

Neonatal Meningitis

***Escherichia coli*, together with group B streptococci, is the most common cause of neonatal meningitis**; 75% of these strains possess the K1 capsular antigen. Although colonization of infants with *Escherichia coli* at the time of delivery is common, disease is relatively infrequent.

Gastroenteritis

Strains of *Escherichia coli* that cause gastroenteritis are subdivided into **five groups**: enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggregative

1. **Gastroenteritis produced by enterotoxigenic *Escherichia coli* (ETEC)** is mediated by heat-labile and heat-stable enterotoxins described previously. The production of both toxins is

plasmid mediated, and maximum virulence is associated with specific adhesive pili. Secretory diarrhea caused by ETEC follows a 1- to 2-day incubation period and persists for an average of 3 to 4 days. Symptoms are characteristically **mild, with cramps, nausea, vomiting, and watery diarrhea.** Disease mediated by either toxin is indistinguishable. Toxin production is not associated with specific serotypes, so detection of toxigenic strains requires tissue culture or animal model assays for toxin activity. Nucleic acid probes have also been used to detect the toxin genes.

2. Enteroinvasive *Escherichia coli* (EIEC) are able to invade and destroy the colonic epithelium, producing a disease characterized by **fever and cramps, with blood and leukocytes in stool specimens.** Disease has been associated with specific O serotypes of *Escherichia coli*; however, serologic classification of isolates cannot reliably identify invasive strains.

3.Enteropathogenic *Escherichia coli* (EPEC) are historically important agents of **childhood diarrhea, particularly in impoverished countries**. Although specific O serotypes have been associated with nursery outbreaks of EPEC diarrhea, serotyping *Escherichia coli* isolated in random or endemic disease is discouraged except in epidemiologic investigations. Disease is caused by the ability of the organism to **adhere to the enterocyte plasma membrane** and cause destruction of the adjacent microvilli. Thus these strains have **also been called enteroadherent** *Escherichia coli*. Two adhesion molecules have been characterized: one encoded on the bacterial chromosome and the other plasmid-mediated. Some strains also produce a Shiga-like toxin.

4. Enterohemorrhagic *Escherichia coli* (EHEC) produce a Shiga-like toxin also called verotoxin, which was so named because the toxin causes a cytopathic effect in the Vero cell line of tissue culture cells. Two verotoxins have been described in EHEC: one homologous to *Shigella*, dysenteriae toxin except for a single amino acid substitution in the A subunit; the other toxin with 60% homology to the *Shigella* toxin. The range of disease caused by EHEC varies **from mild uncomplicated diarrhea to hemorrhagic colitis with severe abdominal pain, bloody diarrhea, and little or no fever. Hemolytic uremic syndrome (acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia) is also associated with this organism.** Serologic classification of isolates has limited usefulness; however, approximately half of EHEC strains are serotype **O157:H7.** Disease is **most prevalent in the warm months of the year, with the greatest incidence in children younger than 5 years of age.** Most cases of epidemic and endemic disease have been attributed to **consumption of undercooked ground beef or other beef products, as well as unpasteurized milk.**

5. Enteroaggregative *Escherichia coli* (EAggEC), originally called enteroadherent *Escherichia coli*, have been implicated as a cause of **persistent diarrhea in infants in developing countries.** The bacteria are characterized by their D-mannose resistant aggregative adherence pattern to HEp-2 tissue culture cells. Expression of the aggregative pattern is mediated by a 60 MDa plasmid.

Laboratory Diagnosis

Specimens suspected of containing enteric gram-negative rods such as *E. coli* are grown initially on a blood agar plate and on a differential medium, such as EMB agar or MacConkey's agar. *E. coli*, which ferments lactose, forms pink colonies, whereas lactose-negative organisms are colorless. On EMB agar, *E. coli* colonies have a characteristic green sheen. Some of the important features that help to distinguish *E. coli* from other lactose-fermenting gram-negative rods are as follows: (1) it produces indole from tryptophan, (2) it decarboxylates lysine, (3) it utilizes acetate as its only source of carbon, and (4) it is motile. *E. coli* **0157:H7 does not ferment sorbitol,** which serves as an important criterion that distinguishes it from other strains of *E. coli*. The isolation of

enterotoxigenic or enteropathogenic *E. coli* from patients with diarrhea is not a routine diagnostic procedure.

Treatment

Treatment of *E. coli* infections **depends on the site of disease and the resistance pattern** of the specific isolate. For example, an **uncomplicated lower urinary tract infection** can be treated for just 1-3 days with oral trimethoprim-sulfamethoxazole or an oral penicillin, eg, ampicillin. However, ***E. coli* sepsis** requires treatment with parenteral antibiotics (eg, a third-generation cephalosporin, such as cefotaxime, with or without an aminoglycoside, such as gentamicin). For the treatment of **neonatal meningitis**, a combination of ampicillin and cefotaxime is usually given. Antibiotic therapy is usually not indicated in *E. coli* diarrheal diseases. However, administration of trimethoprim-sulfamethoxazole or loperamide (Imodium) may shorten the duration of symptoms. Rehydration is typically all that is necessary in this self-limited disease.

Prevention

There is no specific prevention for *E. coli* infections, such as **active or passive immunization.** However, various general measures can be taken to prevent certain infections caused by *E. coli* and other organisms. For example, the incidence of urinary tract infections can be lowered by the judicious use **and prompt withdrawal of catheters** and, in recurrent infections, by **prolonged prophylaxis with urinary antiseptic drugs, eg, nitrofurantoin**. Some cases of sepsis can be prevented by **prompt removal of or switching the site of intravenous lines**. **Traveler's diarrhea can sometimes be prevented by the prophylactic use of doxycycline, ciprofloxacin, trimethoprim-sulfamethoxazole,** or Pepto-Bismol. Caution regarding uncooked foods and unpurified water while traveling in certain countries is also advisable.

VII.5. *Klebsiella-Enterobacter-Serratia* group

These organisms are usually **opportunistic pathogens** that **cause nosocomial infections**, **especially pneumonia and urinary tract infections**. *Klebsiella pneumoniae* is an important respiratory tract pathogen outside hospitals as well.

K. pneumoniae, Enterobacter cloacae, and Serratia marcescens are the species most often involved in human infections. They are frequently found in the **large intestine** but are also present in **soil and water**. These organisms have very similar properties and are usually distinguished on the basis of several biochemical reactions and motility. *K. pneumoniae* has a very large capsule, which gives its colonies a striking mucoid appearance. *S. marcescens* produces red-pigmented colonies.

Pathogenesis & Epidemiology

Of the three organisms, *K. pneumoniae* is most likely to be a primary, nonopportunistic pathogen; this property is related to its **antiphagocytic capsule**. Although this organism is a primary pathogen, patients with *K. pneumoniae* infections frequently have **predisposing conditions such as advanced age, chronic respiratory disease, diabetes, or alcoholism**. The organism is carried in the respiratory tracts of about 10% of normal people, who are prone to pneumonia if host defenses are lowered.

***Enterobacter* and *Serratia* infections are clearly related to hospitalization**, especially to **invasive procedures** such as intravenous catheterization, respiratory intubation, and urinary tract manipulations. In addition, outbreaks of *Serratia* pneumonia have been associated with

contamination of the water in respiratory therapy devices. Prior to the extensive use of these procedures, *S. marcescens* was a harmless organism most frequently isolated from environmental sources such as water.

As with many other gram-negative rods, the pathogenesis of septic shock caused by these organisms is related to the endotoxins in their cell walls.

Clinical Findings

Urinary tract infections and pneumonia are the usual clinical entities associated with these three bacteria, but **bacteremia and secondary spread to other areas** such as the meninges occur. It is difficult to distinguish infections caused by these organisms on clinical grounds, with the exception of pneumonia caused by *Klebsiella*, which produces a thick, bloody sputum ("currant-jelly" sputum) and can progress to necrosis and abscess formation.

There are two other species of *Klebsiella* that cause unusual human infections rarely seen in the developed countries. *Klebsiella ozenae* is associated with atrophic rhinitis, and *Klebsiella rhinoscleromatis* causes a destructive granuloma of the nose and pharynx.

Laboratory Diagnosis

Organisms of this group produce lactose-fermenting (colored) colonies on differential agar such as MacConkey's or EMB, although *Serratia*, which is a late lactose fermenter, can give a negative reaction. These organisms are separated by the use of biochemical tests.

Treatment

Because the antibiotic resistance of these organisms can vary greatly, the choice of drug depends on the results of sensitivity testing. Isolates from hospital-acquired infections are frequently resistant to multiple antibiotics. An aminoglycoside, eg, gentamicin, and a cephalosporin, eg, cefotaxime, are used empirically until the results of testing are known. In severe *Enterobacter* infections, a combination of Imipenem and gentamicin is often used.

Prevention

Some hospital-acquired infections caused by gram-negative rods can be prevented by such general measures as changing the site of intravenous catheters, removing urinary catheters when they are no longer needed, and taking proper care of respiratory therapy devices. There is no vaccine.

VII.6 *Proteus –Providencia-Morganella* group

These organisms primarily cause **urinary tract infections, both community- and hospital-acquired.**

Important Properties These gram-negative rods are distinguished from other members of the *Enterobacteriaceae* by their **ability to produce the enzyme phenylalanine deaminase**. In addition, they produce the enzyme **urease**, which cleaves urea to form NH_3 and CO_2 Certain species are **very motile** and produce a striking **"swarming" effect** on blood agar, characterized by expanding rings (waves) of organisms over the surface of the agar.

The cell wall O antigens of certain strains of *Proteus*, such as OX-2, OX-19, and OX-K, cross-react with antigens of several species of rickettsiae. These *Proteus* antigens can be used in laboratory tests to detect the presence of antibodies against certain rickettsiae in patients' serum. This test, called the **Weil-Felix reaction** after its originators, is being used less frequently as more specific procedures are found. In the past, there were four medically important species of *Proteus*. These species have been renamed: ***Proteus morganii* is now *Morganella morganii*, and *Proteus***

***rettgeri* is now *Providencia rettgeri*.** In the clinical laboratory, these organisms are distinguished from *Proteus vulgaris* and *Proteus mirabilis* on the basis of several biochemical tests.

Pathogenesis & Epidemiology

The organisms are **present in the human colon** as well as **in soil and water**. Their tendency to cause urinary tract infections is probably due to their presence in the colon and to **colonization of the urethra, especially in women**. The vigorous motility of *Proteus* organisms may contribute to their ability to **invade the urinary tract**. Production of the enzyme **urease is an important feature of the pathogenesis of urinary tract infections by this group.** Urease **hydrolyzes the urea in urine to form ammonia, which raises the pH and encourages the formation of stones (calculi)** called "struvite" composed of magnesium ammonium hydroxide. Because alkaline urine also favors growth of the organisms and more extensive renal damage, treatment involves keeping the urine at a low pH.

Clinical Findings

The signs and symptoms of urinary tract infections caused by these organisms cannot be distinguished from those caused by *E. coli* or other members of the *Enterobacteriaceae*. *Proteus* species can also cause pneumonia, wound infections, and septicemia. *P. mirabilis* is the species of *Proteus* that causes most community- and hospital-acquired infections, but *Providencia rettgeri* is emerging as an important agent of nosocomial infections.

Laboratory Diagnosis

These organisms usually are highly motile and produce a **"swarming" overgrowth** on blood agar, which can frustrate efforts to recover pure cultures of other organisms. Growth on blood agar containing phenylethyl alcohol inhibits swarming, thus allowing isolated colonies of *Proteus* and other organisms to be obtained. They **produce non-lactose-fermenting (color-less) colonies on MacConkey's** or EMB agar. *P. vulgaris* and ***P. mirabilis* produce H_2S, which blackens the butt of TSI agar**, whereas neither *M. morganii* nor *Providencia rettgeri* does. *P. mirabilis* is indole-negative, whereas the other three species are indole-positive, a distinction that can be used clinically to guide the choice of antibiotics. These four medically important species are urease-positive. Identification of these sorganisms in the clinical laboratory is based on a variety of biochemical reactions.

Treatment

Most strains are sensitive to aminoglycosides and trimethoprim-sulfamethoxazole, but because individual isolates can vary, antibiotic sensitivity tests should be performed. *P. mirabilis* is the species most **frequently sensitive to ampicillin**. The indole-positive species (*P. vulgaris, M. morganii,* and *P. rettgeri*) are more resistant to antibiotics than *P. mirabilis*, which is indole-negative. The treatment of choice for the indole-positive species is a cephalosporin, eg, cefotaxime. *Providencia rettgeri* is frequently resistant to multiple antibiotics.

Prevention There are no specific preventive measures, but many hospital-acquired urinary tract infections can be prevented by **prompt removal of urinary catheters.**

VIII. PSEUDOMONAS

Clinically important **aerobic gram-negative bacilli** can be artificially classified into four general groups: **(1) facultatively anaerobic fermenters (e.g., Enterobacteriaceae), (2) obligately aerobic nonfermenters (e.g., Pseudomonadaceae), (3) *Haemophilus* and related genera, and (4) unusual bacilli.** Of the bacilli isolated in clinical specimens, 68% to 78% are members of the first group, 12% to 16% are in the second group, 8% to 15% are haemophilic bacilli, and fewer than 1% are classified in the unusual bacilli group.

Pseudomonas and related bacilli are a complex mixture of opportunistic pathogens of plants, animals, and humans. Despite the large number of genera, relatively few are isolated with any frequency. ***Pseudomonas aeruginosa, Xanthomonas maltophilia, Acinetobacter baumannii,* and *Moraxella catarrhalis* represent more than 75% of all isolates.**

PSEUDOMONAS

Pseudomonads are **ubiquitous organisms found in soil, decaying organic matter, vegetation, and water.** They are also, unfortunately, found throughout the **hospital environment** in **moist reservoirs such as food, cut flowers, sinks, toilets, floor mops, respiratory therapy equipment, and even disinfectant solutions. Persistent carriage as part of the normal microbial flora in humans is uncommon** (**less than 6% carriage rate** in healthy individuals), **unless the individual is hospitalized** (38% carriage rate) or is an ambulatory, **immunocompromised host** (78% carriage rate). The broad environmental distribution of *Pseudomonas* is afforded by their **simple growth requirements**. *Pseudomonas* also possess a number of **structural factors and toxins that enhance the virulence potential** of the organism, as well as render them **resistant to most commonly used antibiotics.** Indeed, it is surprising that these organisms are not more common pathogens, with their ubiquitous presence, **ability to grow in virtually any environment, virulence properties, and broad-based antimicrobial resistance.** Instead, *Pseudomonas* **infections are primarily opportunistic** (i.e., restricted to patients with compromised host defenses).

Physiology and Structure

Pseudomonads are straight or **slightly curved gram-negative bacilli** (0.5-1.0 x 1.5-5.0 μm) and **motile by means of polar flagella**. The organisms are **nonfermentative** and use relatively few carbohydrates (e.g., glucose, ribose, gluconate) **by oxidative metabolism**. Oxygen is the terminal electron acceptor, and the **presence of cytochrome oxidase** in *Pseudomonas* is used to differentiate this group from the Enterobacteriaceae. Although these organisms are defined as **obligate aerobes**, anaerobic growth can occur with nitrate used as an alternate electron acceptor. **Some strains appear mucoid** because of the abundance of a polysaccharide capsule; these strains are particularly **common in cystic fibrosis patients**. Some pseudomonads produce **diffusible pigments** (e.g., pyocyanin [blue], fluorescein [yellow], or pyorubin [red-brown]). The genus consists of a number of different species subdivided by biochemical and genetic differences. *Pseudomonas aeruginosa* is the most common clinically significant pseudomonad and the best-characterized member of the genus.

Pathogenesis

Pseudomonads have a number of virulence factors, including structural components, toxins, and enzymes. Defining the role each factor plays in disease caused by these organisms is difficult, and most experts in this field believe ***Pseudomonas* virulence is multifactorial.**

Pili or Fimbriae

These hairlike structures mediate adherence of the bacterium to the respiratory epithelium.

Polysaccharide Capsule

The surface of *P. aeruginosa* is covered with a polysaccharide layer that protects the organism from phagocytosis. This layer can also anchor the bacteria to cell surfaces, particularly in patients with cystic fibrosis or other chronic respiratory diseases who are predisposed to colonization with mucoid strains of *P. aeruginosa*.

Endotoxin

As is true with other gram-negative bacilli, pseudo-monads possess a lipopolysaccharide endotoxin as a major cell wall antigen. The lipid A component of endotoxin mediates the various biological effects of the sepsis syndrome.

Exotoxin A

One of the most important virulence factors produced by pathogenic strains of *P. aeruginosa* is exotoxin A. This toxin blocks eukaryotic cell protein synthesis in a manner similar to that described for diphtheria toxin. However, these toxins are structurally and immunologically different, and exotoxin A is less potent than diphtheria toxin.

Exoenzyme S

This extracellular toxin is produced by one third of the clinical isolates of *P. aeruginosa* and can inhibit protein synthesis. Both exotoxin A and exoenzyme S are ADP-ribosyltransferases, but they are distinguished by the heat stability of exoenzyme S.

Elastase

This enzyme can catalyze the destruction of the elastic fiber in blood vessel walls, resulting in hemorrhagic lesions (ecthyma gangrenosum) associated with disseminated *P. aeruginosa* infections.

Other Proteases

Other proteases have been described in pseudomonads that mediate tissue destruction, inactivation of antibodies, and inhibition of neutrophils.

Phospholipase C

Phospholipase C breaks down lipids and lecithin, facilitating tissue destruction. The exact role of this enzyme in infections of the respiratory and urinary tracts is unclear, although a significant association exists between hemolysin production and disease at these sites.

Epidemiology

Pseudomonads are **opportunistic pathogens present in a variety of environmental habitats.** The ability to isolate these organisms from **moist surfaces** may be limited only by one's interest in searching for the organism. Pseudomonads have **minimal nutritional** requirements, can tolerate a wide **range of temperatures (**4° C to 42° C), and are **resistant to many antibiotics and disinfectants**. Indeed, the simple recovery of *Pseudomonas* from an environmental source (e.g., hospital sink or floor) means very little without epidemiological evidence that the contaminated site is a reservoir for infection. Furthermore, **isolation of *Pseudomonas* from a hospitalized patient is worrisome** but does not normally justify therapeutic intervention without evidence of disease. It is important to note that recovery of *Pseudomonas*, particularly species other than *P. aeruginosa*, from a clinical specimen may represent contamination of the specimen during

collection or laboratory processing. Because these organisms are opportunistic pathogens, the significance of an isolate must be measured by assessing the clinical presentation of the patient.

Clinical Syndromes

Pseudomonas aeruginosa

Bacteremia and endocarditis.

Bacteremia caused by *P. aeruginosa* is clinically indistinguishable from other gram-negative infections, although the mortality rate is higher. This is due in part to the predilection of this organism for **immunocompromised patients** and in part to the inherent virulence of *Pseudmnonas*. *P. aeruginosa* bacteremia is particularly common in patients with **neutropenia, diabetes mellitus, extensive burns, and hematological malignancies**. Most *Pseudmnonas* bacteremias originate **from infections of the lower respiratory tract, urinary tract, and skin and soft tissue** (particularly burn wound infections). Although seen in a minority of patients, characteristic **skin lesions** (**ecthyma gangrenosum**) may develop. The lesions are seen initially as erythematous vesicles that progress to hemorrhage, necrosis, and ulceration. Microscopic examination of the lesion shows abundant organisms with vascular destruction (which explains the hemorrhagic nature of the lesions) and the absence of neutrophils as would be expected in neutropenic patients.

Pseudomonas **endocarditis** is most commonly observed in intravenous drug abusers; the source of infection is drug paraphernalia contaminated with the water-borne organisms. The tricuspid valve is often involved and is associated with a chronic course and a more favorable prognosis compared with infections of the aortic or mitral valves.

***Pulmonary infections*.**

P. aeruginosa infections of the **lower respiratory tract** can range from colonization or benign **tracheobronchitis to severe necrotizing broncho-pneumonia**. Colonization is seen in patients with **cystic fibrosis, other chronic lung diseases, and neutropenia**. *Pseudomonas* pulmonary infection in patients with cystic fibrosis has been associated with exacerbation of the underlying disease, as well as invasive disease in pulmonary parenchyma. Neutropenic and other immunocompromised patients are frequently exposed to *Pseudomonas* following use of **contaminated respiratory therapy equipment**. Invasive disease in this population is characterized by a diffuse, typically bilateral bronchopneumonia with microabscess formation and tissue necrosis. Bacteremia, with an associated high mortality rate, can be observed in severe infections.

***Ear infections*.**

External otitis is most frequently due to *P. aeruginosa*, with swimming (swimmer's ear) a significant risk factor. Although this localized infection can be managed with topical antibiotics and drying agents, a more virulent form of disease (malignant external otitis) can invade the underlying tissues and be life threatening. Aggressive antimicrobial and surgical intervention is required for this latter disease. *P. aeruginosa* is also associated with chronic otitis media.

Burn infections.

P. aeruginosa colonization of a burn wound, **followed by localized vascular damage and tissue necrosis,** and ultimately bacteremia, is not uncommon in patients who have sustained severe burns. The moist surface of the burn and absence of neutrophilic response to tissue invasion predispose patients to *Pseudomonas* infections. Use of topical creams and wound management has controlled *Pseudomonas* colonization with only limited success.

Other infections.

P. aeruginosa is associated with a variety of other infections, including those localized in the gastrointestinal and **urinary tracts, eye, central nervous system, and musculoskeletal system.** The underlying conditions required for most *Pseudomonas* infections are the presence of the organism in a moist reservoir and the circumvention or absence of host defenses (e.g., cutaneous trauma, elimination of normal microbial flora by injudicious use of antibiotics, neutropenia). *Pseudomonas* urinary tract infections are observed in patients with indwelling urinary catheters.

Other Pseudomonads

The *Pseudomonas* species and related organisms (*P. fluorescens* group – *P. aeruginosa, P. fluorescens, P. putida; P. stutzeri* group – *P. stutzeri, P. medocina; P. solanacearum* group – *P. pseudomallei, P. mallei, P. cepacia; Comamonas acidovorans* group; etc.) are all capable of opportunistic infections in immunocompromised patients. The majority of true infections with these organisms have been localized to **the respiratory tract in patients with underlying pulmonary disease** or to the **urinary tract following instrumentation or catheterization**. *P. cepacia* is a particularly common respiratory pathogen in cystic fibrosis patients.

The clinical significance of an isolate is often difficult to assess because specific signs and symptoms of disease may be absent in patients and the organism may be an insignificant waterborne contaminant.

Laboratory Diagnosis

P. aeruginosa grows as **non-lactose-fermenting** (colorless) colonies on MacConkey's or EMB agar. It is oxidase-positive. A typical **metallic sheen** of the growth on TSI agar, coupled with **the blue-green pigment on ordinary nutrient agar** and a **fruity aroma**, is sufficient to make a presumptive diagnosis. The diagnosis is confirmed by biochemical reactions. Identification for epidemiologic purposes is done by bacteriophage or pyocin typing.

Treatment, Prevention, and Control

Antimicrobial therapy for *Pseudomonas* infections is frustrating because the infected patient with compromised host defenses is unable to augment the antibiotic activity, and pseudomonads are **typically resistant to most antibiotics**. Even in susceptible organisms resistance can develop during therapy by the induction of antibiotic inactivating enzymes (e.g., β - lactamases) or the transfer of plasmid-mediated resistance from a resistant organism to a susceptible one. Successful therapy for serious infections generally requires the **combined use of ami-noglycoside and β - lactam antibiotics**, which have documented activity against the isolate. ***P. cepacia*** differs from *P. aeruginosa* and most other *Pseudomonas* species in that the isolates are **generally susceptible to sulfonamide antibiotics**. Augmentation of compromised immune function with hyperimmune globulin and granulocyte transfusions may have beneficial effects with selected patients who have *Pseudomonas* infections.

Attempts to eliminate *Pseudomonas* from the hospital environment are practically useless given the ubiquitous presence of the organism in water supplies. **Effective infection-control practices should concentrate on prevention of contamination of sterile equipment such as respiratory therapy machines, and cross-contamination of patients by medical personnel. The inappropriate use of broad-spectrum antibiotics should be avoided**, because this practice can suppress the normal microbial flora and permit the overgrowth of resistant pseudo-monads.

Mechanisms of Antibiotic Resistance in *Pseudomonas*

Antibiotic	Resistance mechanisms

Penicillins and cephalosporins	β - lactamase hydrolysis, altered binding proteins, decreased permeability
Aminoglycosides	Enzymatic hydrolysis by acetylation, adenylation; decreased permeability; altered ribosomal target
Chloramphenicol	Enzymatic hydrolysis by acetyl – transferase; decreased permeability
Fluoroquinolones	Altered target (DNA gyrase); decreased permeability

IX. VIBRIONACEAE

Members of the *Vibrionaceae* family are **curved or straight bacilli, capable of aerobic or anaerobic growth, oxidase positive, and non – spore-formers.** They are primarily found in water and are well known for their ability to produce **gastrointestinal disease**. The family includes three genera associated with human disease: *Vibrio, Aeromonas*, and *Plesiomonas*.

VIBRIO

The genus *Vibrio* is composed of **gram-negative, curved bacilli** that differ from *Enterobacteriaceae* by their **positive oxidase reaction**, polar flagella, and growth on alkaline media but not acidic media. Species pathogenic for humans are: *V. cholerae, V. paraheamolyticus, V. vulfinicus, V. alginolyticus,* and others.

Physiology and Structure

Vibrio species **can grow aerobically or anaerobically** on a variety of simple media, with a broad temperature range (from 18° C to 37° C) for optimal growth. *Vibrio cholerae*, the best-known member of the genus, can be serologically **subdivided into six groups based on somatic O antigens**. **Most pathogens belong to the O1 group**. **Toxigenic *V. cholerae* non-O1 isolates can also cause human disease,** although they are not associated with epidemics. ***V. cholerae* O1 can be subdivided into two biotypes (el tor and cholerae),** as well as **two serologic subgroups** (ogawa, inaba). Strains with both the ogawa and inaba antigens have been termed hikajima. These groups are important for the epidemiologic classification of isolates. *V. parahaemolyticus* can also be subdivided by differences in the somatic O antigens.

Pathogenesis

The mechanism by which *V. cholerae* causes cholera is well established. The cholera enterotoxin produced by the organism is a complex molecule **(A-B toxin**). The enterotoxin can **bind to specific receptors in the small intestine, enter into the mucosal cells, and effect a series of reactions that result in the rapid secretion of sodium, potassium, and bicarbonate into the intestinal lumen**. Severely infected patients can **lose as much as 1 liter of fluid per hour during the active phase of the disease.** The tremendous loss of fluid would normally flush the organism out of the gastrointestinal tract; however, *V. cholerae* is able to penetrate through the mucus covering the surface of the intestine and adhere to the mucosal cell layer. Nonadherent strains are unable to establish infection. Similarly, toxin-negative strains of *V. cholerae* O1 are avirulent.

Epidemiology

V. cholerae is found in freshwater ponds and estuaries in Asia, the Middle East, Africa, parts of Europe, and along the coastal areas of South, Central, and North America. Although the major reservoir is believed to be **human carriage**, some evidence indicates that infected **crustaceans may also be a significant source of infection**. Disease is **spread by contaminated water and food, most commonly during the warm months**. Person-to-person spread is unusual because a high inoculum (e.g., 10^8 to 10^{10} organisms) is required to establish infection in an individual with normal gastric acidity. Achlorhydria or hypochlorhydria can reduce the infectious dose to 10^3 to 10^5 organisms. Cholera is usually seen in **communities with poor sanitation**. Once the reservoir for this organism is established, elimination is particularly difficult. For that reason sporadic disease has occurred for centuries, and seven major pandemics have been observed since 1817. The current pandemic began in Asia in 1961 with *V. cholerae* O1 (biotype el tor, serotype

inaba) and spread to Africa, Europe, and Oceania in the 1970s and 1980s. Endemic disease with an unrelated strain of *V. cholerae* O1 has been reported sporadically in the Gulf of Mexico since 1973. In 1991 the pandemic strain spread to Peru and subsequently has involved most countries in South and Central America, as well as the United States and Canada. By the end of 1992 more than 600,000 cases had been reported in the Americas. Although cases are reported throughout the year, they are most prevalent during the warm months.

In contrast with *V. cholerae*, ***V. parahaemolyticus, V. vulnificus,*** **and** ***V. alginolyticus*** are halophilic marine vibrios that require salt for growth. These species are **free-living vibrios that inhabit estuaries and coastal waters worldwide**. Because they are also rapidly killed by gastric acids, the infectious dose of all vibrios is generally high. Gastroenteritis with *V. parahaemo-lyticus* and septicemia caused by *V. vulnificus* commonly follow ingestion of raw or impro-perly handled seafood such as oysters. *V. parahaemolyticus* is the major cause of diarrheal disease in Japan, where consumption of raw fish is common. Wound infections caused by *Vibrio* species are usually associated with exposure to seawater or laceration with a seashell.

Clinical Syndromes

Vibrio cholerae

Infection with *V. cholerae* **can range from asymptomatic colonization or mild diarrheal disease to severe, potentially life-threatening diarrhea and vomiting** (2% to 5% of all infections). Severe cholera will occur initially an average of **2 to 3 days after ingestion** of the bacilli, with the abrupt onset of vomiting and severe watery diarrhea. The stool specimens are colorless and odorless, free of protein, and speckled with mucus flecks (**rice-water stools**). The **severe fluid and electrolyte loss can lead to dehydration, metabolic acidosis and vomiting** (bicarbonate loss), and hypokalemia and **hypovolemic shock** (potassium loss) with cardiac arrhythmia and renal failure. The mortality is 60% in untreated patients but less than 1% in patients promptly treated to **replace lost fluids and electrolytes.** Cholera will **spontaneously resolve after a few days of symptoms**. Disease caused by *V. cholerae* biotype cholerae is more severe than with biotype el tor. *V. cholerae* non-O1 causes a gastroinrestinal disease similar to *V. cholerae* O1, although it is generally less severe and has not been associated with epidemic disease.

Vibrio parahaemolyticus

Gastroenteritis caused by *V. parahaemolyticus* can range from self-limiting diarrhea to a cholera-like illness. In general, the disease will present after a 5-hour to 92-hour incubation period (mean 24 hours) with an explosive, watery diarrhea. **No gross blood or mucus is found in stool specimens** except in very severe cases. Headache, abdominal cramps, nausea, vomiting, and low-grade fever may persist for 72 hours or more. Recovery is usually uneventful.

Vibrio vulnificus

V. vulnificus is a particularly virulent *Vibrio* species responsible for rapidly progressive wound infections after exposure to contaminated seawater and septicemia after consumption of raw oysters. **The wound infections are characterized by initial swelling, erythema, and pain,** followed by the development of vesicles or bullae and eventual tissue necrosis. Systemic signs of fever and chills are usually seen. Mortality caused by *V. vulnificus* septicemia can be as high as 50% unless antimicrobial therapy is initiated rapidly. Infections are most severe for patients with hepatic disease, hematopoietic disease, chronic renal failure, or those receiving immunosuppressive drugs.

Treatment, Prevention, and Control

Cholera must be promptly **treated with fluid and electrolyte replacement before massive fluid loss results in hypovolemic shock**. Antibiotic therapy, although of secondary value,

can reduce exotoxin production and more rapidly eliminate the organism. **Tetracycline is the drug of choice, but vibrios are also usually susceptible to erythromycin, chloramphenicol, trimetho-prim-sulfamethoxazole, and the fluoroquinolones**. *V. parahaemolyticus* gastroenteritis is usually a self-limited disease, although antibiotic therapy can be used to supplement fluid and electrolyte therapy in severe infections. *V. vulnificus* wound infections and septicemia must be promptly treated with antibiotic therapy. Tetracycline is the most effective drug in vivo, although some success has been reported with aminoglycosides.

Because vibrios are free-living in freshwater and marine reservoirs and human carriage of *V. cholerae* can range from 1% to 20% in previously infected patients, it is unlikely that the reservoir for this organism will be eradicated. Disease can be controlled effectively only by improved hygiene. This involves adequate sewage management and water purification systems to eliminate contamination of the water supply and appropriate steps to prevent contamination of food.

Although a killed cholera vaccine is available, the protection is short-lived and useful only for individuals who will be in an endemic area for less than 6 months. Currently the vaccine is not recommended for individuals traveling to areas with endemic disease. Tetracycline prophylaxis has also been used to reduce the risk of infection in endemic areas but has not prevented spread of cholera. Because the infectious dose of *V. cholerae* is high, antibiotic prophylaxis is generally unnecessary if appropriate hygiene is used.

X. CAMPYLOBACTER AND HELICOBACTER

The classification of bacteria in these genera has undergone a number of changes since they were first isolated at the beginning of this century. More recently discovered species (e.g., *Campylobacter curvus*, *C. rectus*) were originally classified as *Wolinella* species, and the helicobacters were first placed in the *Campylobacter* genus. The *Campylobacter* and *Helicobacter* species most commonly involved with human infections are discussed further.

X. 1 *Campylobacter*

The genus *Campylobacter*, from the Greek word *campylo* for curved, consists of **comma-shaped, gram-negative bacilli that are oxidase-positive and catalase-positive, motile by means of a polar flagella, and require a microaerophilic atmosphere for growth**. Eleven species and seven subspecies or biovars are now recognized.

Campylobacter jejuni is the most common cause of **bacterial gastroenteritis** in the United States. ***Campylobacter coli*** is responsible for 2% to 5% of campylobacter gastroenteritis, although it is reported to be more common in underdeveloped countries. ***C. lari* and *C. upsaliensis*** have also been associated with diarrheal disease in humans. These species are primarily restricted to the gastrointestinal tract, with bacteremia observed in less than 1% of the infections. In contrast with other species, *Campylobacter fetus* ssp. *fetus* is most commonly responsible for systemic infections such as bacteremia, septic thrombophlebitis, arthritis, septic abortion, and meningitis.

Physiology and Structure

Campylobacter has a **typical gram-negative cell wall structure**. The major antigen of the genus is the lipopolysaccharide of the outer membrane. Serologic heterogeneity of *C. jejuni* isolates is common, with more than **90 different somatic O polysaccharide antigens recognized and 50 capsular and flagellar antigens.**

Recognition of the role of campylobacters in gastrointestinal disease was delayed because the organisms **grow best in an atmosphere of reduced oxygen** (5% to 7%) and **increased carbon dioxide** (5% to 10%). In addition, ***C. jejuni* grows better at 42° C vs. 37° C**. These growth properties have now been exploited in the selective isolation of pathogenic campylobacters in stool specimens. The small size of the organisms (0.3 to 0.6 μm in diameter) has also been used to recover the bacteria by **filtering stool specimens** (campylobacters pass through 0.45 μm filters, whereas other bacteria are retained).

Pathogenesis and Immunity

The major factors associated with the development of disease are the **infectious dose of organisms and the level of specific immunity**. Patients exposed to a large number of organisms, or who lack gastric acids, are more likely to develop disease. Individuals in a population of high endemic disease develop measurable levels of specific serum and secretory antibodies and have less severe disease. Patients with hypogammaglobulinemia have prolonged, severe disease with *C. jejuni*.

The pathogenesis of *C. jejuni* gastrointestinal disease is not completely understood. Disease is characterized by **destruction of the mucosal surfaces of the jejunum** (as implied by the name), ileum, and colon. On gross examination, the **mucosal surface appears edematous and bloody**. Histologic examination reveals **ulceration of the mucosal surface, crypt abscesses** in the epithelial glands, and infiltration into the lamina propria, with neutrophils, mononuclear cells, and eosinophils. This inflammatory process is consistent with invasion of the organisms into the intestinal tissue. **Enterotoxins, cytopathic toxins, and endotoxic activity have been detected in *C. jejuni* isolates**.

***C. fetus* has a propensity to spread from the gastrointestinal tract to the bloodstream** and distal foci. This is particularly common in debilitated and immunocompromised patients such as those with liver disease, diabetes mellitus, chronic alcoholism, or a malignancy. In vitro studies have demonstrated that *C. fetus* is resistant to complement-mediated and antibody-mediated serum killing, whereas *C. jejuni* is rapidly killed. *C. fetus* is covered with a protein (S protein) that prevents complement-mediated killing in serum (inhibits C3b binding to the bacteria).

Epidemiology

Campylobacters are **commensals of cattle, sheep, dogs, cats, rodents, and fowl**. Lifelong **asymptomatic carriage** in animals, following an initial symptomatic phase, represents an important reservoir for human disease. Human infections result from **consumption of contaminated food, milk, or water. Contaminated poultry** is responsible for more than half of the campylobacter infections in developed countries. Food products that neutralize gastric acids (e.g., milk) effectively reduce the infectious dose. Fecal-oral transmission from person to person may also occur, but transmission from food handlers is uncommon.

The actual incidence of campylobacter infections is unknown because disease is not systematically reported to public health officials. However, it has been estimated that more than 2 million *C. jejuni* infections occur annually and such infections are more common than *Salmonella* and *Shigella* infections combined. **Disease is most common in the warm months** but does occur throughout the year. The peak incidence of disease is in young adults. In underdeveloped countries, symptomatic disease occurs in young children, and persistent carriage is observed in adults.

C. fetus infections are relatively uncommon, with fewer than 250 cases reported. In contrast with *C. jejuni*, *C. fetus* infects immunocompromised, elderly individuals.

Clinical Syndromes

C. jejuni infections are seen most commonly as **acute enteritis with diarrhea, malaise, fever, and abdominal pain**. Ten **or more bowel movements per day can occur during the peak of disease**, and grossly **bloody stools** may be present. The disease is generally self-limiting, although symptoms may last for 1 week or longer. The range of clinical manifestations can include colitis, acute abdominal pain, and bacteremia. ***C. fetus* infection is most commonly seen as septicemia** with dissemination to multiple organs, although the initial presentation may be referable to the gastrointestinal tract or abdomen. Endovascular localization is reported.

Treatment, Prevention, and Control

Campylobacters are **susceptible to a wide variety of antibiotics, including erythromycin, tetra-cyclines, aminoglycosides, chloramphenicol, and clindamycin**. Most isolates are **resistant to penicillins, cephalosporins, and sulfonamide antibiotics**. Erythromycin is the antibiotic of choice and is used to treat enteritis when indicated; an aminoglycoside is generally used for systemic infections.

Campylobacter gastroenteritis is **prevented by the proper preparation of food, particularly poultry, consumption of pasteurized milk**, and safeguards to prevent contamination of water supplies. Elimination of campylobacter carriage in animal reservoirs is unlikely.

X.2 *Helicobacter*

In 1982 spiral-shaped bacilli, resembling campylobacters, were observed associated with type B gastritis. The organisms were originally classified as *Campylobacter* but were subsequently reclassified as a new genus, *Helicobacter*. ***Helicobacter pylori* is the species associated with gastritis,** and more recently it has been implicated in **gastric and duodenal ulcers, as well as gastric cancer.** Other bacteria that have now been classified in the genus *Helicobacter* include *H. cinaedi* and *H. fennelliae* (isolated from homosexual men with proctitis, proctocolitis, or enteritis) and *H. mustelae* (isolated from ferrets).

Physiology and Structure

Members of the genus *Helicobacter* are characterized by sequence analysis of 16S rRNA, their cellular fatty acids, **presence of one or more polar, sheathed flagella**, and selected biochemical tests (e.g., positive oxidase and catalase reactions, negative hippurate hydrolysis). *H. pylori* is highly motile (corkscrew motility) and produces an **abundance of urease**.

Pathogenesis and Immunity

A number of factors have been identified as potential virulence factors in *H. pylori* disease. The most important factors are believed to be **urease production** (produces a cloud of ammonia that protects the organism from gastric acids), **motility and mucinase activity** (allows the organism to pass through the mucous layer rapidly), and **adherence factors** (anchors the bacteria at the intracellular junction of enteric cells). The gastric tissue associated with *H. pylori* infection is invariably inflamed with **infiltration of mononuclear cells into the lamina propria.** Antibody response to *H. pylori* infection is common; however, the organism is able to evade elimination in its protected location in the gastric mucosa.

Epidemiology

Serologic studies in the United States have documented that the incidence of *H. pylori* infection in healthy individuals is relatively low during childhood **but increases to approximately 50% in older adults**. Infection appears earlier in individuals in a low socioeconomic class and in developing nations. *H. pylori* is identified in 70% to 100% of patients with **gastritis, gastric ulcers, and duodenal ulcers** but is infrequently isolated from patients without histologic evidence of gastritis. No animal reservoir has been identified and infection via food or water has not been demonstrated. Although the mechanism of transmission is not known, family **clustering has been recognized. Humans are most likely the main reservoir for infection, which is probably spread person to person.**

Clinical Syndromes

The clinical evidence is now overwhelming that *H. pylori* is the etiologic agent for virtually all cases of type B gastritis. Evidence includes **virtually 100% association between gastritis and**

infection with the bacterium, experimental infection in both animals and humans, and histological resolution of pathology when specific therapy is used to eradicate the organism. Strong evidence also implicates *H. pylori* in gastric and duodenal ulcers, where elimination of the organism leads to healing of the ulcers and significantly reduced recurrences. Since gastritis precedes the development of gastric adenocarcinomas, extensive epidemiological and experimental studies are underway to determine the role of *H. pylori* in the pathogenesis of this malignancy.

Treatment, Prevention, and Control

Antibiotics alone are generally ineffective in eradicating *H. pylori*. However, when **antibiotics are combined with bismuth**, successful elimination of the organism has been reported. Current therapy includes the use of **bismuth salt with nitroimidazole and either amoxicillin or tetracycline.** If the organism is found to be resistant to nitroimidazole, then is used **clarytromicin**. Prevention and control of *H. pylori* disease are difficult because the organism is ubiquitous.