CHAPTER 16

Gram-Negative Cocci

CHAPTER CONTENTS

NEISSERIA

Diseases

The genus *Neisseria* contains two important human pathogens: *Neisseria meningitidis* and *Neisseria gonorrhoeae. Neisseria meningitidis* mainly causes meningitis and meningococcemia (Figure 16–1). In the United States, it is the leading cause of death from infection in children. *Neisseria gonorrhoeae* causes gonorrhea (Figure 16–2), the second most common notifiable bacterial disease in the United States (Tables 16–1 and 16–2). It also causes neonatal conjunctivitis (ophthalmia neonatorum) (Figure 16–3) and pelvic inflammatory disease (PID). Note that *N. meningitidis* is also known as the meningococcus (plural, meningococci), and *N. gonorrhoeae* is also known as the gonococcus (plural, gonococci).

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.

Important Properties

Neisseriae are gram-negative cocci that resemble paired kidney beans (Figure 16–4).

(1) *Neisseria meningitidis* (meningococcus) has a prominent **polysaccharide capsule** that enhances virulence by its antiphagocytic action. The capsule also is the immunogen in the vaccine that induces protective antibodies (Table 16–3). Meningococci are divided into at least 13 serologic groups on the basis of the antigenicity of their capsular polysaccharides. Five serotypes cause most cases of meningitis and meningococcemia: A, B, C, Y, and W-135. Serotype A is the leading cause of epidemic meningitis worldwide. Serotype B accounts for most disease in the United States. This is because the group B polysaccharide is not immunogenic in humans and therefore is not part of the vaccines that contain the capsular polysaccharide of the other four groups. In 2014, a vaccine against group B meningococci containing factor H binding protein as the immunogen was approved.

(2) *Neisseria gonorrhoeae* (gonococcus) has no polysaccharide capsule but has multiple serotypes based on the antigenicity of its pilus protein. There is **marked antigenic variation** in the gonococcal pili as a result of chromosomal rearrangement; more than 100 serotypes are known. Gonococci have three outer membrane proteins (proteins I, II, and III). Protein II plays a role in attachment of the organism to cells and varies antigenically as well.

Neisseriae are gram-negative bacteria and contain endotoxin in their outer membrane. Note that the endotoxin of Neisseriae consist of lipo**oligo**saccharide (LOS), in contrast to the lipo**poly**saccharide (LPS) found in enteric gram-negative rods. Both LPS and LOS contain lipid A, but the oligosaccharide part of LOS contains few sugars, whereas the polysaccharide part of LPS contains a long repeating sugar side chain.

The growth of both organisms is inhibited by toxic trace metals and fatty acids found in certain culture media (e.g., blood agar plates). They are therefore cultured on "chocolate" agar containing blood heated to 80°C, which inactivates the inhibitors. Neisseriae are **oxidase-positive** (Figure 16–5) (i.e., they possess the enzyme cytochrome *c*). This is an important laboratory diagnostic test in which colonies exposed to phenylenediamine turn purple or black as a result of oxidation of the reagent by the enzyme (see Figure 16–2).

The genus *Neisseria* is one of several in the family Neisseriaceae. A separate genus contains the organism *Moraxella catarrhalis*, which is part of the normal throat

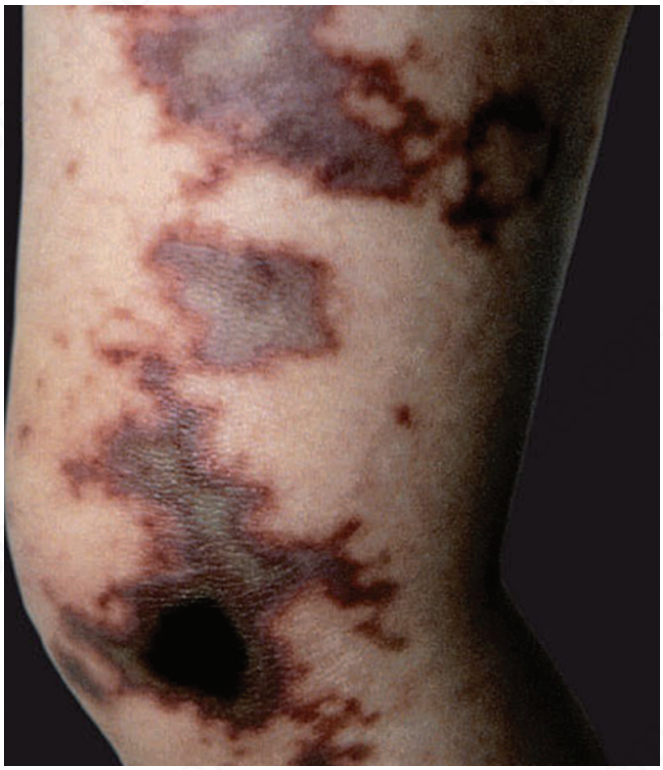

FIGURE 16–1 Meningococcemia. Note purpuric lesions on leg caused by endotoxin-mediated disseminated intravascular coagulation (DIC). (Reproduced with permission from Wolff K, Johnson R (eds): *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 6th ed. New York: McGraw-Hill, 2009. Copyright © 2009 by The McGraw-Hill Companies, Inc.)

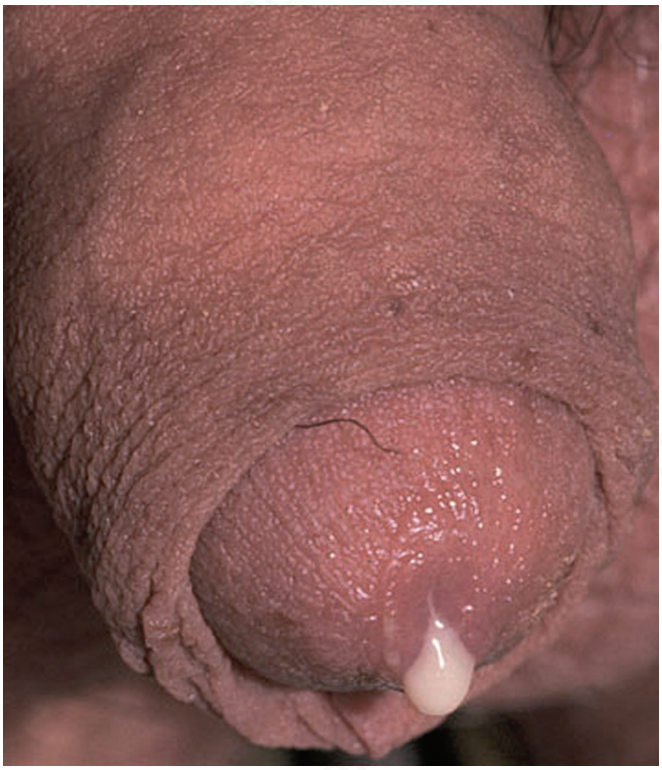

FIGURE 16–2 Gonorrhea. Note purulent urethral discharge caused by *Neisseria gonorrhoeae*. (Reproduced with permission from Wolff K, Johnson R (eds): *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 6th ed. New York: McGraw-Hill, 2009. Copyright © 2009 by The McGraw-Hill Companies, Inc.)

TABLE 16–1 Neisseriae of Medical Importance[1]

Species	Portal of Entry	Polysaccharide Capsule	Maltose Fermentation	β-Lactamase Production	Available Vaccine
N. meningitidis (meningococcus)	Respiratory tract	+	+	None	+
N. gonorrhoeae (gonococcus)	Genital tract	–	–	Some	–

[1]All neisseriae are oxidase-positive.

TABLE 16–2 Important Clinical Features of Neisseriae

Organism	Type of Pathogenesis	Typical Disease	Treatment
N. meningitidis	Pyogenic	Meningitis, meningococcemia	Penicillin G
N. gonorrhoeae	Pyogenic		
	1. Local	Gonorrhea (e.g., urethritis, cervicitis)	Ceftriaxone[1] plus doxycycline[2]
	2. Ascending	Pelvic inflammatory disease	Cefoxitin plus doxycycline[1,2]
	3. Disseminated	Disseminated gonococcal infection	Ceftriaxone[1]
	4. Neonatal	Conjunctivitis (ophthalmia neonatorum)	Ceftriaxone[3]

[1]Other drugs can also be used. See treatment guidelines published by the Centers for Disease Control and Prevention.

[2]Add doxycycline for possible coinfection with *Chlamydia trachomatis*.

[3]For prevention, use erythromycin ointment or silver nitrate drops.

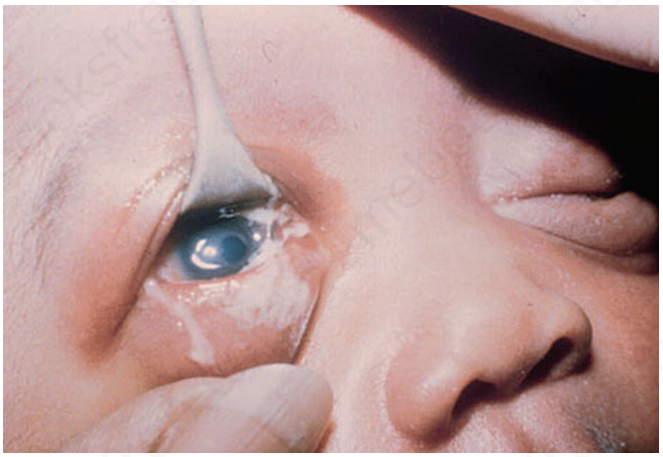

FIGURE 16–3 Neonatal conjunctivitis (ophthalmia neonatorum) caused by *Neisseria gonorrhoeae*. Note purulent exudate, especially on lower right eyelid. The other common cause of neonatal conjunctivitis is *Chlamydia trachomatis*.

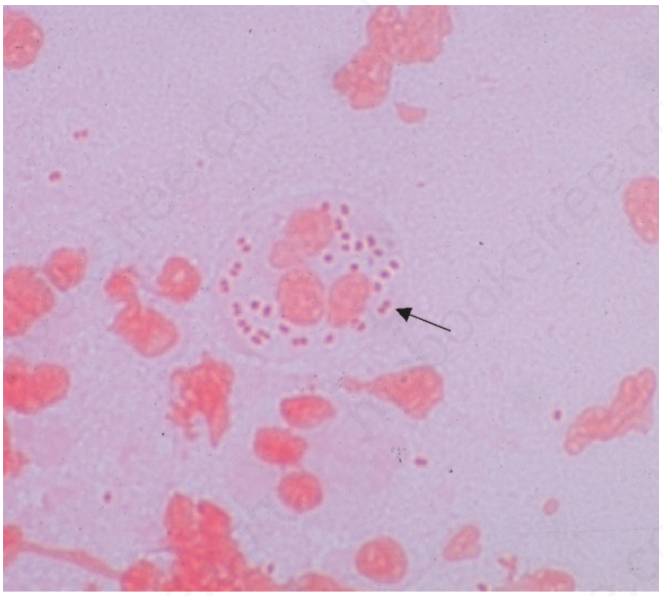

FIGURE 16–4 *Neisseria gonorrhoeae*—Gram stain. Arrow points to typical "kidney bean"–shaped gram-negative diplococci within a neutrophil. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

TABLE 16–3 Properties of the Polysaccharide Capsule of the Meningococcus[1]

(1) Enhances virulence by its antiphagocytic action
(2) Is the antigen that defines the serologic groups
(3) Is the antigen detected in the spinal fluid of patients with meningitis
(4) Is the antigen in the vaccine

[1]The same four features apply to the capsule of the pneumococcus and *Haemophilus influenzae*.

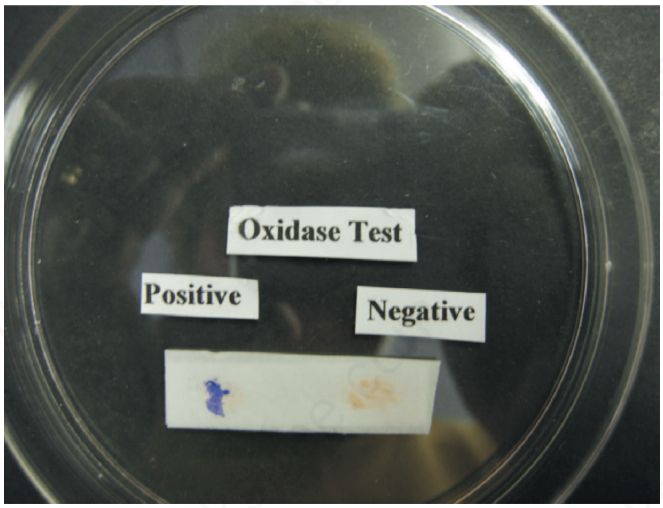

FIGURE 16–5 Oxidase test—A drop of the oxidase reagent was placed on the left and right side of the filter paper. Bacteria from a colony of *Neisseria gonorrhoeae* were rubbed on the drop on the left, and the purple color indicates a positive test (i.e., the organism is oxidase-positive). Bacteria from a colony of *Escherichia coli* were rubbed on the drop on the right, and the absence of a purple color indicates a negative test (i.e., the organism is oxidase-negative). (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

flora but can cause such respiratory tract infections as sinusitis, otitis media, bronchitis, and pneumonia. *Moraxella catarrhalis* and members of other genera, such as *Branhamella, Kingella*, and *Acinetobacter*, are described in Chapter 27. (*Moraxella catarrhalis* is the new name for *Branhamella catarrhalis*.)

1. *Neisseria meningitidis*

Pathogenesis & Epidemiology

Humans are the only natural hosts for meningococci. The organisms are transmitted by **airborne droplets;** they colonize the membranes of the nasopharynx and become part of the transient flora of the upper respiratory tract. Carriers are usually asymptomatic. From the nasopharynx, the organism can enter the bloodstream and spread to specific sites, such as the meninges or joints, or be disseminated throughout the body (meningococcemia).

About 5% of people become chronic carriers and serve as a source of infection for others. The carriage rate can be as high as 35% in people who live in close quarters (e.g., military recruits); this explains the high frequency of outbreaks of meningitis in the armed forces prior to the use of the vaccine. The carriage rate is also high in close (family) contacts of patients. Outbreaks of meningococcal disease also have occurred in college students living in dormitories.

Two organisms cause more than 80% of cases of bacterial meningitis in infants older than 2 months of age: *S. pneumoniae* and *N. meningitidis*. Of these organisms,

meningococci, especially those in group A, are most likely to cause **epidemics of meningitis.** Group B meningococci cause many cases of meningitis in developed countries because it is not present in the capsular polysaccharide vaccine (see "Prevention," later). Overall, *N. meningitidis* ranks second to *S. pneumoniae* as a cause of meningitis but is the most common cause in persons between the ages of 2 and 18 years.

Meningococci have four important virulence factors:

(1) A **polysaccharide capsule** that enables the organism to resist phagocytosis by polymorphonuclear leukocytes (PMNs). The capsule is the immunogen in several commonly used vaccines against meningococci.

(2) **Endotoxin,** which causes fever, shock, and other pathophysiologic changes (in purified form, endotoxin can reproduce many of the clinical manifestations of meningococcemia).

(3) An **immunoglobulin A (IgA) protease** that helps the bacteria attach to the membranes of the upper respiratory tract by cleaving secretory IgA.

(4) **Factor H binding protein** (FHBP) on meningococci binds Factor H, an inhibitor of complement factor C3b. The presence of Factor H on the surface of meningococci reduces the opsonizing activity of C3b and reduces the amount of membrane attack complex produced (see complement action in Chapter 63). FHBP is the immunogen in the vaccine against group B meningococci.

Resistance to disease correlates with the presence of antibody to the capsular polysaccharide. Most carriers develop protective antibody titers within 2 weeks of colonization. Because immunity is group-specific, it is possible to have protective antibodies to one group of organisms yet be susceptible to infection by organisms of the other groups. Complement is an important feature of the host defenses, because people with complement deficiencies, particularly in the **late-acting complement components** (C6–C9), have an increased incidence of meningococcal bacteremia.

Clinical Findings

The two most important manifestations of disease are **meningococcemia** (see Figure 16–1) and **meningitis.** The most severe form of meningococcemia is the life-threatening **Waterhouse–Friderichsen syndrome,** which is characterized by high fever, shock, widespread purpura, disseminated intravascular coagulation, thrombocytopenia, and adrenal insufficiency. Bacteremia can result in the seeding of many organs, especially the meninges. The symptoms of meningococcal meningitis are those of typical bacterial meningitis, namely, fever, headache, stiff neck, and an increased level of PMNs in spinal fluid.

Laboratory Diagnosis

The principal laboratory procedures are smear and culture of blood and spinal fluid samples. A presumptive diagnosis of meningococcal meningitis can be made if gram-negative cocci are seen in a smear of spinal fluid (see Figure 16–4). The organism grows best on chocolate agar incubated at 37°C in a 5% CO_2 atmosphere. A presumptive diagnosis of *Neisseria* can be made if oxidase-positive colonies of gram-negative diplococci are found (see Figure 16–5). The differentiation between *N. meningitidis* and *N. gonorrhoeae* is made on the basis of sugar fermentation: meningococci ferment maltose, whereas gonococci do not (both organisms ferment glucose). Immunofluorescence can also be used to identify these species. Tests for serum antibodies are not useful for clinical diagnosis. However, a procedure that can assist in the rapid diagnosis of meningococcal meningitis is the latex agglutination test, which detects capsular polysaccharide in the spinal fluid.

Treatment

Penicillin G is the treatment of choice for meningococcal infections. A third-generation cephalosporin such as ceftriaxone can also be used. Strains resistant to penicillin have rarely emerged, but sulfonamide resistance is common. In 2007–2008, strains of *N. meningitidis* resistant to ciprofloxacin emerged.

Prevention

Chemoprophylaxis and immunization are both used to prevent meningococcal disease. Either rifampin or ciprofloxacin can be used for prophylaxis in people who have had close contact with the index case. These drugs are preferred because they are efficiently secreted into the saliva, in contrast to penicillin G.

The vaccines against groups A, C, Y, and W-135 meningococci contain the polysaccharide capsule as the immunogen. The vaccine against group B meningococci contains Factor H binding protein as the immunogen.

There are three forms of the polysaccharide vaccine for use in the United States, all of which contain the capsular polysaccharide of groups A, C, Y, and W-135 as the immunogen. There are two forms of the conjugate vaccine: Menactra contains the four polysaccharides conjugated to diphtheria toxoid as the carrier protein, whereas Menveo contains the four polysaccharides conjugated to a nontoxic mutant of diphtheria toxin as the carrier protein. Menomune, the unconjugated vaccine, contains only the four polysaccharides (not conjugated to a carrier protein). The conjugate vaccines induce higher titers of antibodies in children than does the unconjugated vaccine. The vaccines induce similar antibody titers in adults. Note that none of the vaccines contain the group B polysaccharide because it is not immunogenic in humans. A fourth vaccine created for use in the meningitis belt of Africa called MenAfriVac is a conjugate vaccine that contains only the group A polysaccharide.

In general, the conjugate vaccines are preferred over the unconjugated version. The unconjugated vaccine is effective in preventing epidemics of meningitis and in reducing

the carrier rate, especially in military personnel. Travelers to areas where epidemics are occurring should receive the vaccine. College students living in dormitories are encouraged to receive the vaccine. No booster dose is recommended for either form of the vaccine if given after the age of 16 years. The conjugate vaccine is recommended for children at the age of 11 to 12 years, which will reduce the incidence of meningococcal disease in teenagers and young adults. A booster dose is recommended for those who received the conjugate vaccine prior to the age of 16. Vaccine Adverse Events Reports describe several cases of Guillain-Barré syndrome following immunization with Menactra. A causal relationship between the immunization and Guillain-Barré syndrome has not been established.

The vaccine against group B meningococci containing Factor H binding protein as the immunogen was approved in 2014. It induces antibody against the binding protein thereby inhibiting the ability of the bacteria to bind factor H. This enhances the action of complement, an important host defense, because factor H inhibits complement component C3b. Factor H binding protein for use in the vaccine is made by recombinant DNA techniques in *Escherichia coli*. The vaccine is approved for use in people 10 to 25 years of age. In 2015, a second vaccine against group B meningococci containing four surface proteins (fHbp, NadA, NHBA, and PorA) was approved.

2. *Neisseria gonorrhoeae*

Pathogenesis & Epidemiology

Gonococci, like meningococci, cause disease only in humans. The organism is usually transmitted **sexually;** newborns can be infected during birth. Because gonococcus is quite sensitive to dehydration and cool conditions, sexual transmission favors its survival. Gonorrhea is usually symptomatic in men but often asymptomatic in women. Genital tract infections are the most common source of the organism, but anorectal and pharyngeal infections are important sources as well.

Pili constitute one of the most important virulence factors, because they mediate attachment to mucosal cell surfaces and are antiphagocytic. Piliated gonococci are usually virulent, whereas nonpiliated strains are avirulent. Two virulence factors in the cell wall are **endotoxin (lipooligosaccharide, LOS)** and the **outer membrane proteins**. The organism's **IgA protease** can hydrolyze secretory IgA, which could otherwise block attachment to the mucosa. Gonococci have no capsules.

The main host defenses against gonococci are antibodies (IgA and IgG), complement, and neutrophils. Antibody-mediated opsonization and killing within phagocytes occur, but repeated gonococcal infections are common, primarily as a result of antigenic changes of pili and the outer membrane proteins.

Gonococci infect primarily the mucosal surfaces (e.g., the urethra and vagina), but dissemination occurs. Certain strains of gonococci cause disseminated infections more frequently than others. The most important feature of these strains is their resistance to being killed by antibodies and complement. The mechanism of this "serum resistance" is uncertain, but the presence of a porin protein (porin A) in the cell wall, which inactivates the C3b component of complement, appears to play an important role.

The occurrence of a disseminated infection is a function not only of the strain of gonococcus but also of the effectiveness of the host defenses. Persons with a deficiency of the late-acting complement components (C6–C9) are at risk for disseminated infections, as are women during menses and pregnancy. Disseminated infections usually arise from asymptomatic infections, indicating that local inflammation may deter dissemination.

Clinical Findings

Gonococci cause both localized infections, usually in the genital tract, and disseminated infections with seeding of various organs. Gonococci reach these organs via the bloodstream (gonococcal bacteremia).

Gonorrhea in men is characterized primarily by urethritis accompanied by dysuria and a purulent discharge (see Figure 16–2). Epididymitis can occur.

In women, infection is located primarily in the endocervix, causing a purulent vaginal discharge and intermenstrual bleeding (cervicitis). The most frequent complication in women is an ascending infection of the uterine tubes (**salpingitis, PID**), which can result in **sterility** or ectopic pregnancy as a result of scarring of the tubes.

Disseminated gonococcal infections (DGI) commonly manifest as arthritis, tenosynovitis, or pustules in the skin. Disseminated infection is the most common cause of septic arthritis in sexually active adults. The clinical diagnosis of DGI is often difficult to confirm using laboratory tests because the organism is not cultured in more than 50% of cases.

Other infected sites include the anorectal area, throat, and eyes. Anorectal infections occur chiefly in women and homosexual men. They are frequently asymptomatic, but a bloody or purulent discharge (proctitis) can occur. In the throat, pharyngitis occurs, but many patients are asymptomatic. In newborn infants, purulent conjunctivitis (ophthalmia neonatorum) (see Figure 16–3) is the result of gonococcal infection acquired from the mother during passage through the birth canal. The incidence of gonococcal ophthalmia has declined greatly in recent years because of the widespread use of prophylactic erythromycin eye ointment (or silver nitrate) applied shortly after birth. Gonococcal conjunctivitis also occurs in adults as a result of the transfer of organisms from the genitals to the eye.

Other sexually transmitted infections (e.g., syphilis and nongonococcal urethritis caused by *Chlamydia trachomatis*) can coexist with gonorrhea; therefore, appropriate diagnostic and therapeutic measures must be taken.

Laboratory Diagnosis

The diagnosis of urogenital infections depends on Gram staining and culture of the discharge (see Figure 16–4). However, nucleic acid amplification tests are widely used as screening tests (see later).

In **men,** the finding of gram-negative diplococci **within PMNs** in a urethral discharge specimen is sufficient for diagnosis (see Figure 16–4). In **women,** the use of the Gram stain alone can be difficult to interpret; therefore, cultures should be done. Gram stains on cervical specimens can be falsely positive because of the presence of gram-negative diplococci in the normal flora and can be falsely negative because of the inability to see small numbers of gonococci when using the oil immersion lens. Cultures must also be used in diagnosing suspected pharyngitis or anorectal infections.

Specimens from mucosal sites, such as the urethra and cervix, are cultured on Thayer-Martin medium, which is a chocolate agar containing antibiotics (vancomycin, colistin, trimethoprim, and nystatin) to suppress the normal flora. The finding of an oxidase-positive colony (see Figure 16–5) composed of gram-negative diplococci is sufficient to identify the isolate as a member of the genus *Neisseria*. Specific identification of the gonococcus can be made either by its fermentation of glucose (but not maltose) or by fluorescent-antibody staining. Note that specimens from sterile sites, such as blood or joint fluid, can be cultured on chocolate agar without antibiotics because there is no competing normal flora.

Nucleic acid amplification tests, often abbreviated NAAT, detect the presence of gonococcal nucleic acids in patient specimens. These tests are widely used for screening purposes, produce results rapidly, and are highly sensitive and specific. They can be used on urine samples, obviating the need for more invasive collection techniques. Note that serologic tests to determine the presence of antibody to gonococci in the patient's serum are *not* useful for diagnosis.

Treatment

Ceftriaxone is the treatment of choice in uncomplicated gonococcal infections. Azithromycin or ciprofloxacin should be used if the patient is allergic to penicillins or cephalosporins. Because mixed infections with *C. trachomatis* are common, azithromycin or doxycycline should be prescribed also. A follow-up culture should be performed 1 week after completion of treatment to determine whether gonococci are still present. Treatment of complicated gonococcal infections, such as PID, typically requires hospitalization. Treatment regimens are complex and beyond the scope of this book.

Prior to the mid-1950s, all gonococci were highly sensitive to penicillin. Subsequently, isolates emerged with low-level resistance to penicillin and to other antibiotics such as tetracycline and chloramphenicol. This type of resistance is encoded by the bacterial chromosome and is due to reduced uptake of the drug or to altered binding sites rather than to enzymatic degradation of the drug.

Then, in 1976, **penicillinase-producing (PPNG)** strains that exhibited high-level resistance were isolated from patients. Penicillinase is plasmid-encoded. PPNG strains are now common in many areas of the world, including several urban areas in the United States, where approximately 10% of isolates are resistant. Isolates resistant to fluoroquinolones, such as ciprofloxacin, have become a significant problem, and fluoroquinolones are not recommended as treatment.

Prevention

The prevention of gonorrhea involves the use of condoms and the prompt treatment of symptomatic patients and their contacts. Cases of gonorrhea must be reported to the public health department to ensure proper follow-up. A major problem is the detection of asymptomatic carriers. Gonococcal conjunctivitis in newborns is prevented most often by the use of erythromycin ointment. Silver nitrate drops are used less frequently. No vaccine is available.

SELF-ASSESSMENT QUESTIONS

1. Regarding the differences between *N. meningitidis* (meningococci) and *N. gonorrhoeae* (gonococci), which one of the following is the most accurate statement?

(A) Meningococci are oxidase-positive, whereas gonococci are not.
(B) Meningococci have a thick polysaccharide capsule, whereas gonococci do not.
(C) Meningococci have lipid A, whereas gonococci do not.
(D) Meningococci produce penicillinase, whereas gonococci do not.
(E) Meningococci synthesize IgA protease, whereas gonococci do not.

2. Your patient is a 14-year-old girl who was sent home from school because she had a fever of 102°C, a severe headache, and was falling asleep in class. When her fever rose to 104°C, her mother took her to the emergency room, where a blood pressure of 60/20 and several petechial hemorrhages were found. Gram-negative diplococci were seen in a Gram stain of the spinal fluid. Which one of the following is most likely to cause the fever, hypotension, and petechial hemorrhages?

(A) Endotoxin
(B) IgA protease
(C) Oxidase
(D) Pilus protein
(E) Superantigen

3. Regarding the patient in Question 2, which one of the following is the best antibiotic to treat the infection?

(A) Azithromycin
(B) Doxycycline
(C) Penicillin G
(D) Rifampin
(E) Trimethoprim-sulfamethoxazole

4. Regarding the differences between *N. meningitidis* (meningococci) and *N. gonorrhoeae* (gonococci), which one of the following is the most accurate statement?

(A) Humans are the reservoir for both organisms.
(B) Many clinical isolates of meningococci produce β-lactamase, but clinical isolates of gonococci do not.
(C) Meningococci have multiple antigenic types, but gonococci have only one antigenic type.
(D) The conjugate vaccine against gonorrhea contains seven types of the pilus protein as the immunogen.
(E) The main mode of transmission for both organisms is respiratory droplets.

5. Your patient is a 20-year-old man with a urethral exudate. You do a Gram stain of the pus and see gram-negative diplococci with neutrophils. Which one of the following is the best antibiotic to treat the infection?

(A) Ceftriaxone
(B) Gentamicin
(C) Penicillin G
(D) Trimethoprim-sulfamethoxazole
(E) Vancomycin

ANSWERS

1. **(B)**
2. **(A)**
3. **(C)**
4. **(A)**
5. **(A)**

SUMMARIES OF ORGANISMS

Brief summaries of the organisms described in this chapter begin on page 655. 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.