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.

20.1 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.

20.2 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.

20.3 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.

20.4 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.

20.5. 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.

20.6 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.**

21. 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.

Tabel 6. 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

22. 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.

23. 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.

23. 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.

23.2 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.

24. INTRODUCTION TO ANAEROBIC BACTERIA

Anaerobes are characterised by their ability to grow only in an atmosphere containing **less than 20% oxygen**: i.e., they grow poorly if at all in room air. They are a heterogenous group composed of bacteria that can barely grow in 20% oxygen to those that can grow only in less than 0.02% oxygen.

The obligate anaerobes such as *Bacteroides fragilis* and *Clostridium perfringens* require an almost total absence of oxygen. Many anaerobes use nitrogen as terminal electron acceptor rather than oxygen.

The precise reason why the growth of anaerobes is inhibited by oxygen is not understood, but several factors are probably involved. One important aspect is the **production of toxic compounds, such as H_2O_2 and superoxides, and the reduced amount (or absence) of catalase and superoxide dismutase in anaerobes to detoxify them.**

In addition to oxygen concentration, the oxidation-reduction potential (E_h) of a tissue is an important determinant of the growth of anaerobes. **Areas with low E_h such as the periodontal pocket, dental plaque, and colon, support the growth of anaerobes well. Crushing injuries that result in devitalized tissue owing to impaired blood supply produce a low E_h, allowing anaerobes to grow and cause disease.**

Table 7. The anaerobes of medical interest

Morphology	**Gram Stain**	**Genus**
Spore-forming rods	+	Clostridium
	-	None
Non-spore-forming rods	+	*Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus, Propionibacterium*
	-	Bacteroides, Fusobacterium
Non-spore-forming cocci	+	*Peptococcus, Peptostreptococcus, Streptococcus*
	-	Veillonella

Many of the medically important anaerobes are **part of the normal human flora.** As such, they are nonpathogens in their normal habitat and cause disease only when they leave those sites. **The two prominent exceptions to this are *Clostridium botulinum* and *Clostridium tetani,*** the agents of botulism and tetanus, respectively, which are soil organisms. *C. perfringens*, another important human pathogen, is found in the colon and in the soil.

Diseases caused by members of the anaerobic normal flora are characterised by abscesses, which are most frequently located in the brain, lungs, female genital tract, biliary tract, and other intraabdominal sites. Most abscesses contain more than one organism, either multiple anaerobes or a mixture of anaerobes plus facultative anaerobes. It is thought that the facultative anaerobes consume sufficient oxygen to allow the anaerobes to flourish.

Three important findings on physical examination that arouse suspicion of an anaerobic infection are a **foul-smelling discharge**, **gas in tissue, and necrotic tissue**. In addition, infections in the setting of **pulmonary aspiration, bowel surgery, abortion, cancer, or human and animal bites frequently involve anaerobes.**

Two aspects of microbiologic diagnosis of an anaerobic infection are important even before the specimen is cultured:

1. **obtaining the appropriate specimen**
2. **rapidly transporting the specimen under anaerobic conditions to the laboratory.**

An appropriate specimen is one **that does not contain members of the normal flora to confuse the interpretation**. **For example, specimens such blood, pleural fluid, pus, and transtracheal aspirates are appropriate, but sputum and faeces are not.**

In the laboratory, the cultures are handled and incubated under anaerobic conditions. In addition to the usual diagnostic criteria of Gram's stain, morphology, and biochemical reactions, the special technique of gas chromatography is important. In this procedure, organic acids such as formic, acetic, and propionic acid are measured.

In general, surgical drainage of the abscess plus administration of antimicrobial drugs are indicated. **Drugs that are commonly used to treat anaerobic infections are penicillin G, cefoxitin, chloramphenicol, clindamycin, and metronidazole.** Note, however, that many isolates of the important *B. fragilis* produce β-lactamase and so are resistant to penicillin.

24.1 Anaerobic Gram-Positive, Spore-Forming Bacilli

All medically important, anaerobic, gram-positive, spore-forming bacilli are classified in the genus ***Clostridium***. The organisms are ubiquitous, **present in soil, water, sewage, and as part of the normal microbial flora in the gastrointestinal tract of animals and humans**. **Most clostridia are harmless saprophytes**, **although some are well-recognized human pathogens with a clearly documented history of causing diseases such as tetanus (*C. tetani*), botulism (*C. botulinum*), and gas gangrene (*C. perfringens*, *C. novyi*, *C. septicum*, and others).** Clostridia are more commonly **associated with skin and soft tissue infections, food poisoning, and antibiotic-associated diarrhea and colitis**. Their remarkable capacity for causing diseases is attributed to their **ability to survive adverse environmental conditions by spore formation**, rapid rate of growth in a nutritionally enriched, oxygen-deprived environment, and production of numerous histolytic toxins, enterotoxins, and neurotoxins.

CLOSTRIDIUM PERFRINGENS

Physiology and Structure

Clostridium perfringens, the clostridial species most frequently isolated in clinical specimens, either can be **associated with simple colonization or can cause severe, life-threatening disease.** *C. perfringens* is a **large, rectangular, gram-positive bacillus**, with **spores rarely observed** in vivo or following in vitro cultivation. The organism is one of the few **nonmotile clostridia, but rapidly spreading growth on laboratory media** (resembling growth of motile organisms) is characteristic. The organism grows rapidly both in tissues and in culture, is **hemolytic, and is metabolically active**, which facilitates the rapid identification of these organisms in the laboratory. Production of **four major lethal toxins** for *C. perfringens* (alpha, beta, epsilon, and iota toxins) is used to **subdivide isolates into five types (A through E).** Type A *C. perfringens*, the toxin type responsible for most human infections, is further subdivided into many epidemiological serotypes.

Pathogenesis

Clostridium perfringens can cause a spectrum of diseases, **from self-limited gastroenteritis to overwhelming destruction of tissue** (e.g., clostridial myonecrosis) with very

high mortality despite early appropriate medical intervention. This pathogenic potential is attributed to the **numerous toxins and enzymes** produced by this organism. **Alpha toxin**, the most important toxin that is produced by all *C. perfringens*, is a **lecithinase** (phospholipase C) that lyses erythrocytes, platelets, leukocytes, and endothelial cells. Increased vascular permeability with massive hemolysis and bleeding, tissue destruction (as found in **myonecrosis**), **hepatic toxicity, and myocardial dysfunction** (bradycardia, hypotension) are associated with this toxin. **The largest quantities of alpha toxin are produced by *C. perfringens* type A. Beta toxin is responsible for the necrotic lesions in necrotizing enterocolitis** (enteritis necroticans).

Epidemiology

C. perfringens is a **common inhabitant of the intestinal tract of humans and animals and is widely distributed in nature**, particularly in soil and water contaminated with feces. These organisms form spores under adverse environmental conditions and can survive for prolonged periods. Gas gangrene and food poisoning are primarily caused by *C. perfringens* type A, whereas enteritis necroticans is caused by type C.

Clinical Syndromes

Bacteremia

The isolation of *C. perfringens* or other clostridial species in blood cultures can be alarming. However, **more than half of the isolates are clinically insignificant**, representing transient bacteremia or, more likely, contamination of the culture with clostridia colonizing the skin surface. The significance of an isolate must be viewed in the light of other clinical findings.

Myonecrosis (Gas Gangrene)

This life-threatening disease illustrates the full virulence potential of histotoxic clostridia. The onset of disease, characterized by **intense pain, generally begins within 1 week after clostridia are introduced into tissue by trauma or surgery**.

Cellulitis, Fasciitis, and Other Soft Tissue Infections

Clostridial species can **colonize wounds and the skin surface with no clinical consequences**. Indeed, most isolates of *C. perfiingens* and other clostridial species from wound cultures are insignificant. However, these organisms can also initiate **cellulitis or a rapidly progressive, destructive process whereby the organisms spread through fascial planes (fasciitis),** causing suppuration and gas formation.

Food Poisoning

Clostridial food poisoning is characterized by a **short incubation period** (8 to 24 hours); clinical presentation **of abdominal cramps and watery diarrhea** in the absence of fever, nausea, or vomiting; and a clinical course of less than 24 hours. Disease is due to **ingestion of meat products contaminated with large numbers (10^{8-9} organisms) of enterotoxin-producing type A *C. perfringens*.** Clostridia are the third most common cause of food poisoning in the United States (behind *Salmonella* and *S. aureus*).

Enteritis Necroticans

This rare disease is an acute necrotizing process in the small intestine that is characterized by abdominal pain, bloody diarrhea, shock, and peritonitis. The incidence of death approaches 50%. Beta toxin-producing *C.perfringens* type C is responsible for this disease.

Laboratory diagnosis

1. Morphology

a. Vegetative cells. This strongly **gram-positive rod** is the only nonmotile species in the genus. Classically, it has blunt or **square ends.**

(1) Microscopic appearance of individual cells varies **from long rods to coccobacillary** forms depending upon available nutrients and other growth conditions.

(2) Capsules are formed in tissue.

b. Spores. Large, ovoid, centrally located endospores often give the vegetative cell a swollen appearance. **Spores usually are found in soil and intestinal specimens** but only rarely are identified in laboratory cultures or cooked foods.

c. Colonial morphology. Colonies can vary from round to irregular in shape but usually are low, convex, shiny, and semiopaque.

(1) Hemolysis also is variable and may be complete or partially complete.

(2) The bacterium is not a strict anaerobe; it **can tolerate exposure to air for short periods of time.**

2. Antigenic composition. **Five different types of *C. perfringens*, designated A through E**, are based on serologically distinct exotoxins.

3. Metabolic activity. *C. perfringens* is active biochemically, fermenting glucose, maltose, lactose, and sucrose. It produces hydrogen sulfide gas and the proteolytic enzyme, gelatinase. Nitrate reduction, lack of motility, sporulation, lactose fermentation, and lecithinase production differentiate *C. perfringens* from other clostridia.

Treatment, Prevention, and Control

Treatment of systemic *C. perfringens* infections such as fasciitis and myonecrosis requires **aggressive surgical debridement and high-dose penicillin therapy**. The use of antiserum directed against alpha toxin (antitoxin) and treatment in a hyperbaric oxygen chamber (presumably to inhibit growth of the anaerobe) have poorly denned benefits. Despite all therapeutic efforts, the prognosis with these diseases is poor, with mortality reported from 40% to almost 100%. Less serious, localized clostridial diseases can be successfully treated with penicillin, with resistance only rarely reported for species other than *C. perfringens*. Antibiotic therapy for clostridial food poisoning is unnecessary.

Prevention and control of *C. perfringens* infections is difficult because of the ubiquitous distribution of the organisms. Disease requires introduction of the organism into devitalized tissues and maintenance of an anaerobic environment favorable for bacterial growth. Thus most infections can be prevented by proper wound care and judicious use of prophylactic antibiotics.

CLOSTRIDIUM TETANI

Physiology and Structure

Clostridium tetani is a **small, motile, spore-forming bacillus** that frequently stains **gram-negative**. **Round, terminal spores** are produced that give the organisms a **drumstick appearance.** In contrast with *C. perfringens*, *C. tetani* is **difficult to grow in vitro** (because it is sensitive to oxygen toxicity) and is relatively inactive metabolically.

Pathogenesis

Tetanus is caused by a potent, **heat-labile, neurotoxin (tetanospasmin)** that is produced during the stationary phase of growth and released when cell lysis occurs. Tetanospasmin (an A-

B toxin) is synthesized as a single 150,000 d peptide that is cleaved into a light (A equivalent) and a heavy (B equivalent) chain. The two chains are held together by a disulfide bond and noncovalent forces. The carboxy-terminal portion of the heavy (100,000 d) chain binds to gangliosides (GT_1) on neuronal membranes. The light chain of the toxin is then internalized and moves from the peripheral nerve terminals to the central nervous system by retrograde axonal transport. It is released from the postsynaptic dendrites, crosses the synaptic cleft, and is localized within vesicles in the presynaptic nerve terminals. **Tetanospasmin acts by blocking the release of neurotransmitters** (e.g., gamma-aminobutyric acid [GABA], glycine) for inhibitory synapses, thus permitting unregulated excitatory synaptic activity (spastic paralysis) .

C. tetani also produces an oxygen-labile hemolysin (tetanolysin) that is serologically related to some other clostridial hemolysins and streptolysin O. The clinical significance of this enzyme is unknown because it is inhibited by oxygen and serum cholesterol.

Epidemiology

Clostridium tetani is **ubiquitous, found in fertile soil and colonizes the gastrointestinal tract of many animals, including humans.** The vegetative forms of *C. tetani* are **extremely susceptible to oxygen toxicity**, but the organisms sporulate readily and can **survive in nature for prolonged periods**. Disease is **relatively rare in the developed countries because of the high incidence of immunity following vaccination**. However, tetanus is still responsible for **significant mortality in underdeveloped areas** where vaccination is unavailable or medical practices are lax**. Tetanus in neonates and the unprotected elderly is associated with a high mortality rate.** Virtually all patients with tetanus are inadequately immunized. Drug abusers who inject drugs subcutaneously ("skin poppers") are susceptible to tetanus.

Clinical Syndromes

The incubation period for tetanus is variable, ranging from a few days to weeks. The length of the incubation period is directly related to the distance of the primary wound infection from the central nervous system.

Generalized tetanus is the most common form seen. Involvement of the **masseter muscles (trismus or lockjaw) is the presenting sign in the majority of patients**. **The sardonic smile characteristic of sustained trismus is known as "risus sardonicus".** Other early signs include drooling, sweating, irritability, and persistent **back spasms (opisthotonos).** More severe disease is seen with involvement of the autonomic nervous system, with cardiac arrhythmias, fluctuations in blood pressure, profound sweating, and dehydration.

Another form of *C. tetani* disease is **localized tetanus** in which the disease remains confined to the musculature at the site of primary infection. A variant of localized tetanus is **cephalic tetanus** in which the primary site of infection is the head. In contrast with localized tetanus, the prognosis for patients with cephalic tetanus is very poor.

Laboratory diagnosis

1. Morphology. This slender, **gram-positive rod characteristically is motile and a strict anaerobe.** The spores are spherical and terminal, giving the cells a drumstick or tennis-racket appearance; immature spores are oval. Cultured cells form fine filaments of growth, which are easily overlooked. Nonmotile mutants do not swarm, and they form discrete colonies. Complete or partial hemolysis is seen on horse blood agar.

2. Clinical samples are placed on **blood agar** or in cooked meat broth and incubated under anaerobic conditions **for 4 or 5 days**. inoculum is placed near the edge of the blood agar plate to allow swarming of the *C. tetani*. Pure cultures are obtained by subculturing the leading edge of

growth. Nonmotile mutants can be isolated by adding neomycin sulfate to the medium or by carefully heating the specimen to kill non-spore-forming bacteria. Identification is confirmed by biochemical tests.

Treatment, Prevention, and Control

The highest incidence of mortality is in newborns and in patients who experience an incubation period of less than 1 week before the onset of disease.

Treatment of tetanus requires **debridement of the primary wound (**which may appear innocuous), **administration of penicillin, passive immunization with human tetanus immunoglobulin, and vaccination with tetanus toxoid**. Wound care and penicillin therapy eliminate vegetative bacteria that produce toxin, while the antitoxin antibodies bind free tetanospasmin molecules. The toxin bound to nerve endings is protected from antibodies. Thus the toxic effects must be controlled symptomatically until normal regulation of synaptic transmission is restored. Vaccination with a series of three doses of tetanus toxoid and booster doses every 10 years is highly effective in preventing tetanus.

CLOSTRIDIUM BOTULINUM

Microbial Physiology and Structure

Clostridium botulinum, the etiological agent of botulism, is a heterogeneous group of fastidious**, spore-forming, anaerobic bacilli.** Like tetanus toxin, *Clostridium botulinum* toxin is a 150,000 d progenitor protein (A-B toxin) consisting of the neurotoxin subunit (light or A chain) and one or more nontoxic subunits (B or heavy chain). The nontoxic subunit(s) protects the neurotoxin from inactivation by stomach acids.

Pathogenesis

Botulinum is very specific for cholinergic nerves**. The toxin blocks neurotransmission at peripheral cholinergic synapses by preventing release of the neurotransmitter acetylcholine**. Recovery of function requires regeneration of the nerve endings. *C. botulinum* also produces a binary toxin consisting of two components that combine to disrupt vascular permeability.

Epidemiology

Three forms of botulism have been identified: **classical or food-borne, infant, and wound botulism**. Although *C. botulinum* is distributed worldwide, with spores commonly isolated in soil and water samples, disease is relatively uncommon in the developed countries, with most associated with home-canned foods and occasionally preserved fish (type E toxin). The food may or may not appear spoiled, but even a small taste can cause full-blown clinical disease. Botulism in infants is more common and has been associated with the consumption of honey contaminated with botulinal spores.

Clinical Syndromes

Food-Borne Botulism

After consumption of contaminated food and a 1- to 2-day incubation period, the patient develops weakness and dizziness. The initial signs of botulism include **blurred vision** with fixed **dilated pupils, dry mouth** (indicative of the anticholinergic effects of the toxin), **constipation**, and **abdominal pain**. Bilateral, descending weakness of the peripheral muscles develops in progressive disease (flaccid paralysis), with death most commonly attributed to respiratory

paralysis. **Complete recovery** in patients who survive this initial period frequently requires **many months to years** until the affected nerve endings regrow. Mortality, which once approached 70%, has been reduced to 10% with the use of better supportive care, particularly in the management of respiratory complications.

Infant Botulism

Although this disease was first recognized in 1976, it is now the most common form of botulism seen in the United States. In contrast with food-borne botulism, this disease is caused by the in vivo production of neurotoxin by *C. botulinum* colonizing the gastrointestinal tract of young infants. Disease is reported in infants younger than 1 year of age (most between 1 to 6 months) with the initial symptoms nonspecific (e.g., constipation or "failure to thrive"). Progressive disease with flaccid paralysis and respiratory arrest can develop, although the mortality rate in documented infant botulism is very low (1% to 2%). Some infant deaths attributed to other conditions (e.g., sudden infant death syndrome) may be due to botulism.

Wound Botulism

This is the rarest form of botulism in the United States. As the name implies, wound botulism develops from in vivo toxin production by *C. botulinum* in contaminated wounds. The symptoms of disease are identical to those of food-borne disease; however, the incubation period is generally longer (4 days or more), and gastrointestinal symptoms are less prominent.

Laboratory diagnosis

1. Morphology. The **gram-positive rods are large and motile.** The **spores are oval and terminal** or **subterminal.** Colonies are translucent and can be circular or irregularly shaped. The surface of colonies appears granular, and swarming cells may cover the entire culture plate. *C. botulinum* hemolyzes horse erythrocytes.
2. Antigenic composition. *C. botulinum* produces seven serologically distinct neurotoxins, designated A through G. The seven strains are placed in three groups because of some antigenic relationships.

The neurotoxin can be demonstrated in the blood and stools of affected individuals. It can be demonstrated in food by the injection of extracts into mice, and the type can be determined by mouse-protection studies using specific antisera. Reverse hemagglutination and immunofluorescence also are used to identify the toxin. Anaerobic cultures can be prepared on blood agar and egg yolk agar, but the procedure is time-consuming and may not produce viable bacteria.

Treatment, Prevention, and Control

Treatment of botulism requires **adequate ventilatory support, elimination of the organism from the gas-trointestinal tract by the judicious use of gastric lavage and penicillin therapy**, and the use oftrivalent botulinum antitoxin (vs. toxins A, B, and E) to bind toxin circulating in the bloodstream. Ventilatory support has had an unquestioned benefit in significantly reducing mortality.

Prevention of disease involves destruction of the spores in food products (virtually impossible for practical reasons), prevention of spore germination (by maintaining the food in an acid pH or storage at 4° C or colder), or destruction of the preformed toxin (by heating the food for 20 minutes at 80° C). Infant botulism is strongly associated by consumption of honey contaminated with *C. botulinum* spores, so children younger than 1 year of age should not eat honey.

CLOSTRIDIUM DIFFICILE

Until the mid-1970s the clinical importance of *Clostridium difficile* was not appreciated. This organism was infrequently isolated in fecal cultures and rarely associated with human disease. However, systematic studies have now clearly demonstrated that toxin-producing *C. difficile* is responsible for **antibiotic-associated gastrointestinal diseases ranging from relatively benign, self-limiting diarrhea to severe, life-threatening pseudomembranous colitis.**

C. difficile produces **two toxins**: **an enterotoxin** (toxin A) and a **cytotoxin** (toxin B). These toxins are immunologically distinct, although physical separation has proved to be difficult. The enterotoxin is chemotactic for neutrophils, with infiltration of polymorphonuclear neutrophil leukocytes (PMNs) into the ileum, resulting in release of cytokines, hypersecretion of fluid, and hemorrhagic necrosis. The cytotoxin causes depolymerization of actin with destruction of the cellular cytoskeleton both in vivo and in vitro.

C. difficile is **part of the normal intestinal flora in a small proportion of healthy persons and hospitalized patients. Exposure to antibiotics alters the normal enteric flora, permitting the overgrowth of these relatively resistant organisms** or making the patient more susceptible to exogenous acquisition of *C. difficile*. Proliferation of the organisms with localized production of their toxins in the colon leads to disease.

The specific diagnosis of *C. difficile* infection is accomplished by **culture of feces on highly selective media, detection of the cytotoxin** by an in vitro cytotoxicity assay with tissue culture cells, or detection of enterotoxin by immunoassays. The most specific test for *C. difficile* disease is the cytotoxicity assay. Maximal sensitivity for detecting colonization is accomplished by using a combination of diagnostic tests.

Discontinuation of the implicated antibiotic (e.g., ampicillin, clindamycin) is generally **sufficient to alleviate mild disease**. However, **specific therapy with either metronidazole or vancomycin is required** for serious disease. Relapses after completion of therapy may occur in as many as 20% to 30% of patients because spores of *C. difficile* are resistant to antibiotic treatment. **Re treatment with the same antibiotic is frequently successful**. **Prevention is difficult because the organism is commonly isolated in hospital environments**, particularly in areas adjacent to infected patients. **The spores of *C. difficile* are difficult to destroy**; thus the organism **can contaminate an environment for many months** and be a **major source of nosocomial hospital outbreaks** of *C. difficile* disease.

24.2 Anaerobic Non-Spore-Forming Bacteria

A. SUPRAGINGIVAL MICROBES
1. GRAM-POSITIVE BACTERIA
1.1 *Actinomyces*

Actinomyces are irregular gram-positive bacilli and areanaerobic bacteria also commonly found in oral samples.Common members of the *Actinomyces* genus inoral microbiology are *Actinomyces israelii, Actinomyces naeslundii,Actinomyces odontolyticus*, and *Actinomyces viscosus*.The type species of this genus is *Actinomyces bovis*.The shape and size of bacterial cells can vary greatlybut are often found to be irregular branched bacilli with adiameter of 0.2–1.0 μm and a length of about 5.0–10.0 μm.The cells are usually rod-shaped, but can occasionally beclub-

shaped with irregular arrangements including single,paired, chain, clusters, and fence-shaped. The cells produce no spores, show no motility, and also do notproduce conidia. Spider-like microcoloniesor branched mycelia can form on agar plates after18–24 h incubation. These typical spider web-shapedcolonies can help identify the genus of bacteria.

Actinomycetes are normal members of the oral flora and are the dominant bacteria in dental plaque. *A.israelii*, *A. naeslundii*, *A. odontolyticus*, and *A. Viscosus* canbe detected in human dental plaque, dental calculus, andsaliva. The main colonization site of *Actinomyces mai* is inthe gingival sulcus. Clinical and epidemiological investigationsindicate that *A. israelii* can cause actinomycosis,conjunctivitis, lachrymal and other diseases of the face,neck, lung, and abdomen. *A. naeslundii* and *A. mai* can bedetected in clinical samples of gingivitis, periodontitis,pulp periapical infection, and pericoronitis. *A. viscosus* issuspected to be a cariogenic bacterium.

1.2 *Bifidobacterium*

Bifidobacterium is a genus of bacteria with variousforms; nonmotile they are gram-positive, nonsporulating,anaerobic bacilli. Bacteria isolated from the oral cavity belonging to the*Bifidobacterium* spp. include mainly *Bifidobacterium dentium*,*Bifidobacterium breve*, *Bifidobacterium inopinatum*,and *Bifidobacterium denticolenu*. The type species is *B. bifidum*.The bacterial cells are short and thin, with pointedends, and are irregular. They also appear as long cellswith many branches and slightly branching spoonshapedcells. Cells are arranged as single cells, chains,polymer-shaped, V-shaped, or palisade-shaped. Theirdistinct cell morphology can be helpful in differentiating bacteria belonging to this genus. For example, *B. bifidum*appear as flask-shaped cells, while *Bifidobacteriumasteroides* are star-shaped. All members of this genus aregram-positive.*Bifidobacterium* are anaerobes, and most strains cannotgrow under 90% air and 10% CO2. Colonies formed onagar plates are convex, creamy or white, glossy, smooth,neat-edged, sticky, and soft.Originally, *B. dentium* was isolated from pus specimens and named *B. appendicitis*. Later, researchers isolatedsimilar bacteria from the adult dental caries, feces,and vagina, and they were then named *Actinomyces eriksonii*or grouped into *B. adolescentis*.

1.3 *Lactobacillus*

Lactobacillus is a group of anaerobic or microaerobicgram-positive bacilli that do not produce spores. Bacteriaof this genus form part of the normal flora of thehuman oral cavity and intestinal tract. This genus is cariogenic,as they are detected in decayed oral cavity materials.This genus includes 44 species. The common species found in the oral cavityinclude *Lactobacillus acidophilus*, *Lactobacillus salivavius*,*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillusbrevis*, and *Lactobacillus casei*. The shapes and sizes of the bacterial cells can varygreatly. They can be vimineous, stubbed, bent, bacilliform,clavate, club-shaped, etc. However, most *Lactobacillus*cells are fairly regular with no branching. The cellsare square or obtuse at the ends when compared withother gram-positive nonsporulating bacilli. They produceno spores and no capsules and stain gram-positive.Surface culture on a solid medium is best when performedin anaerobic or microaerophilic conditions. However,some species must be cultivated under anaerobicconditions. Some members of this genus can grow withinthe 15–45 °C range, in the presence of 5–10% CO2, whichpromotes bacterial growth. As acid-producing bacteria, lowpH. Rogosa agar is the culture medium of choice for manystrains of *Lactobacillus*. The optimal pH for growth is 5.5–6.2.nontransparent with a diameter from pinprick-sized to2 mm on the agar surface. Smooth colonies are soft, raised,and lustrous, and the edge of the colony is neat. The surfaceof rough colonies is dry, flat, and lackluster, and the edgeis not neat. The bacteria normally do not produce pigment.Lactobacilli can ferment glucose to produce acid,and are negative for catalase, urease, and cytochromeenzyme. Sugar fermentation test and arginine hydrolysis testcan help

identify the genus of bacteria. The bacteria can promote the development of toothdecay, as its detection is significantly increased in deepcaries material.

L. acidophilus is mainly isolated from the gastrointestinaltract of humans and animals, human mouths,and the human vagina. *L. acidophilus* can be isolatedfrom a minority of neonates' mouths. As the childrengrow older, the number of bacteria found in their mouthdecreases gradually, until at age 2, only a very smallamount of *L. acidophilus* can be detected. The main siteof colonization of *L. acidophilus* in the mouth is dentalplaque; it is relatively rare in saliva, on the tongue, orin the gingival sulcus. As it is often found in materialfrom deep caries, *L. acidophilus* is believed to be associatedwith the development of dental caries.*L. acidophilus* colonies can be separated into rough andsmooth type. Hair-like structures can be observed underthe stereomicroscope. Colonies do not produce pigment.

1.4 *Rothia*

Rothia are gram-positive facultative anaerobic bacillithat do not produce spores. *R. dentocariose* is the typespecies of the *Rothia* genus.

1.4.1 Rothia dentocariose

R. dentocariose is a gram-positive bacillus that doesnot produce spores. The bacteria cells can be spherical, pleiomorphic (similarto *Corynebacterium diphtheria*), or filamentous. Cellsstain as gram-negative. The cell diameter is generally1.0 μm, but cells are irregular in shape, and the ends canreach a diameter of 5.0 μm. Cells appear almost filamentousfollowing culture on solid media, while they appearspherical in broth media. *R.dentocariose* does not produce spores or a capsule. Theyare nonmotile and are not acid tolerant. These bacteria are facultative anaerobes. They grow well in an aerobic environment, although primary culturesrequire incubation under anaerobic conditions (80%N2, 10% H2, 10% CO2). The optimal growth temperatureis 35–37 °C. When cultured for 18–24 h under anaerobicconditions, young colonies are always filamentous andappear as spider-like colonies. When inoculated underaerobic conditions, young colonies can reach a diameterof 1 mm. The colony surface is smooth or grainyand often shows an umbrella edge.*R. dentocariose* are detected in the human oral cavity.Its main sites of colonization are the saliva and subgingivalplaque. They are nonpathogenic members of thehuman oral microflora and have no confirmed relationshipto oral infections. As an opportunistic pathogen, ithas been detected from endocarditis samples and otherclinical infected specimens.

2 GRAM-NEGATIVE BACTERIA

2.1 *Leptotrichia*

Leptotrichia is a gram-negative anaerobic bacillus andis a very commonly observed genus in the human oralcavity. For a long time, *Leptotrichia* were consideredopportunistic pathogens until recent reports that indicatedthat they may be pathogenic. *Leptotrichia* can beisolated from the oral cavity and are mainly found inbacterial biofilms. It can also separate from the vaginaand the uterus of pregnant women.The *Leptotrichia* cell measures 0.8–1.5 μm × 5–20 μm.They can be straight or curved rod shapes. The ends ofthe cell (either one or both ends) can be sharp or rounded.Cells normally organize as pairs or in a chain. The cells do not produce spores and arenonmotile. Fresh cultures can be stained gram-positive.Under the light microscope, both gram-negative andgram-positive cells can be observed on a single slide.After culturing in anaerobic blood agar for 1–2 days,*Leptotrichia* can form 1–2 mm, raised, and transparent colonieswith smooth and filamentous edges.Sometimes polymorphous colonies are also formed.*Leptotrichia* grow best under anaerobic conditions.Cultures require 5–10% CO2. The ideal temperature

forculture growth is between 35 °C and 37 °C, while *Leptotrichia*cells stop growing when temperatures drop below25 °C. The ideal pH for culturing these cells is betweenpH 7.0 and 7.4.

2.2 *Veillonella*

Veillonella are gram-negative anaerobic cocci andbelong to the family *Veillonellaceae.* Strains detected inoral cavities include *Veillonella parvula*, *Veillonella atypic*,and *Veillonella dispar*.

Veillonella parvula *subsp.* Parvula*V. parvula* subsp. *parvula* are gram-negative anaerobiccocci and are among the most common bacteria inthe oral cavity. *V. parvula* subsp. *parvula* is a strict anaerobe. Severalstrains require putrescine and cadaverine in their growth medium. The cells are relatively biochemically inactive whentested using classical biochemical tests. They are unableto ferment carbohydrate to acid and do not produce indole. They appear negative with the catalase test butare able to reduce nitrate to nitrite. *V. parvula* subsp. *parvula* is detected in saliva, on thetongue, and in plaques. They are able to utilize lactateproduced by *Streptococcus mutans*, and are thus consideredas beneficial bacteria in dental plaques. They formpart of the normal human gut flora.*V. parvula* subsp. *parvula* require strictly anaerobicconditions, forming small gray-white colonies on the surface of BHI blood agar.

B. SUBGINGIVAL ANAEROBIC MICROBES

1. *Eubacterium*

Eubacterium is a genus of gram-positive nonsporulatingstrictly anaerobic bacilli. Currently, bacteria species detected in the oral cavitythat belong to this genus include *Eubacterium alactolyticum*,*Eubacterium saburreum*, *Eubacterium lentum*, *Eubacteriumlimosum*, *Eubacterium nodatum*, *Eubacterium brachy*,*Eubacterium timidum*, *Eubacterium saphenus*, and *Eubacteriumminutum*.Cells can be homogeneous or polymorphous rodshaped.No spores are produced. Cells are gram-positive,but Gram staining old cultures and cultures that haveproduced acid in the culture medium will yield negativeresults.Eubacteria are strictly anaerobic. Culturing cellscan be difficult due to this bacteria's strict anaerobicdemands, and some strains can only grow in prereducedmedium. The optimum growth temperature is 37 °C,while the optimum pH is 7.0.Eubacteria are chemoheterotrophs and produceenergy from mixed organic acids produced by carbohydratesor protein metabolism. Eubacteria mainly colonize the saliva and plaqueas a member of the normal oral microflora. *Eubacteriumlentum* and *E. limosum* can be detected in the oralcavity. *Eubacterium nodatum*, *E. brachy*, *E. timidum*, *E.saphenus*, and *E. minutum* are new species isolated fromsubgingival plaque of patients with periodontitis andare considered as potential periodontal pathogens.

2.Peptostreptococcus

Peptostreptococcus is the most common gram-positiveanaerobic coccus in the human oral cavity and in theclinic. *Peptostreptococcus anaerobius* and *Peptostreptococcusmicros* are the most commonly encountered speciesin this genus.

2.1 Peptostreptococcus anaerobius

The cells of *P. anaerobius* are gram-positive, spherical, approximately 0.5–0.6 μm in diameter,and arranged in pairs or chains. Cells in early cultureshave been observed to form long chains. The optimal temperature for *P. anaerobius* growth is37 °C, and cells of this species do not grow well at 25 °Cor 30 °C, and do not grow at all at 45 °C. Growth is stimulatedby 0.02% polysorbate-80. *P. anaerobius* cells form pinpoint-like or rounded (about 1 mm in diameter), raised,white, glossy, nontransparent colonies with a smoothsurface, without hemolytic zone. Coloniesformed on the surface of BHI supplemental mediumwithout addition of blood are gray.

In deep glucose agar, *P. anaerobius* can produce a largeamount of gas and generate ammonia from peptone.Dental plaque and gingival sulcus are the main habitatsfor *P. anaerobius* in the oral cavity. Moreover, *P. anaerobius*is often detected in clinical samples of periodontitis,pulpitis, and pericoronitis.

3. *Propionibacterium*

Propionibacterium is a genus of gram-positive polymorphicbacilli that do not sporulate. The genus canbe divided into two groups: one that lives on the skin,including inside the oral and intestinal tracts, namedthe sores and blisters group or *P. acnes*; and another thatlives in dairy products, cheese or green fodder nameddairy group or typical propionibacteria.The cells are polymorphic in form, many have tworounded ends, and others are shaped like C*orynebacteriumdiphtheria*, with one rounded end and one tapered end.Cells are 0.5–0.8 μm in diameter, 1–5 μm in length, andform two branches or branched rods. Cocci are observed in old cultures, arranged as single cells, in pairs, in chains,or in "Y"- or "V"-shaped branched chains. Cells are gram-positive,but some cells can also stain gram-negative.Members of this genus are anaerobic or microaerophilicbacteria. The highest rate of growth takesplace 48 h after inoculation. The Propionibacteria arechemoheterotrophic bacteria that need a complex nutritionalmedium such as BHI agar in order to be cultured.Most species can grow in dextrose broth containing 20%bile or 6.5% NaCl. *Propionibacterium acnes* colonies onthe surface of agar can produce colorful pigmentationincluding white, gray, pink, red, or yellow.

They can also produce some isovalericacid, formic acid, succinic acid, and lactic acid.All the species in this genus can use glucose to produceacid. They test positive for catalase. Species of *Propionibacterium* are distinguished using indole production,nitrate test, esculin hydrolysis, gelatin liquefication,and other biochemical tests such as the fermentation ofsucrose, maltose, and mannitol.

GRAM-NEGATIVE BACTERIA

1. *Bacteroides*

Bacteroides is a genus of gram-negative, obligate anaerobic, nonsporulating bacilli that belong to the family *Bacteroidaceae*. Members of this genus are chemoheterotrophsand can use carbohydrates, peptone, and otherintermediate bacterial metabolites.*Bacteroides* are naturally found in the mouth, tongue, intestinal tract, and vagina. All strains that can toleratebile (20% oxgall salts) fall collectively into the *B. fragilis* group, including *B. fragilis*, *B. thetaiotaomicron*, etc. Thatare detected in cultures from appendicitis, peritonitis and cervicitis clinical specimens. Other species that cannottolerate bile and that produce melanin were reclassified to the genera *Porphyromonas* and *Prevotella*. Cells measure 0.8–1.8 μm × 0.8–1.6 μm when grown in glucose broth, often showing visible vacuoles or darker stain at the ends. Cells are arranged singly or in pairs with no spores. Many strains are encapsulated.

Bacteroides is the most **common cause of serious anaerobic infections, eg, sepsis, peritonitis, and abscesses.** *Bacteroides fragilis* is the most frequent pathogen.

Bacteroides organisms are **anaerobic, non-spore-forming, gram-negative rods**. Of the 22 species of *Bacteroides*, three are human pathogens: ***B. fragilis, Bacteroides melaninogenicus,* and *Bacteroides corrodens*.** *Bacteroides melaninogenicus* was recently renamed ***Prevotella melaninogenica,*** but the former name is still commonly used.

Members of the *B. fragilis* group are the predominant organisms in the human colon, numbering approximately 10^{11}/g of feces, and are found in the **vagina** of approximately 60% of women. *B. melaninogenicus* and *B. corrodens* occur primarily in the **oral cavity**.

Pathogenesis & Epidemiology

Because *Bacteroides* species are part of the normal flora, **infections are endogenous**, usually arising from a break in a mucosal surface, and are not communicable. These organisms cause a variety of infections, such as **local abscesses at the site of a mucosal break, metastatic abscesses by hematogenous spread to distant organs, or lung abscesses by aspiration of oral flora.**

Predisposing factors such as surgery, trauma, and chronic disease play an important role in pathogenesis. Local tissue necrosis, impaired blood supply, and growth of facultative anaerobes at the site contribute to anaerobic infections. The facultative anaerobes, such as *E. coli*, utilize the oxygen, thereby reducing it to a level that allows the anaerobic *Bacteroides* strains to grow. As a result, many anaerobic infections contain a mixed facultative and anaerobic flora. This has important implications for therapy; both the facultative anaerobes and the anaerobes should be treated.

The polysaccharide capsule of *B. fragilis* is an important virulence factor. Many of the symptoms of *Bacteroides* sepsis resemble those of sepsis caused by bacteria with endotoxin, but the lipopolysaccharide of *Bacteroides* is chemically different from the typical endotoxin. No exotoxins have been found.

Clinical Findings

The *B. fragilis* group of organisms is most frequently associated with **intraabdominal infections, either peritonitis or localized abscesses. Pelvic abscesses and bacteremia** occur as well. **Oral, pharyngeal, and pulmonary abscesses** are more commonly caused by B melaninogenicus, a member of the normal oral flora, but *B. fragilis* is found in about 25% of lung abscesses. In general, *B. fragilis* causes disease below the diaphragm whereas *B. melaninogenicus* causes disease above the diaphragm.

Laboratory Diagnosis

Bacteroides species can be isolated anaerobically on blood agar plates containing kanamycin and vancomycin to inhibit unwanted organisms. They are identified by biochemical reactions (eg, sugar fermentations) and by production of certain organic acids (eg, formic, acetic, and propionic acids), which are detected by gas chromatography. *B. melaninogenicus* produces characteristic black colonies.

Treatment

Members of the *B. fragilis* group are **resistant to penicillins, first-generation cephalosporins, and aminoglycosides, making them among the most antibiotic-resistant of the anaerobic bacteria**. Penicillin resistance is the result of β-lactamase production. **Metronidazole is the drug of choice, with cefoxitin, clindamycin, and chloramphenicol** as alternatives. **Aminoglycosides are frequently combined** to treat the facultative gram-negative rods in mixed infections. By contrast, the drug of choice for ***B. melaninogenicus*** **infections is penicillin G**, although β-lactamase producing strains have emerged. However, strains resistant to penicillin have been isolated from patients. Surgical drainage of abscesses usually accompanies antibiotic therapy, but lung abscesses often heal without drainage.

Prevention

Prevention of *Bacteroides* infections centers on perioperative administration of a cephalosporin, frequently cefoxitin, for abdominal or pelvic surgery. There is no vaccine.

2. *Capnocytophaga*

Capnocytophaga are gram-negative, facultative anaerobicbacteria. They were the earliest bacteria to be isolatedand named from the human subgingival plaque. Common *Capnocytophaga*

species are: *Capnocytophaga ochracer*, *Capnocytophaga sputigena*, *Capnocytophaga gingivalis*, *Capnocytophagagranulose*, and *Capnocytophaga heamolytica*.

Capnocytophaga cells are 0.42–0.6 μm × 2.5–2.7 μm insize and shaped like bent rods or filaments, usually withrounded or slightly pointed ends. The length of the cellsvaries. In liquid culture, cells are polymorphic or take ona long, filamentous morphology, and tight clumps canbe observed. The bacteria produce no capsule and nosheath. They do not form spores, have no flagella, buthave sliding motility.*Capnocytophaga* are facultative anaerobes but do notgrow under aerobic conditions. Cultures grow well ina CO2-added anaerobic environment. Primary culturesshould be performed in an aerobic environment with CO2 added.Species in this genus often form colonies of wet, thin,flat, diffuse growth with ragged edges on TS blood agarand BHI blood agar. After 24 h incubation at 35–37 °C,the size of colonies are like pinpricks. After incubationfor 48–96 h, colonies become 2–4 mm in diameter andtake on the appearance of bumps. Some colonies maybecome recessed into the agar. Aside from hemolytic*Capnocytophaga* (which produces β-hemolysis), otherspecies are not hemolytic on blood agar. The concentrationof agar in the medium affects the force of slidingmotility. *Capnocytophaga* cultures can produce a specialsmell, similar to caramel or a bitter almond flavor. Colonies on the agar surface can produce white topink or orange-yellow pigmentation. Centrifuged cellsappear to be an orange-yellow clump.A member of the normal microflora of humans andprimates, this genus is mainly found to colonize theoral cavity. They are common oral bacteria and can beobtained from various parts of the oral cavity, includingplaque, gingival sulcus, saliva and sputum, andthroat specimens. These bacteria are often detected inmixed bacterial infections, such as juvenile periodontitis,infected root canal, dry socket after tooth extraction,oral ulcers, and other clinical specimens. A member of the normal microflora of humans andprimates, this genus is mainly found to colonize theoral cavity. They are common oral bacteria and can beobtained from various parts of the oral cavity, including plaque, gingival sulcus, saliva and sputum, andthroat specimens. These bacteria are often detected inmixed bacterial infections, such as juvenile periodontitis,infected root canal, dry socket after tooth extraction,oral ulcers, and other clinical specimens, and can also be isolated from bacteremia, soft tissue infections, injuriesand abscesses at various locations, cerebrospinal fluid,vaginal, cervical, and amniotic fluid, trachea, and eyes.

3. *Eikenella*

Eikenella is a genus of gram-negative facultative anaerobic bacteria that do not produce spores.

3.1 Eikenella corrodens

E. corrodens was thus named because it produces typical colonies that can erode agar. It is also known as *Bacteroides corrodens* and is the only species in the genus *Eikenella*. Cells stain gram-negative. *E. corrodens* is a facultative anaerobe, and primary cultures require anaerobic conditions or supplementation with 5–10% CO2. It is essential to add hemin (5–25 mg/L) to the culture when grown under aerobic conditions. The optimum growth temperature is from 35 °C to 37 °C, the optimum pH is 7.3, and cultures require sufficient humidity. *E. corrodens* does not grow well in liquid media. *E. corrodens* is biochemically inactive. It does not ferment glucose and other carbohydrates or produce acid. It tests negative for catalase, urease, arginine dehydrogenase, and indole, but is positive for nitrate reduction, as well as oxidase and lysine decarboxylase.

E. corrodens is a member of the normal flora in the human oral cavity and intestinal tract. It can also be isolated from the upper respiratory tract and urogenital tract. As an opportunistic pathogen, it is often associated with other bacterial pathogens to cause mixed bacterial infections, especially in the mouth and respiratory tract. Its detection rate is higher in lesions of active adult

periodontitis and specimens of dry socket after tooth extraction, and it is suspected to be related to periodontitis.

4 *Fusobacterium*

Fusobacterium is a group of gram-negative obligate anaerobic bacteria that do not form spores. They belong to the family *Bacteroidaceae*. Species mainly found in the oral cavity are *Fusobacterium nucleatum*, *Fusobacterium necrophorum*, and *Fusobacterium varium*. Most *Fusobacteria* are spindle-shaped cells. They may also appear polymorphous. Polymorphic *Fusobacteria* can form globular or long filiform cells. *Fusobacterium necrophorum* can take on many other morphologies, including irregular spherical swollen cells and linear cells. The cells are nonmotile, do not form spores, and stain gram-negative. These obligate anaerobes can be grown in aerobic conditions on agar plates when 5–10% CO2 is added to the culture conditions. Their sensitivity to oxygen depends on the specific bacterial species, the quantity of cells inoculated, and the type of culture medium. *Fusobacterium* can be detected in clinical specimens of pus or gangrene infections. *Fusobacterium nucleatum* has a high prevalence in saliva and dental plaque, and is considered to be one of the bacteria involved in mixed infections of periodontitis, root canal infection, and postextraction infection. The type species is *F. nucleatum*. *F. nucleatum* is mainly found on transparent gingiva and the gingival groove, and make up the oral normal flora. They can also be isolated from upper respiratory tract and chest infections, and occasionally from wounds and other sites of infection. *F. nucleatum* has a high rate of detection in destructive periodontal disease and infectious dental pulp, as it assists other pathogens in establishing oral infectious diseases.

5. *Prevotella*

Prevotella is a genus named after the French microbiologist A. R. Prevol. These bacteria belong to the genus *Bacteroides* and include bile-sensitive strains and melanin-producing, sugar metabolizing strains. The main species of *Prevotella* found in the oral cavity are: *Prevotella intermedia*, *Prevotella melaninogenica*, *Prevotella loescheii*, *Prevotella nigrescens*, *Prevotella dentocola*, and *Prevotella corporis*. The most frequently found cell morphology is short bacillus, but long bacilli are sometimes observed. The cells are nonsporulating, nonmotile, and stain gram-negative. Bacteria belonging to this genus are obligate anaerobes, and most cultures require supplementing with hemin and vitamin K. On blood agar, *Prevotella* spp. produce melanin to form black colonies. They are sensitive to bile salt and can thus be distinguished from other bacteroides that can tolerate bile salts. *Prevotella* are dominant bacteria in the human gingival groove and are also the suspected pathogens behind periodontitis. For *P.intermedia* the site of colonization is the gingival sulcus, but cells also can be found in saliva, dental calculus, and other plaque specimens. Previous research has shown that this species is the main pathogenic bacteria in pregnancy related gingivitis. In addition, the bacteria can also be isolated from pericoronitis, the focus of infection after tooth extraction, infected root canals, infections of the head and neck, and pleural infection. *P. intermedia* is occasionally isolated from blood, abdominal, or pelvic specimens.

6. *Porphyromonas*

In 1998, three species of *Bacteroides* were found to have significantly different biological characteristics than other *Bacteroides* species. However, they were similar in their ability to ferment carbohydrates to produce melanin. These species, namely *Bacteroides saccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodentalis* have been placed into a new genus: *Porphyromonas*. Members of this genus most commonly found in the oral cavity are *Porphyromonas gingivalis* and *Porphyromonas endodentalis*. Broth cultured cells are typically small rods approximately 0.5–0.8 × 1.0–3.5 μm in size, but occasionally cells can be found that

measure 4–6 μm long. These bacteria produce no spores, are nonmotile, and are gram-negative. *Porphyromonas* are obligate anaerobes with an optimum growth temperature of 37 °C. On blood agar plates, they can form colonies 1–3 mm in diameter that are protuberant, lustrous, and with smooth surface (very few with rough surface). The primary endpoint product in PYG medium is butyric acid and acetic acid. There is a small amount of propionic acid, isobutyric acid, and isoamyl propionate produced. The type species is *Porphyromonas asaccharolytica*.