CHAPTER

18 Gram-Negative Rods Related to the Enteric Tract

CHAPTER CONTENTS

INTRODUCTION

Gram-negative rods are a large group of diverse organisms (Figures 18–1, 18–2, and 19–1). In this book, these bacteria are subdivided into three clinically relevant categories, each

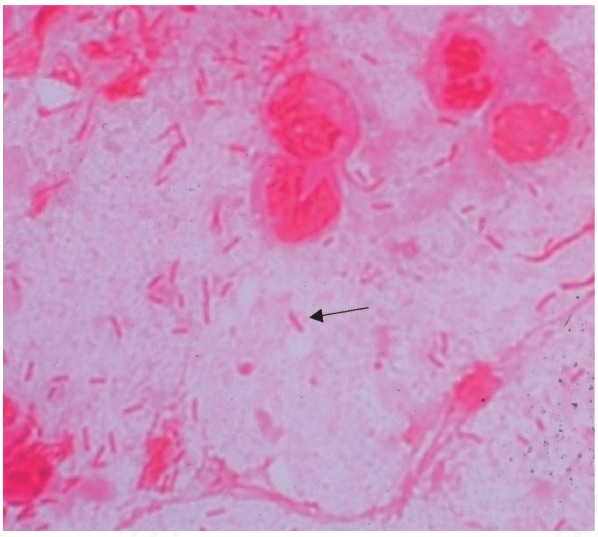

FIGURE 18–1 *Escherichia coli*—Gram stain. Arrow points to a gram-negative rod. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

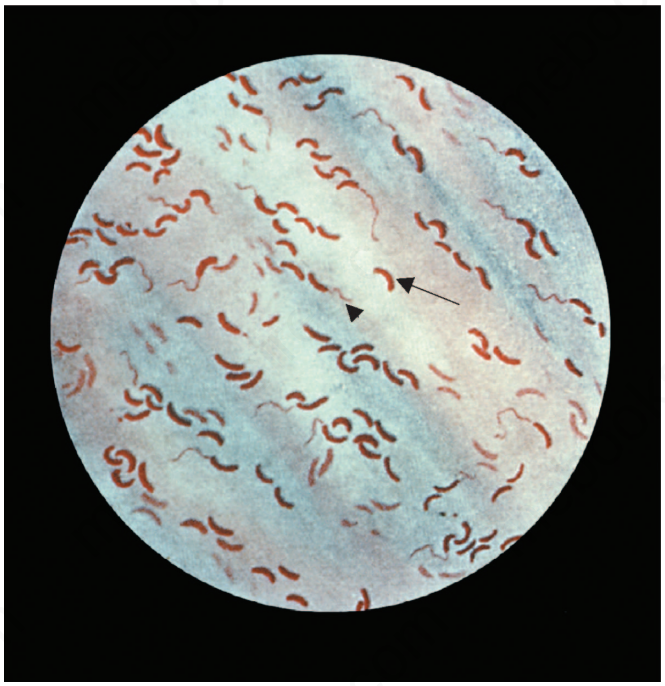

FIGURE 18–2 *Vibrio cholerae*—Gram stain. Long arrow points to a curved gram-negative rod. Arrowhead points to a flagellum at one end of a curved gram-negative rod. (Source: Public Health Image Library, Centers for Disease Control and Prevention.)

TABLE 18–1 Categories of Gram-Negative Rods

Chapter	Source of Site of Infection	Genus
18	Enteric tract	
	1. Both within and outside	*Escherichia, Salmonella*
	2. Primarily within	*Shigella, Vibrio, Campylobacter, Helicobacter*
	3. Outside only	*Klebsiella–Enterobacter–Serratia* group, *Proteus–Providencia–Morganella* group, *Pseudomonas, Bacteroides, Prevotella, Fusobacterium*
19	Respiratory tract	*Haemophilus, Legionella, Bordetella*
20	Animal sources	*Brucella, Francisella, Pasteurella, Yersinia*

in a separate chapter, according to whether the organism is related primarily to the enteric or the respiratory tract or to animal sources (Table 18–1). Although this approach leads to some overlap, it should be helpful because it allows general concepts to be emphasized.

Gram-negative rods related to the enteric tract include a large number of genera. These genera have therefore been divided into three groups depending on the major anatomic location of disease, namely, (1) pathogens both within and outside the enteric tract, (2) pathogens primarily within the enteric tract, and (3) pathogens outside the enteric tract (see Table 18–1).

The frequency with which the organisms related to the enteric tract cause disease in the United States is shown in Table 18–2. *Salmonella, Shigella*, and *Campylobacter* are frequent pathogens in the gastrointestinal tract, whereas *Escherichia, Vibrio*, and *Yersinia* are less so. Enterotoxigenic strains of *Escherichia coli* are a common cause of diarrhea in developing countries but are less common in the United States. The medically important gram-negative rods that cause diarrhea are described in Table 18–3. Urinary tract infections are caused primarily by *E. coli;* the other organisms occur less commonly. The medically important gram-negative rods that cause urinary tract infections are described in Table 18–4.

Additional information regarding the clinical aspects of infections caused by the organisms in this chapter is provided in Part IX entitled Infectious Diseases beginning on page 593.

TABLE 18–2 Frequency of Diseases Caused in the United States by Gram-Negative Rods Related to the Enteric Tract

Site of Infection	Frequent Pathogens	Less-Frequent Pathogens
Enteric tract	*Salmonella, Shigella, Campylobacter*	*Escherichia, Vibrio, Yersinia*
Urinary tract	*Escherichia*	*Enterobacter, Klebsiella, Proteus, Pseudomonas*

Patients infected with such enteric pathogens as *Shigella, Salmonella, Campylobacter*, and *Yersinia* have a high incidence of certain autoimmune diseases such as reactive arthritis and Reiter's syndrome (see Chapter 66). In addition, infection with *Campylobacter jejuni* predisposes to Guillain-Barré syndrome.

Before describing the specific organisms, it is appropriate to describe the family Enterobacteriaceae, to which many of these gram-negative rods belong.

ENTEROBACTERIACEAE & RELATED ORGANISMS

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. The organisms and some of the diseases they cause are listed in Table 18–5.

Features common to all members of this heterogeneous family are their anatomic location and 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 (i.e., 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.*[1]

Pseudomonas aeruginosa, a significant cause of urinary tract infection and sepsis in hospitalized patients, does not ferment glucose or reduce nitrates and is oxidase-positive.

[1]The other less frequently isolated organisms in this group are members of the following genera: Achromobacter, Acinetobacter, Alcaligenes, Eikenella, Flavobacterium, Kingella, and Moraxella; see Chapter 27.

TABLE 18–3 Gram-Negative Rods Causing Diarrhea

Species	Fever	Leukocytes in Stool	Infective Dose	Typical Bacteriologic or Epidemiologic Findings
Enterotoxin-mediated				
1. *Escherichia coli*	–	–	?	Ferments lactose
2. *Vibrio cholerae*	–	–	10^7	Comma-shaped bacteria
Invasive-inflammatory				
1. *Salmonella* (e.g., *S. enterica*)	+	+	10^5	Does not ferment lactose
2. *Shigella* (e.g., *S. dysenteriae*)	+	+	10^2	Does not ferment lactose
3. *Campylobacter jejuni*	+	+	10^4	Comma- or S-shaped bacteria; growth at 42°C
4. *E. coli* (enteropathic strains)	+	+	?	
5. *E. coli* O157:H7	+	+/–	?	Transmitted by undercooked hamburger; causes hemolytic-uremic syndrome
Mechanism uncertain				
1. *Vibrio parahaemolyticus*[1]	+	+	?	Transmitted by seafood
2. *Yersinia enterocolitica*[1]	+	+	10^8	Usually transmitted from pets (e.g., puppies)

[1]Some strains produce enterotoxin, but its pathogenic role is not clear.

TABLE 18–4 Gram-Negative Rods Causing Urinary Tract Infection[1] or Sepsis[2]

Species	Lactose Fermented	Features of the Organism
Escherichia coli	+	Colonies show green sheen on EMB agars
Enterobacter cloacae	+	Causes nosocomial infections and often drug-resistant
Klebsiella pneumoniae	+	Has large mucoid capsule and hence viscous colonies
Serratia marcescens	–	Red pigment produced; causes nosocomial infections and often drug resistant
Proteus mirabilis	–	Motility causes "swarming" on agar; produces urease
Pseudomonas aeruginosa	–	Blue-green pigment and fruity odor produced; causes nosocomial infections and often drug-resistant

EMB = eosin–methylene blue.

[1]Diagnosed by quantitative culture of urine.

[2]Diagnosed by culture of blood or pus.

TABLE 18–5 Diseases Caused by Members of the Enterobacteriaceae

Major Pathogen	Representative Diseases	Minor Related Genera
Escherichia	Urinary tract infection, traveler's diarrhea, neonatal meningitis	
Shigella	Dysentery	
Salmonella	Typhoid fever, enterocolitis	*Arizona, Citrobacter, Edwardsiella*
Klebsiella	Pneumonia, urinary tract infection	
Enterobacter	Pneumonia, urinary tract infection	*Hafnia*
Serratia	Pneumonia, urinary tract infection	
Proteus	Urinary tract infection	*Providencia, Morganella*
Yersinia	Plague, enterocolitis, mesenteric adenitis	

In contrast to the Enterobacteriaceae, it is a strict aerobe and derives its energy from oxidation, not fermentation.

Pathogenesis

All members of the Enterobacteriaceae, being gram-negative, contain endotoxin in their cell walls. In addition, several exotoxins are produced (e.g., *E. coli* and *Vibrio cholerae* secrete exotoxins, called *enterotoxins*, that activate adenylate cyclase within the cells of the small intestine, causing diarrhea) (see Chapter 7). In addition, *E. coli* O157 produces Shiga toxin that causes hemolytic-uremic syndrome (HUS).

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 (see Figure 2–6). 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 (e.g., 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.

TABLE 18–6 Lactose Fermentation by Members of the Enterobacteriaceae and Related Organisms

Lactose Fermentation	Organisms
Occurs	*Escherichia, Klebsiella, Enterobacter*
Does not occur	*Shigella, Salmonella, Proteus, Pseudomonas*
Occurs slowly	*Serratia, Vibrio*

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 (Table 18–6). On these media, the non–lactose fermenters (e.g., *Salmonella* and *Shigella*) form colorless colonies, whereas the lactose fermenters (e.g., *E. coli*) form colored colonies. On EMB agar, *E. coli* colonies have a characteristic **green sheen.** 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 performed prior to the definitive identification procedures. The rationale for the use of these media and the reactions of several important organisms are presented in the box titled "Agar Media for Enteric Gram-Negative Rods" and in Table 18–7. The results of the screening process are

TABLE 18–7 Triple Sugar Iron (TSI) Agar Reactions

Reactions[1]				
Slant	**Butt**	**Gas**	**H_2S**	**Representative Genera**
Acid	Acid	+	–	*Escherichia, Enterobacter, Klebsiella*
Alkaline	Acid	–	–	*Shigella, Serratia*
Alkaline	Acid	+	+	*Salmonella, Proteus*
Alkaline	Alkaline	–	–	*Pseudomonas*[2]

[1]Acid production causes the phenol red indicator to turn yellow; the indicator is red under alkaline conditions. The presence of black FeS in the butt indicates H_2S production. Not every species within the various genera will give the above appearance on TSI agar. For example, some *Serratia* strains can ferment lactose slowly and give an acid reaction on the slant.

[2]*Pseudomonas,* although not a member of the Enterobacteriaceae, is included in this table because its reaction on TSI agar is a useful diagnostic criterion.

AGAR MEDIA FOR ENTERIC GRAM-NEGATIVE RODS

Triple Sugar Iron (TSI) Agar

The important components of this medium are ferrous sulfate and the three sugars glucose, lactose, and sucrose. Glucose is present in one-tenth the concentration of the other two sugars. The medium in the tube has a solid, poorly oxygenated area on the bottom, called the butt, and an angled, well-oxygenated area on top, called the slant. The organism is inoculated into the butt and across the surface of the slant.

The interpretation of the test results is as follows: (1) If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in the butt and on the slant. Some organisms generate gases, which produce bubbles in the butt. (2) If lactose is not fermented but the small amount of glucose is, the oxygen-deficient butt will be yellow, but on the slant, the acid will be oxidized to CO_2 and H_2O by the organism and the slant will be red (neutral or alkaline). (3) If neither lactose nor glucose is fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of the production of ammonia from the oxidative deamination of amino acids. (4) If H_2S is produced, the black color of ferrous sulfide is seen.

The reactions of some of the important organisms are presented in Table 18–7. Because several organisms can give the same reaction, TSI agar is only a screening device.

Urea Agar

The import ant components of this medium are urea and the pH indicator phenol red. If the organism produces urease, the urea is hydrolyzed to NH_3 and CO_2. Ammonia turns the medium alkaline, and the color of the phenol red changes from light orange to reddish purple. The important organisms that are urease-positive are *Proteus* species and *K. pneumoniae*.

often sufficient to identify the genus of an organism; however, an array of 20 or more biochemical tests is required to identify the species.

Another valuable piece of information used to identify some of these organisms is their motility, which is dependent on the presence of flagella. *Proteus* species are very motile and characteristically **swarm** over the blood agar plate, obscuring the colonies of other organisms. Motility is also an important diagnostic criterion in the differentiation of *Enterobacter cloacae*, which is motile, from *K. pneumoniae*, which is nonmotile.

If the results of the screening tests suggest the presence of a *Salmonella* or *Shigella* strain, an agglutination test can be used to identify the genus of the organism and to determine whether it is a member of group A, B, C, or D.

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, *E. coli* is identified by its ability to ferment lactose with the production of acid and gas, its ability to grow at 44.5°C, and its characteristic colony type on EMB agar. 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 the twentieth 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 (e.g., some penicillins and cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, quinolones, and sulfonamides). The specific choice usually depends on 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. For example, plasmid-encoded New Delhi metallo-β-lactamase causes resistance to penicillins, cephalosporins, monobactams, and carbapenems.

PATHOGENS BOTH WITHIN & OUTSIDE THE ENTERIC TRACT

ESCHERICHIA

Diseases

Escherichia coli is the most common cause of urinary tract infection 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.

Important Properties

Escherichia coli is a straight gram-negative rod (see Figure 18–1), in contrast to the curved gram-negative rods of the genera *Vibrio*, *Campylobacter*, and *Helicobacter*.

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

Escherichia 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 (e.g., O55 and O111 cause outbreaks of neonatal diarrhea).

Pathogenesis

The reservoir of *E. coli* includes both humans and animals. The source of the *E. coli* that causes urinary tract infections is the patient's own colonic flora that colonizes the urogenital area. The source of the *E. coli* that causes neonatal meningitis is the mother's birth canal; the infection is acquired during birth. In contrast, the *E. coli* that causes traveler's diarrhea is acquired by ingestion of food or water contaminated with human feces. Note that the main reservoir of enterohemorrhagic *E. coli* O157 is cattle and the organism is acquired in undercooked beef, for example, hamburgers.

Escherichia coli has several clearly identified components that contribute to its ability to cause disease: pili, a capsule, endotoxin, and three exotoxins (enterotoxins), two that cause watery diarrhea and one that causes bloody diarrhea and hemolytic–uremic syndrome.

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* (ETEC) can produce either or both of two enterotoxins.

(1) The heat-labile toxin (LT) acts by stimulating **adenylate cyclase.** Both LT and cholera toxin act by catalyzing the addition of adenosine diphosphate-ribose (a process called ADP-ribosylation) to the G protein that stimulates the cyclase. This irreversibly activates the cyclase. The resultant increase in intracellular cyclic adenosine monophosphate (AMP) concentration stimulates cyclic AMP–dependent protein kinase, which phosphorylates ion transporters in the membrane. The transporters export ions, which cause an outpouring of fluid, potassium, and chloride from the enterocytes into the lumen of the gut, resulting in watery diarrhea. Note that cholera toxin has the same mode of action.

(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, nonbloody diarrhea. However, certain strains of *E. coli* are enteropathic (enteroinvasive) 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* (i.e., those with the O157:H7 serotype) (STEC) also cause bloody diarrhea by producing an exotoxin called **Shiga toxin,** so called because it is very similar to that produced by *Shigella* species. Shiga toxin acts by removing an adenine from the large (28S) ribosomal RNA, thereby stopping protein synthesis. Shiga toxin is encoded by temperate (lysogenic) bacteriophages. Shiga toxin is also called verotoxin because it has a cytopathic effect on Vero (monkey) cells in culture.

These O157:H7 strains are associated with outbreaks of bloody diarrhea following ingestion of undercooked hamburger, often at fast-food restaurants. The bacteria on the surface of the hamburger are killed by the cooking, but those in the interior, which is undercooked, survive. Also, direct contact with animals (e.g., visits to farms and petting zoos) has resulted in bloody diarrhea caused by O157:H7 strains. *E. coli* O157 has a low ID_{50} of approximately 100 organisms.

Some patients with bloody diarrhea caused by O157:H7 strains also have a life-threatening complication called **hemolytic–uremic syndrome (HUS),** which occurs when Shiga toxin enters the bloodstream. This syndrome consists of hemolytic anemia, thrombocytopenia, and acute renal

TABLE 18–8 Clinical Aspects of *Escherichia coli*

Clinical Finding/Disease	Major Pathogenetic Factor	Main Laboratory Result
Findings within the intestinal tract		
Watery, nonbloody diarrhea (traveler's diarrhea)	Enterotoxin that increases cyclic AMP	No RBC or WBC in stool
Bloody diarrhea caused by *E. coli* O-157; hemolytic–uremic syndrome (HUS)	Shiga toxin (verotoxin) inhibits protein synthesis	RBC in stool; schistocytes in blood smear
Findings outside of intestinal tract		
Urinary tract infection	Gal-gal pili bind to bladder mucosa	WBC in urine, positive urine culture
Neonatal meningitis	K-1 capsular polysaccharide is antiphagocytic	WBC in spinal fluid, positive CSF culture
Sepsis, especially in hospital	Endotoxin induces fever, hypotension, and DIC	Leukocytosis, positive blood culture

AMP = adenosine monophosphate; CSF = cerebrospinal fluid; DIC = disseminated intravascular coagulation; RBC = red blood cell; WBC = white blood cell.

failure. The hemolytic anemia and renal failure occur because there are receptors for Shiga toxin on the surface of the endothelium of small blood vessels and on the surface of kidney epithelium. Death of the endothelial cells of small blood vessels results in a microangiopathic hemolytic anemia in which the red cells passing through the damaged area become grossly distorted (schistocytes) and then lyse. Thrombocytopenia occurs because platelets adhere to the damaged endothelial surface. Death of the kidney epithelial cells leads to renal failure. Treatment of diarrhea caused by O157:H7 strains with antibiotics, such as ciprofloxacin, increases the risk of developing HUS by increasing the amount of Shiga toxin released by the dying bacteria.

Urinary Tract Infections

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**). These pili are also called P fimbria or pyelonephritis-associated pili (PAP).

Cranberry juice contains flavonoids that inhibit the binding of pili to receptors and may be useful in the prevention of recurrent urinary tract infections. The motility of *E. coli* may aid its ability to ascend the urethra into the bladder and ascend the ureter into the kidney.

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. For example, *E. coli* strains that cause neonatal meningitis usually have a specific capsular type called the K1 antigen. The endotoxin of *E. coli* is the cell wall lipopolysaccharide, which causes several features of gram-negative sepsis such as fever, hypotension, and disseminated intravascular coagulation.

Th-17 helper T cells that produce interleukin-17 are an important host defense against sepsis caused by enteric bacteria such as *E. coli* and *Klebsiella*. Patients infected with human immunodeficiency virus (HIV) experience a loss of Th-17 cells and are predisposed to sepsis caused by *E. coli* and *Klebsiella*.

Clinical Findings

Escherichia coli causes a variety of diseases both within and outside the intestinal tract. The main clinical findings, the major pathogenetic factors, and the main laboratory results are described in Table 18–8.

(1) Clinical findings within the intestinal tract:

Diarrhea caused by **enterotoxigenic** *E. coli* **(ETEC)** is usually **watery,** nonbloody, self-limited, and of short duration (1–3 days). It is frequently associated with travel (traveler's diarrhea, or "turista").[2]

Infection with enterohemorrhagic *E. coli* **(EHEC),** on the other hand, results in a dysentery-like syndrome characterized by **bloody diarrhea,** abdominal cramping, and fever similar to that caused by *Shigella*.

The O157:H7 strains of *E. coli* **(STEC)** also cause bloody diarrhea, which can be complicated by **HUS.** This syndrome is characterized by kidney failure, hemolytic anemia, and thrombocytopenia. The hemolytic anemia is caused by exotoxin-induced capillary damage, which results in damage to the red cells as they pass through the capillaries. These distorted, fragmented red cells called **schistocytes** can be seen on blood smear and are characteristic of a microangiopathic hemolytic anemia.

In 2011, an outbreak of diarrhea and HUS in Germany was caused by a Shiga toxin–producing strain of *E. coli* that was typed as O104:H4, not O157:H7. This indicates that strains of *E. coli* other than O157:H7 can also cause HUS.

[2]Enterotoxigenic *E. coli* is the most common cause of traveler's diarrhea, but other bacteria (e.g., *Salmonella, Shigella, Campylobacter,* and *Vibrio* species), viruses such as Norwalk virus, and protozoa such as *Giardia and Cryptosporidium* species are also involved.

HUS occurs particularly in children who have been treated with fluoroquinolones or other antibiotics for their diarrhea. For this reason, antibiotics should not be used to treat diarrhea caused by EHEC.

(2) Clinical findings outside of the intestinal tract:

Escherichia coli is the leading cause of community-acquired **urinary tract infections.** These infections occur primarily in women; this finding is attributed to three features that facilitate ascending infection into the bladder, namely, a short urethra, the proximity of the urethra to the anus, and colonization of the vagina by members of the fecal flora. It is also the most frequent cause of nosocomial (hospital-acquired) urinary tract infections, which occur equally frequently in both men and women and are associated with the use of indwelling urinary catheters. Urinary tract infections can be limited to the bladder or extend up the collecting system to the kidneys. If only the bladder is involved, the disease is called *cystitis*, whereas infection of the kidney is called *pyelonephritis*. The most prominent symptoms of cystitis are pain (dysuria) and frequency of urination; patients are usually afebrile. Pyelonephritis is characterized by fever, flank pain, and costovertebral angle tenderness; dysuria and frequency may or may not occur.

Escherichia coli is also a major cause, along with the group B streptococci, of **meningitis** and sepsis in neonates. Exposure of the newborn to *E. coli* and group B streptococci occurs during birth as a result of colonization of the vagina by these organisms in approximately 25% of pregnant women. *Escherichia coli* is the organism isolated most frequently from patients with hospital-acquired sepsis, which arises primarily from urinary, biliary, or peritoneal infections. Peritonitis is usually a mixed infection caused by *E. coli* or other facultative enteric gram-negative rod plus anaerobic members of the colonic flora such as *Bacteroides* and *Fusobacterium*.

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. *Escherichia 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 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 uses acetate as its only source of carbon, and (4) it is motile. *Escherichia coli* O157: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 (cystitis) can be treated using oral trimethoprim-sulfamethoxazole or nitrofurantoin. Pyelonephritis can be treated with ciprofloxacin or ceftriaxone. However, *E. coli* sepsis requires treatment with parenteral antibiotics (e.g., 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 (e.g., nitrofurantoin or trimethoprim-sulfamethoxazole). The use of cranberry juice to prevent recurrent urinary tract infections appears to be based on the ability of flavonoids in the juice to inhibit the binding of the pili of the uropathic strains of *E. coli* to the bladder epithelium rather than to acidification of the urine, which was the previous explanation.

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. Ingestion of uncooked foods and unpurified water should be avoided while traveling in certain countries.

SALMONELLA

Diseases

Salmonella species cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections such as osteomyelitis. They are one of the most common causes of bacterial enterocolitis in the United States.

Important Properties

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,

TABLE 18–9 Comparison of Important Features of *Salmonella* and *Shigella*

Feature	*Shigella*	*Salmonella* Except *Salmonella typhi*	*Salmonella typhi*
Reservoir	Humans	Animals, especially poultry and eggs	Humans
Infectious dose (ID_{50})	Low[1]	High	High
Diarrhea as a prominent feature	Yes	Yes	No
Invasion of bloodstream	No	Yes	Yes
Chronic carrier state	No	Infrequent	Yes
Lactose fermentation	No	No	No
H_2S production	No	Yes	Yes
Vaccine available	No	No	Yes

[1]An organism with a low ID_{50} requires very few bacteria to cause disease.

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. Only one of the two H proteins is synthesized at any one time, depending on which gene sequence is in the correct alignment for transcription into mRNA. The Vi antigens (capsular polysaccharides) are antiphagocytic and are an important virulence factor for *S. typhi*, the agent of typhoid fever. The Vi antigens are also used for the serotyping of *S. typhi* in the clinical laboratory.

There are three methods for naming the salmonellae. Ewing divides the genus into three species: *S. typhi, Salmonella choleraesuis*, and *Salmonella enteritidis*. In this scheme there is one serotype in each of the first two species and 1500 serotypes in the third. 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. The third approach to naming the salmonellae is based on relatedness determined by DNA hybridization analysis. In this scheme, *S. typhi* is not a distinct species but is classified as *Salmonella enterica* serotype (or serovar) *typhi*. All three of these naming systems are in current use.

Clinically, the *Salmonella* species are often thought of in two distinct categories, namely, the typhoidal species (i.e., those that cause typhoid fever) and the nontyphoidal species (i.e., those that cause diarrhea [enterocolitis] and metastatic infections, such as osteomyelitis). The typhoidal species are *S. typhi* and *S. paratyphi*. The nontyphoidal species are the many serotypes of *S. enterica*. Of the serotypes, *S. enterica* serotype *choleraesuis* is the species most often involved in metastatic infections.

Pathogenesis & Epidemiology

The three types of *Salmonella* infections (enterocolitis, enteric fevers, and septicemia) have different pathogenic 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. Neutrophils limit 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. Various properties of salmonellae and shigellae are compared in Table 18–9. 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 caused by endotoxin. Survival and growth of the organism within phagosomes 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.

(3) **Septicemia** accounts for only about 5% to 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 seen 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. *Salmonella* are also an important cause of vascular graft infections.

The epidemiology of *Salmonella* infections is related to the ingestion of food and water contaminated by human and animal wastes. *Salmonella typhi*, the cause of typhoid fever, is **transmitted only by humans,** but all other species have a significant animal as well as human reservoir. Human sources are either persons who temporarily excrete the organism during or shortly after an attack of enterocolitis or chronic carriers who excrete the organism for years. The most frequent **animal source is poultry and eggs,** but meat products that are inadequately cooked have been implicated as well. Dogs and other pets, including turtles, snakes, lizards, and iguanas, are additional sources.

Clinical Findings

After an incubation period of 12 to 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. HIV-infected individuals, especially those with a low CD4 count, have a much greater number of *Salmonella* infections, including more severe diarrhea and more serious metastatic infections than those who are not infected with HIV. *Salmonella typhimurium* is the most common species of *Salmonella* to cause enterocolitis in the United States, but almost every species has been involved.

In typhoid fever, caused by *S. typhi*, and in enteric fever, caused by organisms such as *S. 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. Diarrhea may occur early but usually disappears by the time the fever and bacteremia occur. After the first week, as the bacteremia becomes sustained, high fever, delirium, tender abdomen, and enlarged spleen occur. **Rose spots** (i.e., rose-colored macules on the abdomen) are associated with typhoid fever but occur only rarely. Leukopenia and anemia are often seen. Liver function tests are often abnormal, indicating hepatic involvement.

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. Bone marrow cultures are often positive. Stool cultures may also be positive, especially in chronic carriers in whom the organism is secreted in the bile into the intestinal tract.

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 into serogroup A, B, C, D, or E based on its O antigen. Definitive serotyping of the O, H, and Vi antigens is performed 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 for septicemia and disseminated abscesses. Plasmid-mediated antibiotic resistance is common, and antibiotic sensitivity tests should be done. Drugs that retard intestinal motility (i.e., 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 when 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, handwashing 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 contains the Vi capsular polysaccharide of *S. typhi* (given intramuscularly), and the other contains a live, attenuated strain (Ty21a) of *S. typhi* (given orally). The two vaccines are equally effective. The vaccine is recommended for those who will travel or reside in high-risk areas and for those whose occupation brings them in contact with the organism. A new conjugate vaccine against typhoid fever containing the capsular polysaccharide (Vi) antigen coupled to a carrier protein is safe and immunogenic in young children but is not available in the United States at this time.

PATHOGENS PRIMARILY WITHIN THE ENTERIC TRACT

SHIGELLA

Disease

Shigella species cause enterocolitis. Enterocolitis caused by *Shigella* is often called bacillary dysentery. The term *dysentery* refers to bloody diarrhea.

Important Properties

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.

Pathogenesis & Epidemiology

Shigellae are the most effective pathogens among the enteric bacteria. They have a **very low ID_{50}** (see page 31). Ingestion of as few as 100 organisms causes disease, whereas at least 10^5 *V. cholerae* or *Salmonella* organisms are required to produce symptoms. Various properties of shigellae and salmonellae are compared in Table 18–9.

Shigellosis is only a **human disease** (i.e., there is no animal reservoir). The organism is transmitted by the fecal–oral route. The four Fs—fingers, flies, food, and feces—are the principal factors in transmission. Foodborne outbreaks outnumber waterborne outbreaks by 2 to 1. Outbreaks occur in day care nurseries and in mental hospitals, where **fecal–oral** transmission is likely to occur. Children younger than 10 years account for approximately half of *Shigella*-positive stool cultures. There is no prolonged carrier state with *Shigella* infections, unlike that seen with *S. typhi* infections.

Shigellae, which cause disease almost exclusively in the gastrointestinal tract, produce bloody diarrhea (dysentery) by invading the cells of the mucosa of the distal ileum and colon. Local inflammation accompanied by ulceration occurs, but the organisms rarely penetrate through the wall or enter the bloodstream, unlike salmonellae. Although some strains produce an enterotoxin (called *Shiga toxin*), 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. Shiga toxins are encoded by lysogenic bacteriophages. Shiga toxins very similar to those produced by *Shigella* are produced by enterohemorrhagic *E. coli* O157:H7 strains that cause enterocolitis and HUS.

Clinical Findings

After an incubation period of 1 to 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 the United States 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 neutrophils 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 also cause diarrhea without neutrophils 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 (e.g., 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 (handwashing by food handlers). There is no vaccine, and prophylactic antibiotics are not recommended.

VIBRIO

Diseases

Vibrio cholerae, the major pathogen in this genus, is the cause of cholera. *Vibrio parahaemolyticus* causes diarrhea associated with eating raw or improperly cooked seafood. *Vibrio vulnificus* causes cellulitis and sepsis. Important features of pathogenesis by *V. cholerae, C. jejuni*, and *Helicobacter pylori* are described in Table 18–10.

Important Properties

Vibrios are curved, **comma-shaped,** gram-negative rods (see Figure 18–2). *V. cholerae* is divided into two groups according to the nature of its O cell wall antigen. Members of the O1 group cause epidemic disease, whereas non-O1 organisms either cause sporadic disease or are nonpathogens. The O1 organisms have two biotypes, called classic and El Tor, and three serotypes, called Ogawa, Inaba, and Hikojima. (Biotypes are based on differences in biochemical reactions, whereas serotypes are based on antigenic differences.) These features are used to characterize isolates in epidemiologic investigations. Serogroup O139 organisms, which caused a major epidemic in 1992, are identified by their reaction to antisera to the O139 polysaccharide antigens (O antigen).

Vibrio parahaemolyticus and *V. vulnificus* are **marine organisms;** they live primarily in the ocean, especially in warm salt water. They are **halophilic** (i.e., they require a high NaCl concentration to grow).

1. *Vibrio cholerae*

Pathogenesis & Epidemiology

Vibrio cholerae is transmitted by **fecal contamination** of water and food, primarily from human sources. Human carriers are frequently asymptomatic and include individuals who are either in the incubation period or convalescing. The main animal reservoirs are marine shellfish, such as shrimp and oysters. Ingestion of these without adequate cooking can transmit the disease.

A major epidemic of cholera, which spanned the 1960s and 1970s, began in Southeast Asia and spread over three continents to areas of Africa, Europe, and the rest of Asia. Another epidemic of cholera began in Peru in 1991 and has spread to many countries in Central and South America. The organism isolated most frequently was the El Tor biotype of O1 *V. cholerae*, usually of the Ogawa serotype. The factors that predispose to epidemics are poor sanitation, malnutrition, overcrowding, and inadequate medical services. Quarantine measures failed to prevent the spread of the disease because there were many asymptomatic carriers. In 1992, *V. cholerae* serogroup O139 emerged and caused a widespread epidemic of cholera in India and Bangladesh.

The pathogenesis of cholera is dependent on colonization of the small intestine by the organism and secretion of enterotoxin. For colonization to occur, large numbers of bacteria must be ingested because the organism is particularly sensitive to stomach acid. Persons with little or no stomach acid, such as those taking antacids or those who have had gastrectomy, are much more susceptible. Adherence to the cells of the brush border of the gut, which is a requirement for colonization, is related to secretion of the bacterial enzyme mucinase, which dissolves the protective glycoprotein coating over the intestinal cells.

After adhering, the organism multiplies and secretes an **enterotoxin** called choleragen (cholera toxin). This exotoxin can reproduce the symptoms of cholera even in the absence of the *Vibrio* organisms. The mode of action of

TABLE 18–10 Important Features of Pathogenesis by Curved Gram-Negative Rods Affecting the Gastrointestinal Tract

Organism	Type of Pathogenesis	Typical Disease	Site of Infection	Main Approach to Therapy
Vibrio cholerae	Toxigenic	Watery diarrhea	Small intestine	Fluid replacement
Campylobacter jejuni	Inflammatory	Bloody diarrhea	Colon	Antibiotics[1]
Helicobacter pylori	Inflammatory	Gastritis; peptic ulcer	Stomach; duodenum	Antibiotics[1]

[1]See text for specific antibiotics.

cholera toxin is described in the next paragraph and in Figure 7–3 in the chapter on bacterial pathogenesis.

Choleragen consists of an A (active) subunit and a B (binding) subunit. The B subunit, which is a pentamer composed of five identical proteins, binds to a ganglioside receptor on the surface of the enterocyte. The A subunit is inserted into the cytosol, where it catalyzes the addition of ADP-ribose to the G_s protein (G_s is the stimulatory G protein). This locks the G_s protein in the "on" position, which causes the persistent stimulation of **adenylate cyclase.** The resulting overproduction of cyclic AMP activates cyclic AMP–dependent protein kinase, an enzyme that phosphorylates ion transporters in the cell membrane, resulting in the loss of water and ions from the cell. The watery efflux enters the lumen of the gut, resulting in a massive watery diarrhea that contains neither neutrophils nor red blood cells. Morbidity and death are due to **dehydration** and **electrolyte imbalance.** However, if treatment is instituted promptly, the disease runs a self-limited course in up to 7 days.

The genes for cholera toxin and other virulence factors are carried on a single-stranded DNA bacteriophage called CTX. Lysogenic conversion of non–toxin-producing strains to toxin-producing ones can occur when the CTX phage transduces these genes. The pili that attach the organism to the gut mucosa are the receptors for the phage.

Non-O1 *V. cholerae* is an occasional cause of diarrhea associated with eating shellfish obtained from the coastal waters of the United States.

Clinical Findings

Watery diarrhea in large volumes is the hallmark of cholera. There are no red blood cells or white blood cells in the stool. **Rice-water stool** is the term often applied to the nonbloody effluent. There is no abdominal pain, and subsequent symptoms are referable to the marked dehydration. The loss of fluid and electrolytes leads to cardiac and renal failure. Acidosis and hypokalemia also occur as a result of loss of bicarbonate and potassium in the stool. The mortality rate without treatment is 40%.

Laboratory Diagnosis

The approach to laboratory diagnosis depends on the situation. During an epidemic, a clinical judgment is made and there is little need for the laboratory. In an area where the disease is endemic or for the detection of carriers, a variety of selective media[3] that are not in common use in the United States are used in the laboratory.

For diagnosis of sporadic cases in this country, a culture of the diarrhea stool containing *V. cholerae* will show colorless colonies on MacConkey's agar because lactose is fermented slowly. The organism is oxidase-positive, which distinguishes it from members of the Enterobacteriaceae. On TSI agar, an acid slant and an acid butt without gas or H_2S are seen because the organism ferments sucrose. A presumptive diagnosis of *V. cholerae* can be confirmed by agglutination of the organism by polyvalent O1 or non-O1 antiserum. A retrospective diagnosis can be made serologically by detecting a rise in antibody titer in acute- and convalescent-phase sera.

Treatment

Treatment consists of prompt, adequate replacement of water and electrolytes, either orally or intravenously. Glucose is added to the solution to enhance the uptake of water and electrolytes. Antibiotics such as tetracycline are not necessary, but they do shorten the duration of symptoms and reduce the time of excretion of the organisms.

Prevention

Prevention is achieved mainly by public health measures that ensure a clean water and food supply. There are two oral vaccines. One, called Dukoral, contains killed whole cells of the O-1 strain plus recombinant cholera toxin subunit B. Antibodies induced by the vaccine prevent ingested *V. cholerae* from attaching to the intestinal mucosa and neutralize any cholera toxin that is produced. The second vaccine is a killed whole cell vaccine called Shanchol. It contains both O-1 and O-159 strains and was reported to be very effective in field trials in 2014. As of this writing, neither vaccine is available in the United States. The injectable killed vaccine is no longer in use.

The use of tetracycline for prevention is effective in close contacts but does not prevent the spread of a major epidemic. Prompt detection of carriers is important in limiting outbreaks.

2. *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is a marine organism transmitted by **ingestion of raw or undercooked seafood,** especially shellfish such as oysters. It is a major cause of diarrhea in Japan, where raw fish is eaten in large quantities, but is an infrequent pathogen in the United States, although several outbreaks have occurred aboard cruise ships in the Caribbean. Little is known about its pathogenesis, except that an enterotoxin similar to choleragen is secreted and limited invasion sometimes occurs.

The clinical picture caused by *V. parahaemolyticus* varies from mild to quite severe watery diarrhea, nausea and vomiting, abdominal cramps, and fever. The illness is self-limited, lasting about 3 days. *Vibrio parahaemolyticus* is distinguished from *V. cholerae* mainly on the basis of growth in NaCl: *V. parahaemolyticus* grows in 8% NaCl solution (as befits a marine organism), whereas *V. cholerae*

[3] Media such as thiosulfate-citrate-bile salts agar or tellurite-taurocholate-gelatin are used.

does not. No specific treatment is indicated, because the disease is relatively mild and self-limited. Disease can be prevented by proper refrigeration and cooking of seafood.

3. *Vibrio vulnificus*

Vibrio vulnificus is also a marine organism (i.e., it is found in warm salt waters such as the Caribbean Sea). It causes severe skin and soft tissue infections (**cellulitis**), **especially in shellfish handlers**, who often sustain skin wounds. It can also cause a rapidly fatal **septicemia in immunocompromised people who have eaten raw shellfish** containing the organism. Hemorrhagic bullae in the skin often occur in patients with sepsis caused by *V. vulnificus*. Chronic liver disease (e.g., cirrhosis) predisposes to severe infections. The recommended treatment is doxycycline.

CAMPYLOBACTER

Diseases

Campylobacter jejuni is a frequent cause of enterocolitis, especially in children. *C. jejuni* infection is a common antecedent to Guillain-Barré syndrome. Other *Campylobacter* species are rare causes of systemic infection, particularly bacteremia.

Important Properties

Campylobacters are curved, gram-negative rods that appear either **comma**- or **S-shaped.** They are **microaerophilic,** growing best in 5% oxygen rather than in the 20% present in the atmosphere. *C. jejuni* grows well at 42°C, whereas *Campylobacter intestinalis*[4] does not—an observation that is useful in microbiologic diagnosis.

Pathogenesis & Epidemiology

Domestic animals such as cattle, chickens, and dogs serve as a source of the organisms for humans. Transmission is usually **fecal–oral.** Food and water contaminated with animal feces are the major sources of human infection. Foods, such as poultry, meat, and unpasteurized milk, are commonly involved. Puppies with diarrhea are a common source for children. Human-to-human transmission occurs but is less frequent than animal-to-human transmission. *Campylobacter jejuni* is a major cause of diarrhea in the United States; it was recovered in 4.6% of patients with diarrhea, compared with 2.3% and 1% for *Salmonella* and *Shigella*, respectively. *Campylobacter jejuni* is the leading cause of diarrhea associated with consumption of unpasteurized milk.

Features of pathogenesis by *Campylobacter* are described in Table 18–10. Inflammation of the intestinal mucosa often occurs, accompanied by blood in stools. Systemic infections (e.g., bacteremia) occur most often in neonates or debilitated adults.

Clinical Findings

Enterocolitis, caused primarily by *C. jejuni*, begins as watery, foul-smelling diarrhea followed by bloody stools accompanied by fever and severe abdominal pain. Systemic infections, most commonly bacteremia, are caused more often by *C. intestinalis*. The symptoms of bacteremia (e.g., fever and malaise) are associated with no specific physical findings.

Gastrointestinal infection with *C. jejuni* is associated with Guillain-Barré syndrome, the most common cause of acute neuromuscular paralysis. Guillain-Barré syndrome is an autoimmune disease attributed to the formation of antibodies against *C. jejuni* that cross-react with antigens on neurons (see Chapter 66). Infection with *Campylobacter* is also associated with two other autoimmune diseases: reactive arthritis and Reiter's syndrome. These are also described in Chapter 66.

Laboratory Diagnosis

If the patient has diarrhea, a stool specimen is cultured on a blood agar plate containing antibiotics[5] that inhibit most other fecal flora.

The plate is incubated at 42°C in a microaerophilic atmosphere containing 5% oxygen and 10% carbon dioxide, which favors the growth of *C. jejuni*. It is identified by failure to grow at 25°C, oxidase positivity, and sensitivity to nalidixic acid. Unlike *Shigella* and *Salmonella*, lactose fermentation is not used as a distinguishing feature. If bacteremia is suspected, a blood culture incubated under standard temperature and atmospheric conditions will reveal the growth of the characteristically comma- or S-shaped, motile, gram-negative rods. Identification of the organism as *C. intestinalis* is confirmed by its failure to grow at 42°C, its ability to grow at 25°C, and its resistance to nalidixic acid.

Treatment

Erythromycin or ciprofloxacin is used successfully in *C. jejuni* enterocolitis. The treatment of choice for *C. intestinalis* bacteremia is an aminoglycoside.

Prevention

There is no vaccine or other specific preventive measure. Proper sewage disposal and personal hygiene (handwashing) are important.

[4] Also known as *Campylobacter* fetus subsp. fetus.

[5] For example, Skirrow's medium contains vancomycin, trimethoprim, cephalothin, polymyxin, and amphotericin B.

HELICOBACTER

Diseases

Helicobacter pylori causes gastritis and peptic ulcers. Infection with *H. pylori* is a risk factor for gastric carcinoma and is linked to mucosal-associated lymphoid tissue (MALT) lymphomas.

Important Properties

Helicobacters are curved gram-negative rods similar in appearance to campylobacters, but because they differ sufficiently in certain biochemical and flagellar characteristics, they are classified as a separate genus. In particular, helicobacters are strongly urease-positive, whereas campylobacters are urease-negative.

Pathogenesis & Epidemiology

Helicobacter pylori attaches to the mucus-secreting cells of the gastric mucosa. The production of large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa. Loss of the protective mucus coating predisposes to gastritis and peptic ulcer (see Table 18–10). The ammonia also neutralizes stomach acid, allowing the organism to survive. Epidemiologically, most patients with these diseases show *H. pylori* in biopsy specimens of the gastric epithelium.

The natural habitat of *H. pylori* is the human stomach, and it is probably acquired by ingestion. However, it has not been isolated from stool, food, water, or animals. Person-to-person transmission probably occurs because there is clustering of infection within families. The rate of infection with *H. pylori* in developing countries is very high—a finding that is in accord with the high rate of gastric carcinoma in those countries.

MALT lymphomas are B-cell tumors located typically in the stomach, but they occur elsewhere in the gastrointestinal tract as well. *Helicobacter pylori* is often found in the MALT lesion, and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma. Antibiotic treatment directed against the organism often causes the tumor to regress.

Clinical Findings

Gastritis and peptic ulcer are characterized by recurrent pain in the upper abdomen, frequently accompanied by bleeding into the gastrointestinal tract. No bacteremia or disseminated disease occurs.

Laboratory Diagnosis

The organism can be seen on Gram-stained smears of biopsy specimens of the gastric mucosa. It can be cultured on the same media as campylobacters. In contrast to *C. jejuni, H. pylori* is urease-positive. Urease production is the basis for a noninvasive diagnostic test called the "urea breath" test. In this test, radiolabeled urea is ingested. If the organism is present, urease will cleave the ingested urea, radiolabeled CO_2 is evolved, and the radioactivity is detected in the breath.

A test for *Helicobacter* antigen in the stool can be used for diagnosis and for confirmation that treatment has eliminated the organism. The presence of IgG antibodies in the patient's serum can also be used as evidence of infection.

Treatment & Prevention

The concept that underlies the choice of drugs is to use antibiotics to eliminate *Helicobacter* plus a drug to reduce gastric acidity. A combination of two antibiotics is used because resistance, especially to metronidazole, has emerged. Treatment of duodenal ulcers with antibiotics (e.g., amoxicillin and metronidazole) and bismuth salts (Pepto-Bismol) results in a greatly decreased recurrence rate. Tetracycline can be used instead of amoxicillin. There is no vaccine or other specific preventive measure.

PATHOGENS OUTSIDE THE ENTERIC TRACT

KLEBSIELLA–ENTEROBACTER–SERRATIA GROUP

Diseases

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.

Important Properties

Klebsiella 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. *Klebsiella pneumoniae* has a **very large polysaccharide capsule,** which gives its colonies a striking mucoid appearance. *Serratia marcescens* produces **red-pigmented colonies** (Figure 18–3).

Pathogenesis & Epidemiology

Of the three organisms, *K. pneumoniae* is most likely to be a primary, nonopportunistic pathogen; this property is

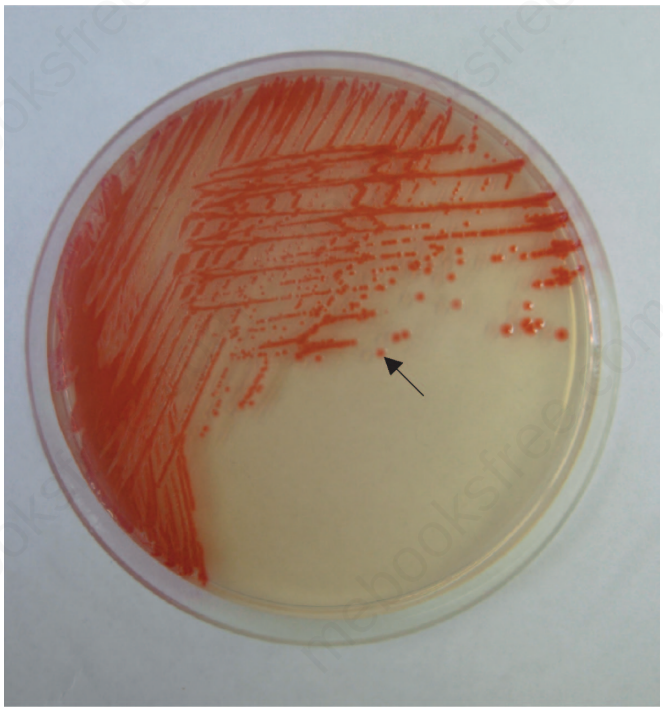

FIGURE 18–3 *Serratia marcescens*—red-pigmented colonies. Arrow points to a red-pigmented colony of *S. marcescens*. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

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 tract of about 10% of healthy 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.

Serratia also causes endocarditis in users of injection drugs. 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 and liver 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, mucoid, 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 United States. *Klebsiella ozaenae* 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 produce a negative reaction. These organisms are differentiated 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. Carbapenem-resistant strains are an important cause of hospital-acquired infections and are resistant to almost all known antibiotics. An aminoglycoside (e.g., gentamicin) and a cephalosporin (e.g., 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.

PROTEUS–PROVIDENCIA–MORGANELLA GROUP

Diseases

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,

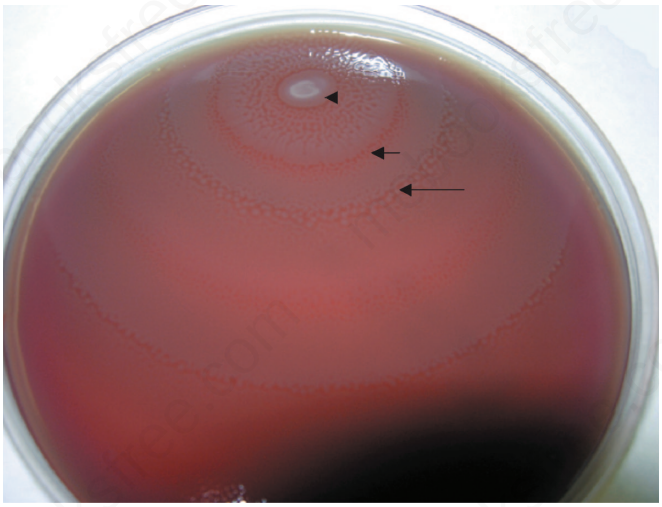

FIGURE 18–4 *Proteus* species—swarming motility on blood agar. Arrowhead points to the site where *Proteus* bacteria were placed on the blood agar. Short arrow points to the edge of the first ring of swarming motility. Long arrow points to the edge of the second ring of swarming motility. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

characterized by expanding rings (waves) of organisms over the surface of the agar (Figure 18–4).

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 developed.

In the past, there were four medically important species of *Proteus*. However, molecular studies of DNA relatedness showed that two of the four were significantly different. 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, producing an alkaline urine. This encourages the formation of stones (calculi) called **"struvite"** composed of magnesium ammonium phosphate. Struvite stones often manifest, as staghorn calculi in the renal pelvis. They obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone. 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. *Proteus mirabilis* is the species of *Proteus* that causes most community- and hospital-acquired infections, but *P. 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 (colorless) colonies on MacConkey's or EMB agar. *Proteus vulgaris* and *P. mirabilis* produce H_2S, which blackens the butt of TSI agar, whereas neither *M. morganii* nor *P. rettgeri* does. *Proteus 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 organisms 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. *Proteus 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 is *P. mirabilis*, which is indole-negative. The treatment of choice for the indole-positive species is a cephalosporin (e.g., cefotaxime). *Proteus 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.

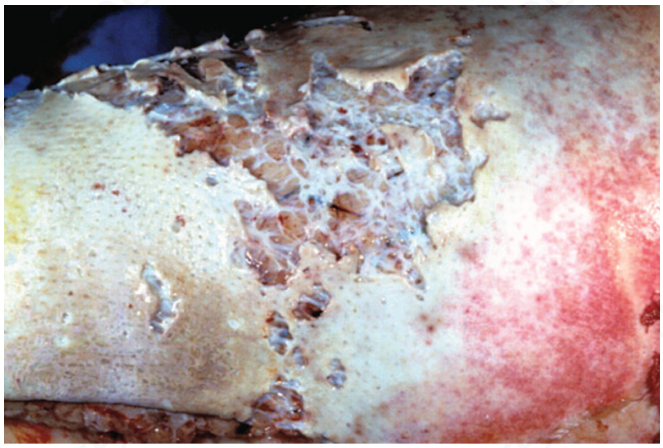

FIGURE 18–5 Cellulitis caused by *Pseudomonas aeruginosa*. Note the blue-green color of the pus in the burn wound infection. (Used with permission from Dr. Robert L. Sheridan.)

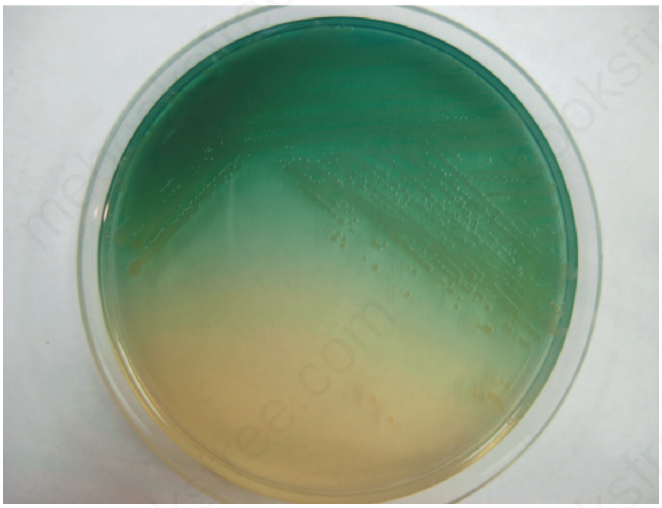

FIGURE 18–6 *Pseudomonas aeruginosa*—blue-green pigment. Blue-green pigment (pyocyanin) produced by *P. aeruginosa* diffuses into the agar. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

PSEUDOMONAS

Diseases

Pseudomonas aeruginosa causes infections (e.g., sepsis, pneumonia, and urinary tract infections) primarily in patients with lowered host defenses. It also causes chronic lower respiratory tract infections in patients with cystic fibrosis, wound infections (cellulitis) in burn patients (Figure 18–5), and malignant otitis externa in diabetic patients. It is the most common cause of ventilator-associated pneumonia. (*P. aeruginosa* is also known as *Burkholderia aeruginosa*.) *Pseudomonas cepacia* (renamed *Burkholderia cepacia*) and *Pseudomonas maltophilia* (renamed *Xanthomonas maltophilia* and now called *Stenotrophomonas maltophilia*) also cause these infections, but much less frequently. *Pseudomonas pseudomallei* (also known as *Burkholderia pseudomallei*), the cause of melioidosis, is described in Chapter 27.

Important Properties

Pseudomonads are gram-negative rods that resemble the members of the Enterobacteriaceae but differ in that they are strict aerobes (i.e., they derive their energy only by oxidation of sugars rather than by fermentation). Because they do not ferment glucose, they are called **nonfermenters,** in contrast to the members of the Enterobacteriaceae, which do ferment glucose. Oxidation involves electron transport by cytochrome c (i.e., they are **oxidase-positive**).

Pseudomonads are able to grow in **water** containing only traces of nutrients (e.g., tap water), and this favors their persistence in the hospital environment. *Pseudomonas aeruginosa* and *B. cepacia* have a remarkable ability to withstand disinfectants; this accounts in part for their role in hospital-acquired infections. They have been found growing in hexachlorophene-containing soap solutions, in antiseptics, and in detergents.

Pseudomonas aeruginosa produces two pigments useful in clinical and laboratory diagnosis: (1) **pyocyanin,** which can **color the pus in a wound blue,** and (2) pyoverdin (fluorescein), a yellow-green pigment that fluoresces under ultraviolet light, a property that can be used in the early detection of skin infection in burn patients. In the laboratory, these **pigments diffuse into the agar, imparting a blue-green color** that is useful in identification. *Pseudomonas aeruginosa* is the only species of *Pseudomonas* that synthesizes pyocyanin (Figure 18–6).

Strains of *P. aeruginosa* isolated from cystic fibrosis patients have a prominent slime layer (glycocalyx), which gives their colonies a very mucoid appearance. The slime layer mediates adherence of the organism to mucous membranes of the respiratory tract and prevents antibody from binding to the organism.

Pathogenesis & Epidemiology

Pseudomonas aeruginosa is found chiefly in soil and water, although approximately 10% of people carry it in the normal flora of the colon. It is found on the skin in moist areas and can colonize the upper respiratory tract of hospitalized patients. Its ability to grow in simple aqueous solutions has resulted in contamination of respiratory therapy and anesthesia equipment, intravenous fluids, and even distilled water.

Pseudomonas aeruginosa is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those with extensive burns), in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed; in those with neutrophil counts of less than 500/μL;

and in those with indwelling catheters. It causes 10% to 20% of hospital-acquired infections and, in many hospitals, is the most common cause of gram-negative nosocomial pneumonia, especially ventilator-associated pneumonia.

Pathogenesis is based on multiple virulence factors: endotoxin, exotoxins, and enzymes. Its endotoxin, like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock. The best known of the exotoxins is exotoxin A, which causes tissue necrosis. It inhibits eukaryotic protein synthesis by the same mechanism as diphtheria exotoxin, namely, ADP-ribosylation of elongation factor-2. It also produces enzymes, such as elastase and proteases that are histotoxic and facilitate invasion of the organism into the bloodstream. Pyocyanin damages the cilia and mucosal cells of the respiratory tract.

Strains of *P. aeruginosa* that have a "type III secretion system" are significantly more virulent than those that do not. This secretion system transfers the exotoxin from the bacterium directly into the adjacent human cell, which allows the toxin to avoid neutralizing antibody. Type III secretion systems are mediated by transport pumps in the bacterial cell membrane. Of the four exoenzymes known to be transported by this secretion system, Exo S is the one most clearly associated with virulence. Exo S has several modes of action, the most important of which is ADP-ribosylation of a Ras protein, leading to damage to the cytoskeleton.

Clinical Findings

Pseudomonas aeruginosa can cause infections virtually anywhere in the body, but urinary tract infections, pneumonia (especially in **cystic fibrosis** patients), and wound infections (especially burns) (see Figure 18–5) predominate. It is an important cause of hospital-acquired pneumonia, especially in those undergoing mechanical ventilation (ventilator-associated pneumonia). From these sites, the organism can enter the blood, causing sepsis. The bacteria can spread to the skin, where they cause black, necrotic lesions called **ecthyma gangrenosum** (Figure 18–7). Patients with *P. aeruginosa* sepsis have a mortality rate of greater than 50%. It is an important cause of endocarditis in intravenous drug users.

Severe external otitis (malignant otitis externa) and other skin lesions (e.g., folliculitis) occur in users of swimming pools and hot tubs (hot tub folliculitis) in which the chlorination is inadequate. *Pseudomonas aeruginosa* is the most common cause of osteomyelitisof the foot in those who sustain puncture wounds through the soles of gym shoes. Corneal infections caused by *P. aeruginosa* are seen in contact lens users.

Laboratory Diagnosis

Pseudomonas aeruginosa grows as non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. It is

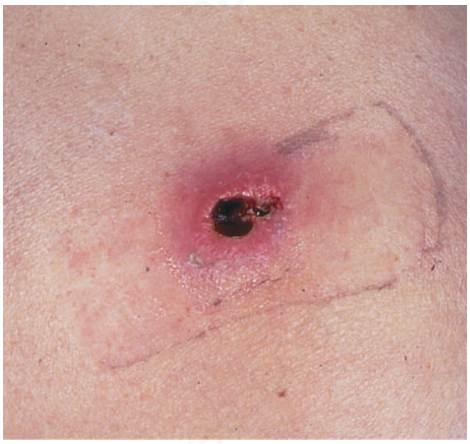

FIGURE 18–7 Ecthyma gangrenosum. Necrotic skin lesion caused by *Pseudomonas aeruginosa.* (Reproduced with permission from Wolff K, Johnson R, Saavedra A (eds): *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 7th ed. New York: McGraw-Hill, 2013. Copyright © 2013 by The McGraw-Hill Companies, Inc.)

oxidase-positive. A typical metallic sheen of the growth on TSI agar, coupled with the blue-green pigment on ordinary nutrient agar (see Figure 18–6), and a fruity aroma are sufficient to make a presumptive diagnosis. The diagnosis is confirmed by biochemical reactions. Identification for epidemiologic purposes is done by bacteriophage or pyocin[6] typing.

Treatment

Because *P. aeruginosa* is **resistant to many antibiotics,** treatment must be tailored to the sensitivity of each isolate and monitored frequently; resistant strains can emerge during therapy. The treatment of choice is an antipseudomonal penicillin (e.g., piperacillin/tazobactam or ticarcillin/clavulanate) plus an aminoglycoside (e.g., gentamicin or amikacin).Ceftazidime is also effective.For infections caused by highly resistant strains, colistin (polymyxin E) is useful. The drug of choice for urinary tract infections is ciprofloxacin. The drug of choice for infections caused by *B. cepacia* and *S. maltophilia* is trimethoprim-sulfamethoxazole.

Prevention

Prevention of *P. aeruginosa* infections involves keeping neutrophil counts above 500/µL, removing indwelling catheters promptly, taking special care of burned skin, and taking other similar measures to limit infection in patients with reduced host defenses.

[6]A pyocin is a type of bacteriocin produced by *P. aeruginosa.* Different strains produce various pyocins, which can serve to distinguish the organisms.

BACTEROIDES & PREVOTELLA

Diseases

Members of the genus *Bacteroides* are the most common cause of serious anaerobic infections (e.g., sepsis, peritonitis, and abscesses). *Bacteroides fragilis* is the most frequent pathogen. *Prevotella melaninogenica* is also an important pathogen. It was formerly known as *Bacteroides melaninogenicus*, and both names are still encountered.

Important Properties

Bacteroides and *Prevotella* organisms are anaerobic, non–spore-forming, gram-negative rods. Of the many species of *Bacteroides*, two are human pathogens: *B. fragilis*[7] and *Bacteroides corrodens*.

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. *Prevotella melaninogenica* and *B. corrodens* occur primarily in the oral cavity.

Pathogenesis & Epidemiology

Because *Bacteroides* and *Prevotella* 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* and *Prevotella* 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. The host response to the capsule plays a major role in abscess formation. Note also that the endotoxin of *B. fragilis* contains a variant lipid A that is missing one of the fatty acids and consequently is 1000-fold less active than the typical endotoxin of bacteria such as *Neisseria meningitidis*.

Enzymes such as hyaluronidase, collagenase, and phospholipase are produced and contribute to tissue damage. Enterotoxin-producing strain of *B. fragilis* can cause diarrhea in both children and adults.

Clinical Findings

The *B. fragilis* group of organisms is most frequently associated with intra-abdominal infections, either peritonitis or localized abscesses. Pelvic abscesses, necrotizing fasciitis, and bacteremia occur as well. Abscesses of the mouth, pharynx, brain, and lung are more commonly caused by *P. melaninogenica*, 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 *P. melaninogenica* causes disease above the diaphragm. *Prevotella intermedia* is an important cause of gingivitis, periodontitis, and dental abscess.

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 (e.g., sugar fermentations) and by production of certain organic acids (e.g., formic, acetic, and propionic acids), which are detected by gas chromatography. *Prevotella melaninogenica* produces characteristic black colonies (Figure 18–8).

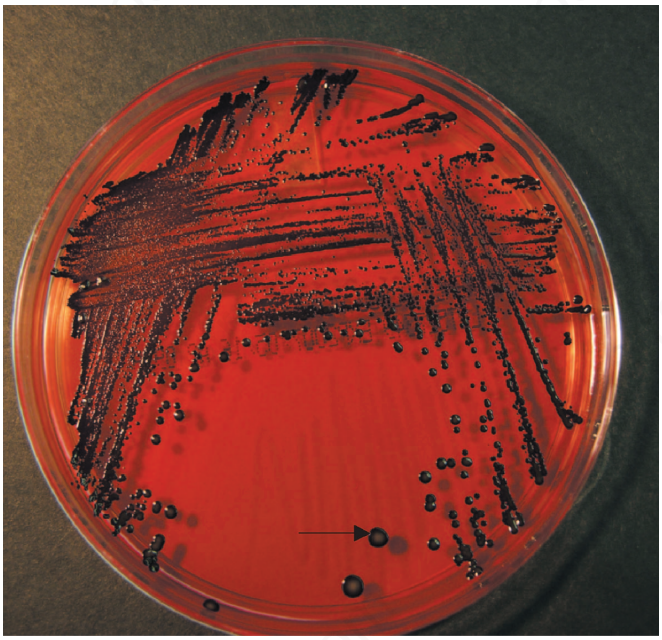

FIGURE 18–8 *Prevotella melaninogenica*—black pigmented colonies. Arrow points to a black pigmented colony of *P. melaninogenica*. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

[7] *B. fragilis* is divided into five subspecies, the most important of which is *B. fragilis* subsp. *fragilis*. The other four subspecies are *B. fragilis* subsp. *distasonis*, *ovatus*, *thetaiotamicron*, and *vulgatus*. It is proper, therefore, to speak of the *B. fragilis* group rather than simply *B. fragilis*.

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. The drug of choice for *P. melaninogenica* infections is either metronidazole or clindamycin. β-Lactamase–producing strains of *P. melaninogenica* have been isolated from patients. Surgical drainage of abscesses usually accompanies antibiotic therapy, but lung abscesses often heal without drainage.

Prevention

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

FUSOBACTERIUM

Fusobacterium species are long, anaerobic gram-negative rods with pointed ends (Figure 18–9). They are part of the human normal flora of the mouth, colon, and female genital tract and are isolated from brain, pulmonary, intra-abdominal, and pelvic abscesses. They are frequently found in mixed infections with other anaerobes and facultative anaerobes.

Fusobacterium nucleatum occurs, along with various spirochetes, in cases of Vincent's angina (trench mouth), which is characterized by a necrotizing ulcerative gingivitis. *Fusobacterium necrophorum* causes Lemierre's disease, which is an anaerobic infection of the posterior pharyngeal space accompanied by thrombophlebitis of the internal jugular vein and metastatic infectious emboli to the lung.

The laboratory diagnosis is made by culturing the organism anaerobically. The drug of choice for *Fusobacterium* infections is either penicillin G, clindamycin, or metronidazole. There is no vaccine.

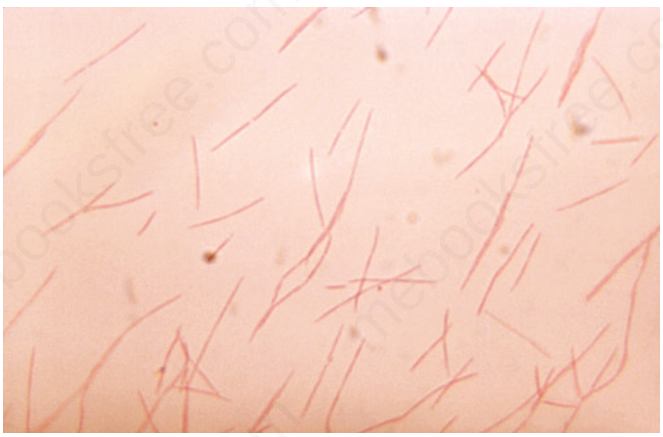

FIGURE 18–9 *Fusobacterium nucleatum*—Gram stain. Note the long, thin gram-negative rods with pointed ends. (Source: Dr. V.R. Dowell, Jr. Public Health Image Library, Centers for Disease Control and Prevention.)

SELF-ASSESSMENT QUESTIONS

1. Your patient is a 75-year-old man with an indwelling urinary catheter following prostatectomy for prostate cancer. He now has the sudden onset of fever to 40°C, blood pressure of 70/40, and a pulse of 140. You draw several blood cultures, and the laboratory reports that all are positive for a gram-negative rod that forms red pigmented colonies. Which one of the following bacteria is the most likely cause of this infection?
   (A) *Escherichia coli*
   (B) *Klebsiella pneumoniae*
   (C) *Proteus mirabilis*
   (D) *Pseudomonas aeruginosa*
   (E) *Serratia marcescens*
2. You're a public health epidemiologist who is called to investigate an outbreak of bloody diarrhea in 16 people. You find that it is associated with eating rare hamburgers in a particular fast-food restaurant. A culture of the remaining uncooked hamburger grows a gram-negative rod that produces a dark purple colony on EMB agar, which is evidence that it ferments lactose. Which one of the following bacteria is the most likely cause of this outbreak?
   (A) *Escherichia coli*
   (B) *Salmonella enterica*
   (C) *Salmonella typhi*
   (D) *Shigella dysenteriae*
   (E) *Vibrio cholerae*
3. Your patient has third-degree burns over most of his body. He was doing well until 2 days ago, when he spiked a fever, and his dressings revealed pus that had a blue-green color. Gram stain of the pus revealed a gram-negative rod that formed colorless colonies on EMB agar. Which one of the following bacteria is the most likely cause of this infection?
   (A) *Campylobacter jejuni*
   (B) *Escherichia coli*
   (C) *Haemophilus influenzae*
   (D) *Pseudomonas aeruginosa*
   (E) *Salmonella enterica*
4. Regarding the patient in Question 3, which one of the following is the best combination of antibiotics to treat the infection?
   (A) Azithromycin plus gentamicin
   (B) Doxycycline plus gentamicin
   (C) Metronidazole plus gentamicin
   (D) Piperacillin/tazobactam plus gentamicin
   (E) Vancomycin plus gentamicin

5. Regarding the members of the family Enterobacteriaceae, which one of the following is the most accurate?

(A) All members of the family are anaerobic, which means they must be cultured in the absence of oxygen.
(B) All members of the family ferment lactose, which is an important diagnostic criterion in the clinical laboratory.
(C) All members of the family have endotoxin, an important pathogenetic factor.
(D) All members of the family produce an enterotoxin, which ADP-ribosylates a G protein in human enterocytes.

6. You're on a summer program working in a clinic in a small village in Ecuador. There is an outbreak of cholera, and your patient has massive diarrhea and a blood pressure of 70/40. Which one of the following would be the most appropriate action to take?

(A) Administer antimotility drugs to diminish the diarrhea.
(B) Administer intravenous saline to replenish volume.
(C) Administer tetracycline to kill the organism.
(D) Perform stool cultures and fecal leukocyte tests to make an accurate diagnosis.

7. Your patient is a 20-year-old woman with diarrhea. She has just returned to the United States from a 3-week trip to Peru, where she ate some raw shellfish at the farewell party. She now has severe watery diarrhea, perhaps 20 bowel movements a day, and is feeling quite weak and dizzy. Her stool is guaiac-negative, a test that determines whether there is blood in the stool. A Gram stain of the stool reveals curved gram-negative rods. Culture of the stool on MacConkey's agar shows colorless colonies. Which one of the following bacteria is the most likely cause of this infection?

(A) *Escherichia coli*
(B) *Helicobacter pylori*
(C) *Proteus mirabilis*
(D) *Pseudomonas aeruginosa*
(E) *Vibrio cholerae*

8. Your patient is a 6-year-old boy with bloody diarrhea for the past 2 days accompanied by fever to 40°C and vomiting. He has a pet corn snake. Blood culture and stool culture from the boy and stool culture from the snake (taken very carefully!) revealed the same organism. The cultures grew a gram-negative rod that formed colorless colonies on EMB agar. Which one of the following bacteria is the most likely cause of this infection?

(A) *Helicobacter pylori*
(B) *Proteus mirabilis*
(C) *Salmonella enterica*
(D) *Shigella dysenteriae*
(E) *Vibrio cholerae*

9. Your patient is a 25-year-old woman with pain on urination and cloudy urine but no fever or flank pain. She has not been hospitalized. You think she probably has cystitis, an infection of the urinary bladder. A Gram stain of the urine reveals gram-negative rods. Culture of the urine on EMB agar shows colorless colonies, and a urease test was positive. Swarming motility was noted on the blood agar plate. Which one of the following bacteria is the most likely cause of this infection?

(A) *Escherichia coli*
(B) *Helicobacter pylori*
(C) *Proteus mirabilis*
(D) *Pseudomonas aeruginosa*
(E) *Serratia marcescens*

10. Your patient has abdominal pain, and a mass is discovered in the left lower quadrant. Upon laparotomy (surgical opening of the abdomen), an abscess is found. Culture of the pus revealed *Bacteroides fragilis*. Regarding this organism, which one of the following is the most accurate?

(A) A stage in the life cycle of *Bacteroides fragilis* involves forming spores in the soil.
(B) *Bacteroides fragilis* is an anaerobic gram-negative rod whose natural habitat is the human colon.
(C) *Bacteroides fragilis* produces black colonies when grown on blood agar.
(D) Pathogenesis by *Bacteroides fragilis* involves an exotoxin that increases cyclic AMP by ADP-ribosylation of a G protein.
(E) The toxoid vaccine should be administered to prevent disease caused by *Bacteroides fragilis*.

11. Regarding the patient in Question 10, which one of the following is the best antibiotic to treat the infection?

(A) Doxycycline
(B) Gentamicin
(C) Metronidazole
(D) Penicillin G
(E) Rifampin

12. Your patient in the gastrointestinal clinic is a 50-year-old insurance salesman with what he describes as a "sour stomach" for several months. Antacids relieve the symptoms. After taking a complete history and doing a physical examination, you discuss the case with your resident, who suggests doing a urea breath test, which tests for the presence of urease. Which one of the following bacteria does the resident think is the most likely cause of the patient's disease?

(A) *Helicobacter pylori*
(B) *Proteus mirabilis*
(C) *Salmonella enterica*
(D) *Serratia marcescens*
(E) *Shigella dysenteriae*

13. Your patient is a 35-year-old woman with epilepsy who had a grand-mal seizure about 2 months ago. She comes to see you now because she has been coughing up foul-smelling sputum for the past week. Chest X-ray reveals a cavity with an air-fluid level. Gram stain of the sputum reveals gram-negative rods, and culture reveals black colonies that grow on blood agar only in the absence of air. Which one of the following bacteria is the most likely cause of this infection?

(A) *Bacteroides fragilis*
(B) *Campylobacter jejuni*
(C) *Klebsiella pneumoniae*
(D) *Prevotella melaninogenica*
(E) *Proteus mirabilis*

ANSWERS

1. **(E)**
2. **(A)**
3. **(D)**
4. **(D)**
5. **(C)**
6. **(B)**
7. **(E)**

8. **(C)**
9. **(C)**
10. **(B)**
11. **(C)**
12. **(A)**
13. **(D)**

SUMMARIES OF ORGANISMS

Brief summaries of the organisms described in this chapter begin on page 658. Please consult these summaries for a rapid review of the essential material.

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Clinical Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 713. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.